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26th World's Poultry Congress

BOOK OF ABSTRACTS 2022

Abstracts submitted in 2020 and 2021 and selected in 2022

Organized by

French Branch of the World's Poultry Science Association

The 26th World's Poultry Congress - 07-11 August 2022

Publisher French Branch of the World's Poultry Science Association

Editors Dr Michèle TIXIER-BOICHARD, chair of 26th WPC Dr Michel DUCLOS, Chairman of the Scientific Committee of 26th WPC

Professional Congress Organizer (PCO) – Colloquium Group (Paris, France)

Welcome Page

Dear participants to the 26th WPC, dear readers,

In the context of the COVID-19 pandemics, which emerged in 2020 and is still present, the highest priority of the French Branch of WPSA and the organizing committee of the 26th World's Poultry Congress has been to organise a successful congress, avoiding health hazards and welcoming participants from all continents. As a consequence, the event planned in 2020 had to be postponed to 2021 and again to 2022.

We are grateful to our sponsors and to the invited speakers who maintained their contribution despite this complex background.

This Book of Abstracts gathers all abstracts that were submitted in 2020 or 2021 and accepted in 2022. They are ordered by sessions, starting with the selected short oral communications presented in Paris 7-11 August 2022, followed by the electronic posters, that will be available on screen and on the congress platform, **https://viewr.wpcparis2022.com/**, together with the webinars which took place between September 2021 and May 2022.

We hope you will enjoy and remember this congress.

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OBJECT-ORIENTED SESSIONS

Adapting poultry production to climate change

Selected short communications

QUANTIFICATION OF THE EFFECT OF FEEDING STRATEGIES ON THE CARBON EMISSIONS OF A BRAZILIAN BROILER PRODUCTION SYSTEM.

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In addition to feed driving the cost of production of broiler systems, carbon footprint of live broiler production is also known to be highly related to feed. Although feeding strategies largely differ depending on countries, regulation, and product standards (e.g., with animal by-products, antibiotic-free, organic, all vegetal diet), few data has been yet collected to quantify the effect of feeding strategy on carbon emissions of live broiler production. A study was conducted with a major Brazilian broiler producer to quantify the greenhouse gas emissions of a conventional broiler production system. A first objective was to simulate the carbon emissions in kg CO2 equivalent/kg live weight at farm gate, and identify opportunities for reducing carbon footprint. The second objective was to quantify the impact of using all vegetal feed program, as compared to a conventional feed program with animal by-products, while assuming no effect of feed on broiler performance. The carbon emissions were estimated following the IPCC guidelines and using the GFLI database, using the economic allocation with land use change for each ingredient. The simulation was completed using a standard feed program, composed of 4 feed phases. The average performance was 3kg of live weight at 45 days of age, with a feed conversion ratio of 1.70 kg weight gain per kg of feed used. The diets were corn and soybean meal based, and consisted of synthetic amino acids, and enzymes. The results indicated that feed carbon footprint contributed to more than 90% of total carbon emissions in kg CO2 equivalent/kg live weight. Using poultry fat rather than soya oil allowed to reduce carbon footprint by 9% when maintaining fat level at 2% of feed diet, because the carbon emission factor of poultry fat is almost 4 times as low as soya oil. Supplying feed diets with no animal by-product resulted in a 28% increase of broiler carbon emissions, as compared to feed program based on vegetal ingredients only. The increase in carbon emissions was mostly due to the use of soya oil and the increased used of soybean meal as a substitute of animal meal. Overall, the study demonstrated that deciding feeding strategy is a main driver of carbon emissions in intensive live broiler production systems, and ingredient sourcing can reduce the carbon emissions in a more significant manner than improving performance.

EVALUATION OF WATER INTAKE, FEED INTAKE AND PROCESSING TRAITS OF FOUR COMMERCIAL BROILER LINES USING A LOW FLOW WATER MONITORING SYSTEM

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Water scarcity is a present and growing global concern, as population growth and industrialization place strain on the available freshwater resources necessary to provide for both human needs and agricultural applications. To guarantee a sustainable and agriculturally secure future, production efficiencies involving water usage must improve. Being that, globally, poultry is the most consumed meat, it is important that water usage and efficiency in broilers be audited. This study set out to evaluate, using a novel low flow water monitoring system, the water conversion ratio (WCR) of four contemporary broiler strains. Day old chicks from four commercial lines were placed sex separate (n=25 per line/sex) in floor pens. Live performance characteristics including body weight, water intake (WI), feed intake (FI), WCR and FCR were recorded weekly through 8 weeks. Carcass traits were evaluated at two market ages (day 43 and 56). Differences in WI and WCR were observed between broiler lines. WCR of the strains ranged from 2.81 to 2.89 and 3.23 to 3.38 at market age of 43 and 56 days, respectively. Per bird WI, in kilograms, ranged from 8.56 to 9.89 and 13.9 to 15.67 at market age of 43 and 56 days, respectively. Parts weights were evaluated in terms of water intake (a) to weight (g), or part water conversion ratio (PWCR). Differences in PWCR were observed between strains in the breast, wing, and leg. These methods of evaluation were successful in characterizing the water consumption and efficiencies of four contemporary broiler strains. The WI and WCR values for each respective strain were recorded with differences being observed between each strain. These findings serve as baseline for performance standards and may indicate that little selection pressure has been employed on water efficiency related traits in broiler populations.

GROWTH PERFORMANCE REDUCTION AND BEHAVIOURAL ADAPTATIONS IN HEAT STRESSED CONVENTIONAL AND THAI NATIVE BROILERS

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Heat stress is the complex of events resulting from adaptation and damage when, due to high environmental temperature, the body's capabilities to control internal temperature fail. Climate changes make heat stress in commercial poultry a problem expected to worsen in the coming decades. Broilers kept above the upper critical temperature aim behaviour at dissipating heat rather than at the intake of feed. Of this adaptation to cope with heat stress, both less feed intake to decrease metabolic heat and dissipating heat behaviour are thought to reduce body weight gain. It is however, not known whether the reduction in growth performance in broilers can be fully attributed to the reduction of feed intake. The aim of the current experiment was to investigate the growth performance in broilers subjected to the following treatments: 1. ad libitum (AL) feeding and housed under heat stress (HS-AL) conditions; 2. AL fed and housed under temperature controlled (TC-AL) conditions; and 3. Pair-fed with HS-AL and housed under temperature controlled (TC-PF) conditions. It is considered of scientific interest to subject these treatments to both modern genotype birds (Ross 308) and Thai native broilers (Korat broilers). The experiment had a 2x3 factorial design with 6 replicates (10 birds/pen) per treatment, pen was considered an experimental unit. During the experiment, ambient temperature ranged between 28.2 °C (SD 2.0, 8.00 h) and 35.0 °C (SD 3.4, 16.00 h). At 08.00, cloacal temperature (CT) was similar between treatments (i.e. ~ 41.5 °C) and genotypes but at 16.00 h CT was increased to 43.2 °C only in HS-AL in Ross 308 birds (p = < 0.01). Feed conversion ratio (FCR) was affected by housing in both Ross 308 and Korat birds (P=0.017) and FCR in the TC-PF Ross 308 birds was similar to their counterparts housed under heat stressed conditions (i.e. the HS-AL Ross 308 birds). In contrast, FCR in the TC-PF Korat birds was higher ($P \le 0.005$) compared to their counterparts housed under HS conditions (HS-AL, Korat). The HS conventional broilers showed clear behavioural responses (high respiratory rate and gular fluttering) compared to Korat broilers. At 16.00 h, conventional broilers were observed in flaccid recumbency while Korat broilers were foraging at that time. The TC-PF Korat broilers showed huddling and increased foraging behaviour. It was concluded that the behavioural responses in Ross 308 birds did not negatively affect the feed conversion ratio.

SUPPLEMENTATION OF POSTBIOTIC LACTOBACILLUS INGLUVIEI C37 IMPROVES GROWTH PERFORMANCE AND EXPRESSION OF GENE-RELATED ANTIOXIDANT ENZYMES AND GUT BARRIERS IN BROILERS SUBJECTED TO HEAT STRESS

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Heat stress (HS) is recognized as a critical environmental stressor that reduces performance, health and well-being of farm animals including poultry. HS increases the generation and accumulation of reactive oxygen species and free radicals in the cells more than the ability of the body's defense mechanisms, thus causing damage cells including out barrier integrity led to impair nutrient absorption and tremendous economic losses in worldwide poultry production. This study aimed to evaluate the efficacy of dead cell L. ingluviei C37 (DC-LIC37) on growth performance, gene-related antioxidant enzymes and gut barriers in broilers exposed to heat stress. A total of 360 male broilers Ross 308 (1-d-old) were allocated into 6 groups in a CRD: thermoneutral zone (TNZ) and received basal diet (control group). While the 2-6 groups were exposed to chronic HS at $32 \pm 1^{\circ}$ C for 5 h daily, started at the age of 15 d until the end of the experiment and received one of the 5 following diets: basal diet (NC group); basal diet + zinc bacitracin at 0.05 g/kg (PC group). The other 3 groups were supplemented with different levels of DC-LIC37 as follows: basal diet + DC-LIC37 at levels 107, 108 and 109 CFU/kg, respectively. At 42 d, the liver and jejunal mucosa of each treatment were collected for the expression of gene-related antioxidant enzymes and gut barriers. All data were analyzed by SPSS software, and comparisons of the means were identified using Tukey' HSD and compared between groups with orthogonal contrasts. Differences were considered to be statistically significant at P < 0.05. The result revealed that supplementation of DC-LIC37 at 107 CFU/kg can improve BW gain, FI, European production efficiency factor and European broiler index of broilers under HS similar to TNZ (P>0.05). In addition, DC-LIC37 can upregulate jejunal gut barrier integrity (MUC-2, JAM and OCCLDN) and antioxidant enzyme (SOD, CAT and GPx) mRNA expression levels. This suggests that DC-LIC37 has the potential to mitigate the deleterious effects of HS and could be useful for future use as a feed additive in broiler industry.

Adapting poultry production to climate change

Posters

OFFERING A COMBINATION OF PLANT EXTRACTS MITIGATES THE ADVERSE EFFECTS OF HEAT STRESS ON BROILER BEHAVIOR

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Rising global temperatures increasingly challenge broiler health and welfare. In the face of heat stress, broilers activate heat dissipation mechanisms, which penalise their productive potential (Renaudeau et al., 2019). Recording behavioral responses to heat stress reflects their capacity to cope with a stressful condition (Keeling and Jensen, 2002). Plant secondary metabolites ameliorate the adverse effects of heat stress via their antioxidant and anti-inflammatory activities, mediating cellular protection (Hu et al., 2019). Herein, we aimed at investigating the effects of offering a combination of extracts deriving from Curcuma longa and Scutellaria baicalensis (AxionFeedstim®) on broiler behavior upon heat stress exposure. Day-old male (128) Ross 308 chicks were allocated to 32 cages and were offered commercial starter (d0-10) grower (d10-21) and finisher (d21-35) diets supplemented (S) or not (C) with AxionFeedstim®. During the finisher period, they were exposed to constant heat stress (30°C). Behavior measurements were carried prior to the onset (d0), and over the acute (d3) and adaptation (d10) phases of heat stress. A specific ethogram was used to record the frequency of open beaks, wing spreading, activity (standing/sitting), feed and water consumption and preening. Logistic regression was applied to analyse the differences in behavioral frequencies (R Studio version 1.3.1093). Birds showed increased frequency of open beaks and wing spreading over both acute and adaptation phases in comparison to thermoneutral conditions, as expected (P<0.001). Birds were more affected during the adaptation than the acute phase, showing reduced capacity to tolerate continuous heat stress exposure at older ages. During the acute phase, the S group showed lower frequency of open beaks (P<0.001) and wing spreading (P<0.001) than the C group. During the adaptation period, previously observed effects persisted, but differences between C and S groups were less pronounced for open beaks (P < 0.05), than wing spreading (P<0.01). Feed and water intake, standing, sitting and preening frequencies were variably affected during the heat stress periods and did not differ significantly between groups. In conclusion, birds showed reduced capacity to adapt to a constant heat stress model over time. Offering AxionFeedstim® reduced the frequency of heat stress related behaviors such as open beaks and wing spreading, effects being more pronounced during the acute phase of heat stress.

ESTIMATION OF GREENHOUSE GAS EMISSIONS FROM POULTRY PRODUCTION IN THE REPUBLIC OF CROATIA FROM 1990 TO 2020

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Due to the harmful effects of greenhouse gases on the climate, many countries, including the Republic of Croatia, have committed to monitoring and gradually reducing greenhouse gas emissions. Livestock production as one of the significant factors of total greenhouse gas emissions is also subject to this obligation. IPCC guidelines were used for calculation and poultry production was divided in 5 categories (laying hens, broilers, turkey, geese and ducks). Methane emissions and direct and indirect nitrogen oxide emissions from manure management were calculated. Methane emissions from enteric fermentation for poultry were not calculated because it is considered that due to the specifics of their digestive system and the types of feed they are fed, methane emissions from enteric fermentation are negligible. Poultry production contributed to methane emission on average with 0,94% and to nitrogen oxide emission on average with 21,10%. Overall, poultry production in Croatia in the given time period emitted 1597,75 kt CO 2eq which is 2,51% of the total emission from livestock production and 0,27% of the total emission of greenhouse gasses emitted in Croatia. This result shows that poultry production has a lower impact on environment than other livestock productions, but despite that, further research is needed on improving feed digestibility and genetic predispositions of animals in order to maintain the current production while reducing greenhouse gas emission.

THE EFFECT OF OXIDATIVE STRESS AT THE WARM SEASON IN FREE-RANGE POULTRY CHICKENS RAISED IN FIELD CONDITIONS

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Introduction. The animal welfare requirements and the growing interest of the consumer in high-quality food have resulted in the production of outdoor chicken systems. Climate changes and the heat stress of the expanded summer period were suggested to influence the meat quality, a hypothesis that is under consideration. 8-isoprostane is biomarker of lipid peroxidation and oxidative stress. The aim of this study was to estimate the effect of oxidative stress at the warm season in free-range broiler chickens raised in field conditions compared to the meat quality [1-3]. Material and Methods. The study took place during 2019-2021 in PINDOS poultry farms and subjects were divided in groups according to the season as follows: warm group (summer-autumn; wG, n=90) and cold group (winterspring; cG, n=90) seasons, respectively. Free-range broilers (Sasso genotype, slowgrowing) were fed a standard diet according to dietary standards and reared indoors and outdoors (13 birds/m2 indoors and 15 birds/m2 forage paddock) for 67 days. Plasma samples were stored at -80 oC. Biochemical analysis was determined using established methods and 8-iso-PGF2a measured using a competitive ELISA. Data were analyzed using SPSS 21.0. Results. 8-iso-PGF2a plasma levels of summer and autumn groups appeared significantly different between winter and spring respectively. Plasma levels of 8-iso-PGF2a were significantly higher (p < 0.001) in wG group (2.4±0.5 ng/ml) compared to the cG group $(1.5\pm0.6 \text{ ng/ml})$. No significant differences (p>0.05) were observed in Body Weight (BW), Feed Conversion Ratio (FCR) and European Production Efficiency Factor (EPEF) between 2 groups. Conclusion. The levels of 8-iso-PGF2a in free-range broiler chickens raised in field conditions were significantly higher at the warm season compared to the cold season, suggesting higher levels of lipid peroxidation and oxidative stress caused by high temperature exposure. However, this observation wasn't correlated with meat quality and productivity under our conditions.

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Acknowledgments. This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE –INNOVATE (project code: T1EDK-03939)

THE EFFECT OF MANIPULATING DIETARY ACID BINDING CAPACITY LEVEL BY INCLUSION OF A MIX OF ORGANIC ACID SALTS ON GROWTH PERFORMANCE, GASTROINTESTINAL TRACT TRAITS, LITTER AND TIBIA QUALITY IN BROILERS UNDER HEAT STRESS

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Poultry diets have high alkalinity characteristics due to a considerable amount of plant-based protein and calcium carbonate, compromising the intestine's ability to keep an acidity level that can support the growth of beneficial intestinal microbiota. Acid binding capacity (ABC) defines as the amount of acid required to reduce the pH of feed to a certain level, which can be considered a possible approach to optimize the gastrointestinal tract (GIT) environment. Selacid BC[™] (Selko, The Netherlands) comprises various organic acid salts, acting as a low-ABC substitution for limestone and sodium bicarbonate. Heat stress has been reported to reduce the secretion of digestive enzymes and nutrient absorption. Adjusting ABC may offset the adverse impact of heat stress on growth performance. This trial aimed to evaluate the impact of the Selacid BC on growth performance, GIT traits, litter and tibia quality in heat-stressed broilers. A total of 1680 male Ross 308 chicks were distributed into four dietary treatments with ten replications. Starting from d7, heat stress involved exposure to 26 °C up to 35°C with 50% relative humidity for 8 hours. Four dietary treatments contained 0.0, 0.2, 0.5 and 0.8% of Selacid BC with 18, 48 and 78 meg/kg calculated ABC reduction, respectively. Birds were sampled for GIT and tibia analysis on d12 and d41, litter quality on d40, and carcass yield on d42. The results showed a tendency of quadratic response on body weight (BW) on d28, in which 0.2, 0.5 and 0.8% groups had 19.6, 26.4, and 7.2 g heavier BW, respectively than the 0.0% group (P = 0.079). Selacid BC did not affect feed intake, FCR, mortality and morbidity, carcass yield, BW on d10 and d40 (P > 0.05). The pH of crop and ceca on d12 was reduced, and the pH of gizzard was increased by adding every Selacid BC level ($P \leq 0.05$). The ileum's length on d12 was increased quadratically by 2.3, 3.4, 0.3 cm, and the duodenum's length on d40 was increased guadratically by 2.0, 1.6 and 0.5 cm, respectively, in 0.2, 0.5, and 0.8% than 0.0% Selacid BC group. Litter moisture showed a tendency to be reduced quadratically by 1.4, 2.2, and 0.14%, respectively, compared to the 0.0% Selacid BC group (P = 0.099). Tibia breaking strength and litter pH were not affected by Selacid BC. In conclusion, ABC reduction by adding either 0.2 or 0.5% Selacid BC showed a tendency to improve growth performance, GIT traits and litter moisture in heat-stressed broilers without compromising tibia quality.

Diversity of production systems and services delivered to humans

Selected short communications

GROWTH PERFORMANCE OF SIX LOCAL GERMAN CHICKEN BREEDS TOWARDS SUSTAINABLE USE OF REGIONAL ANIMAL GENETIC RESOURCES

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Local chicken breeds have been displaced by high performing hybrids and many of them are threatened with extinction worldwide. The objective of this project is to develop an alternative approach to poultry production that, on the one hand, addresses the interests of organic farming in a wide range of regionally-related products and, on the other hand, supports the maintenance of genetic diversity in chickens. The concept is based on the use of crossing local chicken breeds with high-performing genotypes. We included six breeds: East Frisian Gull (EFG) & Ramelsloher (RAM); Altsteirer (ALT) & Augsburger (AUG) and Malines (MAL) & Bielefelder (BIE). Based on their geographic origin, chickens are kept at three different research locations. In the absence of reliable performance data, we first recorded the growth rates of these breeds. Mean female live weights at week of life 24 ranged from 1587.7 \pm 191.0 g (mean \pm SD) in AUG to 2918.9 \pm 314.1 g in MAL. For males, live weights ranged from 2022.5 \pm 140.5 g in EFG to 4097.5 \pm 466.3 g in MAL. Considering two ages for slaughtering of males (see abstract Müsseler et al.), average daily weight gains were calculated from hatch to week of life 14 (LW1-14) and for week of life 14 to 18 (LW14-18) by breed and sex. For females, the mean daily weight gains for LW1-14 ranged from 10.5 ± 0.8 g in EFG to 19.6 \pm 2.1 g in MAL and for LW14-18 from 8.6 \pm 2.0 g in EFG to 18.1 \pm 4.5 g in MAL. Higher mean daily weight gains were observed for the males in all breeds. For LW1-14, it was lowest in AUG (13.7 \pm 1.7 g) and highest in MAL (24.4 \pm 3.2 a), while in LW14-18 the lowest mean daily weight gain was observed for EFG $(14.2 \pm 2.4 \text{ g})$ und the highest for MAL $(29.3 \pm 7.1 \text{ g})$. Within each breed, significant differences were found in the calculated mean daily weight gains between males and females in both periods (p < 0.01). By comparing mean daily weight gains of two breeds at each location including both sexes no significant differences were found between ALT and AUG (pLW1-14 = 0.21; pLW14-28 =0.21), while the other breeds differed significantly from each other (EFG vs. RAM; p < 0.01; BIE vs. MAL; p < 0.01). The results of ongoing performance testing will indicate the suitability for use of each breed in terms of weight gain, feed conversion ratio and production efficiency. In the further course of the project, these results will be used to select the best animals of the respective breed for crossbreeding with broiler or layer parents.

EFFECTS OF REDUCING DIETARY CRUDE PROTEIN UNDER TWO DIFFERENT REARING CONDITIONS ON PERFORMANCE, FOOT PAD DERMATITIS, MEAT YIELDS & ENVIRONMENTAL IMPACTS OF BROILER CHICKENS

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Lowering dietary crude protein (CP) has multiple proven benefits on environmental impacts & welfare indicators of broiler chickens. This study investigated the effects of low CP diets under two rearing conditions on growth performance, foot pad dermatitis (FPD), meat yields & environmental impacts. A total of 1,305 male Ross 308 was randomly allocated to a 3×2 factorial design with 3 CP levels (Control: CTR, CTR -1 pt and CTR -2 pts) and 2 rearing conditions (Optimal: O and suboptimal: S) as experimental factors. Each treatment was replicated 5 times. Dietary CP levels were 21.8, 20.3, 18.3 & 17.3%, in the starter, grower, finisher 1 & 2 CTR diets, respectively. Low CP treatments were formulated by reducing CP level by 1 or 2 pts in all phases. Soybean meal was gradually replaced by wheat and feed-grade amino acids (AA). All indispensable AA were adequately supplied at the level of assumed requirement, digestible Lys and AMEn were kept constant across treatments. Changes in rearing conditions were achieved by using Paracox vaccine at 0 and 9 d of age, removing coccidiostats, replacing wood shaving by long straw as bedding material and increasing density from 15.4 to 18.1 bird/m² for S kept birds. A two-way ANOVA was used to compare groups and Pvalues < 0.05 were deemed significant. Birds kept in S conditions had significantly lower 0-42 d growth performance (ADG -4.6 g/d, ADFI -5.74 g/d, corrected FCR +6 pts) and higher FDP scores than their O kept counterparts. Dietary CP had no significant effect on overall growth performance, irrespective of the rearing condition. Reducing CP level by 2 pts improved significantly mean FDP scores (+0.41 pt). Birds fed CTR -2 pts diets tended to have lower growth performance on the 0-21 d period (e.g. ADG -3.6 g/d) and had significantly lower breast meat yields (-0.8 pts) irrespective of the rearing conditions. According to our internal tool, reducing CP showed a positive impact on environmental footprint per ton of chicken meat produced in a dose-dependent manner (e.g. climate change -5.7%, energy use -3.6%, land use -5.5% for CTR -1 pt vs CTR). Summarizing, the present study confirms the importance of technical management and demonstrates that reducing CP improves welfare and environmental outcomes of broiler production without compromising on growth performance. Future research may be directed towards the alleviation of the negative effect of -2 pts CP diets on starting performance and breast meat yield.

Early management of broilers

Selected short communications

EGGSHELL MICROBIOLOGY AND QUALITY OF CHICK EMBRYOS FROM EGGS SANITIZED WITH CLOVE ESSENTIAL OIL

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Sanitizers based on natural compounds can be valuable in sanitizing hatching eggs. Therefore, the aim of this study was to evaluate whether the sanitization of hatching eggs with clove essential oil in the preincubation phase affects the eggshell microbiota and quality of chick embryos. The present study was approved by the Ethics Committee on Animal Use of the University of Brasília under opinion No. 33/2019. Hatching eggs (n = 1,460) from 37-week-old broiler breeder hens of the CPK line were randomly distributed into four treatments in the preincubation phase (nonsanitized; grain alcohol to a concentration of 93.5%; clove essential oil to a concentration of 0.39%; paraformaldehyde to a concentration of 6 g/m^3). The setters were operated at a mean temperature of 37.7°C, a mean relative humidity of 60%, and with automatic turning every hour at a 45° angle for the first 18 days of incubation. Starting on day 19, the setters were operated at a mean temperature of 36.6°C and a 65% relative humidity. The experiment followed a completely randomized design. Analyzes were performed with SAS Studio University Edition (Inst. Inc., Cary, NC, USA). The data were analyzed by analysis of variance (PROC GLM), and means were compared using Tukey's test. Statistical significance was considered at p < 0.05. The count of total aerobic mesophilic bacteria was significantly lower (p < 0.0001) after spraying with clove essential oil $(2.30 \pm 0.24 \log 10 \text{ CFU/mL})$ than on nonsanitized eggs $(3.49 \pm 0.34 \log 10)$ CFU/mL) or on eggs sprayed with grain alcohol ($3.09 \pm 0.14 \log 10 \text{ CFU/mL}$) but did not differ significantly (p > 0.05) from the count in the paraformaldehyde group $(2.23 \pm 0.29 \log 10 \text{ CFU/mL})$. The lengths and weights of embryos at 18 days of incubation were measured to assess the effects of treatments on bird quality. The lengths of embryos (mean = 15.30 ± 1.41 cm), wings, beaks, and leg were not significantly different (p > 0.05) among the treatments. The absolute weights of the embryos (mean = 29.75 ± 3.02 g) and residual yolks (mean = 11.79 ± 2.58 q) were not affected (p > 0.05) by the sanitization treatments. Length and weight measurements showed that the quality of the embryos was not affected. Clove essential oil reduces the number of bacteria in the eggshell and can be used as a sanitizer for incubating eggs safely for the birds.

IMPACT OF A MULTI-SPECIES CONTAMINATION MODEL SIMULATING COMMERCIAL HATCHING CONDITIONS ON 7-DAY PERFORMANCE OF BROILER CHICKENS

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In commercial hatcheries, formaldehyde fumigation has been utilized to control the microbial load in the hatch cabinet environment. Laboratory-based challenge models simulating contamination in commercial hatch cabinets are essential to evaluate the efficacy of alternatives to formaldehyde fumigation prior to commercial application. Previously, an egg surface contamination model was developed for use in small-scale hatch cabinets (225 eggs/cabinet) to replicate contamination associated with rupturing non-viable embryonated eggs that occur in commercial-scale hatch cabinets (>10,000 eggs/cabinet). For the model, multiple hatchery-relevant bacterial and fungal species were isolated from non-viable embryonated egg homogenates. From the homogenate, a multi-species pathogen mix (PM) was prepared and used to simulate contamination in commercial hatch cabinets in a laboratory setting. PM application to the blunt end of the eggshell at DOE19 altered the composition of the microbial bloom during the hatching phase and microbial recovery from the gastrointestinal tract (GIT), fluff samples, and chick rinse samples at DOH as compared to the nonchallenged control (NC). The purpose of the present study was to evaluate the effects of the PM model with and without formaldehyde fumigation on 7-day performance and microbial recovery from the hatching environment, chick fluff, and GIT. Treatment groups included: 1) NC, 2) PM control, 3) PM + formaldehyde. For all three experiments, application of PM challenge at DOE19 increased overall microbial load in the hatching environment and significantly (P<0.05) increased Gram-negative bacterial recovery from GIT at DOH compared to NC. Formaldehyde fumigation during the hatching phase inhibited microbial replication related to PM application, reflected by reduced enteric and environmental contamination. There were no statistical differences for 7-day body weight gain or FCR across treatment groups (Exp 1-3). These data suggest that the PM model could be used to assess alternatives to formaldehyde fumigation to mitigate the microbial bloom in hatch cabinets and alter enteric colonization at hatch but may not be appropriate for performance evaluations.

PREHATCH APPLICATION OF ENTEROCOCCUS FAECIUM AND LACTOBACILLUS ANIMALIS TO BROILERS.

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Early application of probiotics may be beneficial since application prior to establishment of the intestinal flora is expected to result in increased colonization of the intestinal tract of the chickens. In ovo injections is currently used as a method for prehatch application, despite reduced hatchability. However, some hatcheries do not use in ovo injection technology and others prefer alternatives to avoid the reduced hatchability. Therefore, we investigated several non-invasive prehatch application methods for two probiotics and evaluated the success of colonization of the intestinal tract at different time points in relation to hatch. E. faecium (NCIMB11181) and L. animalis (DSM33570) were applied (cfu: 1×107- 6×108) on fertilized eggs at day 16-18 of incubation by either dipping, placing a drop or spraying on the eggshell. In a separate trial, E. faecium were applied in the drinking water for the broiler breeders and the colonization of the offspring were evaluated. Re-isolation of the probiotic from the yolk sac and intestine of the chickens was evaluated either before hatch (prehatch) or right after hatch (post hatch). It was found, that up to 20% of the fetuses were colonized prehatch depending on the application method and probiotic bacteria used. When using spray application with either E. faecium or L. animalis, post hatch sampling revealed that 100% or 54% of the chickens were colonized, respectively. Additionally, E. faecium was found to be transmitted vertically from the mother hen to 20% of the offspring when sampling prehatch. When sampling post hatch 33% of the offspring became colonized. The most successful method of application was spray application with E. faecium were 9% of the fetuses where colonized prehatch and 100% became colonized post hatch. Therefore, it is suggested that prehatch application by spray of this specific E. faecium probiotic on the shell maybe used as an easy-to-use, non-invasive method for early life colonization of the chicken gut.

EFFECTS OF IN OVO INJECTION OF L-ARGININE AND SELENIUM NANOPARTICLES ON PERFORMANCE, NUTRIENT COMPOSITION AND SELENIUM DEPOSITION IN KORAT CHICKENS

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Korat chicken is a slow-growing chicken established by crossbreeding male Thai native chicken and female Suranaree University of Technology breeder line. Korat chicken can reach a market size at 1.2 kg in both sexes at 60-65 days of age. The aim of this study was to evaluate effect of in ovo injection of L-arginine (L-Arg) and selenium nanoparticles (SeNPs) into the amnion at 18 days of incubation on performance, nutrient composition and selenium deposition in Korat chicken. A total of 960 fertilized Korat chicken eggs (based on 80% hatching rate) with similar weights $(48.75 \pm 0.49 \text{ g})$ were distributed in a completely randomized design with 4 treatment groups: a non-injected control group (NC) and 3 groups of 1% L-Arg solution (IOF-1), 0.3 μ g SeNPs solution (IOF-2), and 1% L-Arg + 0.3 μ g SeNPs solution (IOF-3) injection. Each treatment contained 4 replicates of 60 fertilized eggs each. After hatch, chicks were weighed and placed randomly to each treatment group with 4 replicates per group (40 birds per replicate). The chickens were fed ad libitum until 63 days of age. The results showed that in ovo injection at 18 days of incubation had no negative effect on hatchability, hatched weight, final body weight, average daily gain (ADG) and average daily feed intake (ADFI) (P>0.05). However, feed conversion ratio (FCR) was lower in IOF-2 and IOF-3 groups than other groups (P < 0.01). In ovo injection at 18 days of incubation had no effect on ash, fat and collagen contents in breast meat (P>0.05). However, protein content in breast meat was higher in IOF-3 group than other groups (P<0.01). In ovo injection with SeNPs (IOF-2 and IOF-3 groups) at 18 days of incubation had no toxicology of SeNPs in liver, kidney and breast meat when compared with NC and IOF-1 groups. Our results indicate that IOF-3 injection into amnion at 18 days of incubation could reduce FCR and increase protein content without residue nanoparticles in Korat chicken breast meat.

Early management of broilers

Posters
ALGAL SUPPLEMENTATION FROM SACCHARINA LATISSIMA AFFECTS CHICK QUALITY, GUT DEVELOPMENT AND GROWTH PERFORMANCE IN BROILER CHICKS

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Bioactive compounds from brown algae, especially laminarin and fucoidan have previously improved growth rate and gut architecture in young chicks and is suggested as a potential prebiotic. This study aimed to determine if dietary supplementation of algae rich in laminarin either in the breeder hen diet or in the chick diet could enhance chick quality, growth performance and gastrointestinal tract development in broiler chicks. An experiment was conducted using a splitplot model where eggs from 45 hens fed three different treatments; control (C), algal extract (AE) and algal meal (AM) from Saccharina latissima were hatched individually on-farm. After hatch, 255 chicks were quality scored, id-marked and distributed into 24 pens and two chick treatments; C and AE. The chicks had access to feed as soon as they were placed in their pen. Chicks were individually weighed at day 3, 7, 14 and 37. At day seven, two chicks per pen were killed to assess organ development and histological examination of small intestine. The data was analysed with PROC FREQ and PROC MIXED in SAS ver. 9.4. The model included chick treatment, hen treatment, hatching time and hen treatment× chick treatment as fixed factors and pen and egg number \times hen treatment as random factors. The results showed that fewer chicks from hens fed AM than chicks from hens fed AE had maximum quality score (37.3 vs. 58.11%; P<0.05). Chicks from hens fed AM also had a lower villus height/crypt depth ratio at day 7 than chicks from hens fed AE (5.6 vs. 6.8; P<0.05). In addition, chicks from hens fed AE had higher body weight at day 7 than chicks from hens fed C (187.8 vs 176.9 g; P<0.05). Using AE as chick treatment resulted in higher (P<0.05) body weight at day 3, 7 and 37 compared to chicks fed C. At day 37, chicks fed AE weighed 3037 g compared to 2925 g for chicks fed C. No effect of chick treatment on histological parameters were observed, however, the relative weight of the small intestine (g/g body weight) was higher in chicks fed C than AE at day 7 (62.4 vs 60.4; P<0.05), which was mainly explained by a higher body weight of AE fed chicks. In conclusion, supplementation with AE but not AM in broiler breeder diets may promote chick quality, gut development and early growth in their chicks whereas supplementation with AE directly to the chick diet may stimulate lifetime growth performance.

THE EFFECT OF SELECTED IN OVO GREEN LIGHT PHOTOSTIMULATION PERIODS ON POST-HATCH BROILER GROWTH AND SOMATOTROPIC AXIS ACTIVITY

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In ovo broiler green light photo stimulation accelerated post hatch body and muscle growth. In addition, recent studies conducted in our lab indicated that targeted in ovo green light (GL) photostimulation during the last days of broiler egg incubation increases embryonic expression of the somatotropic axis, similar to in ovo green light photostimulation from embryonic day (ED) 0 to the end of incubation. The aim of this study was to examine the effect of selected in ovo GL photostimulation periods on post-hatch broiler growth, 420 fertile broiler eggs were divided into 7 treatment groups: the first incubated in the dark (standard conditions) as a negative control; the second incubated under monochromatic GL from ED0-ED20 (positive control); the third group incubated under monochromatic GL light from ED15-ED20; the fourth, fifth and sixth groups were incubated under monochromatic GL on ED16, ED17 and ED18, respectively; and the seventh group was incubated under monochromatic GL from ED18-ED20. All illumination was provided intermittently using LED lamps. After hatch, all chicks were transferred to a controlled room under standard rearing conditions. The group incubated under green light from ED18 until hatch showed similar results to the positive control group in body weights, as well as breast muscle weights (as % of body weights), and an elevation in the somatotropic axis activity during the experiment. We suggest that broiler embryos can be exposed to in ovo GL photostimulation from ED18 until hatch (hatching period), and still exhibit the same performance as obtained by photostimulation from day 0 of incubation.

THE BENEFITS OF ENCAPSULATED BUTYRIC ACID ON BROILER PERFORMANCE AND INTESTINAL MORPHOLOGY

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Butyric acid and its salt forms are widely used as feed supplements in poultry to promote intestinal health. Encapsulation technologies are crucial for slow release, allowing beneficial effects along the intestinal tract. Harnessing these technologies and implementing the most effective dosages might be of particular interest for young chicks, as they have a shorter intestinal tract and faster digesta passage. To assess the impact of encapsulated butyrates on growth performance and intestinal morphology in broilers, 221 male Ross 308 broilers were allocated to 13 pen repeats (17 birds/pen). There were 5 treatments: control feed (T1) with no butyrate supplementation (NC), the NC supplemented with either encapsulated butyrates BP1 or BP2 (modulating speed of release using ButiPEARL[™] technologies, Kemin Europa N.V.) added at 300 g/ton to the starter, grower and finisher diets (T2 and T4, respectively). Additional treatments (T3 and T5) received during the starter phase 1000 g/ton and during the grower and finisher phase 300 g/ton of the products. BP1 at lower dosage during starter phase (T2) numerically increased body weight compared to the NC (2586 and 2549 grams, P>0,05). However, when BP2 was provided during the starter phase at 300 g/ton (T4), the birds showed at day 35 a significantly better FCR compared to the NC (1,38 and 1,40, P=0,009). Interestingly, FCR for BP1 at higher dosage (T3) showed no difference compared to BP2 at lower dosage (T4) (P>0.05), which implies a higher efficacy at lower dosage of BP2 compared to BP1. By contrast, a higher dosage of BP2 (T5) did not improve the FCR compared to the NC. A low supplementation of BP2 (T4) had a beneficial influence at the villus height (VH) compared to the NC (850,7 µm and 819,4 µm, P=0,07). Even a stronger beneficial effect on the VH was noticed in the group with higher dosage of BP1 (T3) compared to the NC (892,10 μ m and 819, 41 μ m, P=0,0035). In conclusion, during the starter phase, strategies using either the higher dosage of BP1 or the lower dosage of BP2 both improved overall growth performance and intestinal morphology. Histological evaluation showed that implementing BP1 at higher dosage during the starter phase improves the villus height compared to the NC and even BP2 at lower dosage (T4) had beneficial effects on the VH. These differences between BP1 and BP2 show the importance of using the right dosage of encapsulated butyrate to deliver all expected benefits.

EFFECT OF THREE LEVELS OF DIETARY CALCIUM ON THE PRODUCTIVE RESPONSE AND TIBIA ASH OF 7-DAY-OLD EGG-TYPE PULLETS

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A study was carried out to test the hypothesis that the level of calcium in the diet can be reduced without affecting the productive indicators, relative organ weight and tibia ash content in the pre-initial phase from of 0 to 7-day-old egg-type pullets. The objective of the study was to determine the effect of three levels of calcium in the diet on productive indicators and tibia ash in the pre-initial phase from of 0 to 7-day-old egg-type pullets. It was carried out in the experimental unit of the R & D Nutrition Laboratory of the Faculty of Veterinary of the National University "San Luis Gonzaga". A total of five hundred four 1-day-old Lohman eggtype pullets were used, distributed under a complete random design. The treatments were three levels of calcium: 065, 0.85 and 1.05% with 4 repetitions each. Live weight, feed intake, feed conversion, relative weight of liver and gizzard, gallbladder weight, metatarsal length and % of tibia ash were evaluated, as well as the pH of the feed used. The data of production and tibia measurements were subjected to ANOVA using the general linear model (GLM) of SAS. Differences among means were tested by the Tukey method. The results indicated that calcium levels did not significantly affect (P>0.05) live weight, feed intake, feed conversion index, relative weight of liver and gizzard, metatarsal length and percentage of ash at 7 days of age. Live weights at 7 days were: 68.72, 68.58 and 68.69 g / chick, metatarsal length: 34, 33 and 34.5 mm and tibia ash content: 57.16, 57 and 56.30% for levels of 0.65%, 0.85 and 1.05% calcium respectively. The pH of the food was increased as the calcium level increased, obtaining values of 5.82, 5.96 and 6.11 for the levels of 0.65, 0.85 and 1.05% of calcium in the diet. Additional studies are required such as reevaluations related to the relationship of calcium and phosphorus, as well as evaluating up to 21 days of age to better specify and adapt to the conditions of the poultry industry, however, the results obtained represent a baseline to design subsequent studies. It is concluded that it is possible to reduce calcium levels in the pre-initial diet up to 0.65%, without affecting the productive response, it also allows to reduce calcium carbonate and create space in the formula to improve nutritional density and reduce the pH of the diet of egg-type pullets, which under commercial conditions would be very useful in nutritional management. Keywords: egg-type pullets calcium diet ash

INVESTIGATING THE POST HATCH GROWTH RESPONSE AND DEVELOPMENT OF BROILER CHICKENS FED EITHER A HIGH FAT OR HIGH CARBOHYDRATE DIET

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Dietary fats and carbohydrates i.e., starch and sugars are the main calorie contributors in poultry diets, and their distinctly different catabolic pathways in avian species are considered a major determinant of their utilization. In this study. The possible differences in the growth performance and development of broiler chickens fed either dietary fats or carbohydrates as a primary energy source were investigated. Two practical diets; high fat (HF, 7.88% crude fat and 20.56% sugars + starch) and high carbohydrate (HC, 3.5% crude fat, and 33.27% sugars + starch) were formulated to meet the requirement of starter (1-21d) and grower (22-35d) chickens and fed to one hundred eighty 1d-old unsexed chicks (5 replicates, 18 chicks per replicate) in a completely randomized design. Daily feed intake, DFI, daily live weight gain, DLWG, and Feed conversion ratio, FCR was estimated at the end of the starter and grower phases, and feed cost/kg body weight gain of chickens was calculated. On d1, 3, 5, 7, 11, 14, 21, and 35, one chicken per replicate was slaughtered and weights and lengths of the empty gastrointestinal tract and small intestinal segments were measured, while proventriculus, gizzard, pancreas, and breast were harvested and weighed. Feed and excreta were collected from d25-28 and the coefficient of energy utilization of both diets was determined. Data were analyzed in JASP; studentized t-test at a0.05. In the starter phase, 17.18% higher DLWG (P=0.003) and 8.08% higher DFI (P<0.001) were recorded for chickens on the HF diet over the HC group. A more efficient FCR was also recorded for chickens on the HF diet compared to chickens on the HC diet $(2.84\pm0.74 \text{ vs } 3.20\pm1.02, P=0.03)$. In the grower phase, chickens on the HF diet recorded an 11.6% decline in DFI compared to chickens on the HC diet, while chickens on both diets had similar DLWG and FCR. Similar feed cost/kg body weight gain was also recorded for chickens on both HF and HC diets. Relative weights and lengths of the empty gastrointestinal tract, small intestinal segments, as well as proventriculus, gizzard, pancreas, and breast weights were also unaffected by the dietary treatments. Although a higher coefficient of energy utilization was recorded for the HF diet compared to the HC diet (0.74 \pm 0.02 vs 0.67 \pm 0.03, P <0.001), the superior growth performance of chickens on the HF diet up to 21d did not carry over into the grower phase.

USE OF MORINGA OLEIFERA LEAVES IN BROILERS PRODUCTION CHAIN: EFFECT ON HATCHABILITY OF SASSO BREEDER EGGS AND DAY-OLD CHICK SERUM LIPIDS

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This study was designed to investigate the in ovo and or dietary effects of Moringa oleifera leaves or a combination of both on hatchability and serum lipids of dayold chicks. Hatching eggs (n=2400) collected at 31, 43, and 55 weeks from Sasso broiler breeders fed with or without Moringa oleifera leaves were incubated at standard incubation conditions. On day 18, both eggs were candled and those with evidence of living embryos were divided into 2 new groups of 360 eggs each. Moringa oleifera extract was injected into the air chamber of one group. After extract inoculation, eggs were transferred from the turning trays to hatching baskets. Between 456 and 510 h of incubation, hatching events such as external pipping and hatch were monitored every 3 h. Hatched chicks were recorded and weighed. Eighty newly chicks from each group were used for liver, heart and yolk sac weight; and for serum lipids determination. General Linear Models procedure was used to analyze data. Each batch of set eggs was considered as a repetition. When means were statistically different, then they were further compared using Tukey's test based on P<0.05. Results indicated that in ovo inoculation of extract reduced external pipping duration. Chick weights increased with Moringa oleifera leaves used in broiler breeders' diet but hatchability was greater in all groups treated with this material. Liver relative weights of day-old chick showed noremarkable changes while in ovo injection of extract reduced heart weights. Yolk sac utilization was better with Moringa oleifera leaves, regardless of the stage where this material was used. Serum concentration of total cholesterol and LDL cholesterol decreased with in ovo treatment while HDL cholesterol increased. These results suggest that Moringa oleifera leaves had positive effects on hatchability.

Keywords: Moringa oleifera, broiler breeders, in ovo, hatchability, serum lipids.

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Genetics x environment interactions and epigenetics

Selected short communications

EVALUATING THE EFFECT OF DIFFERENT BROILER BREEDER FEEDING PROGRAMS ON THE BROILER GENERATION VIA RNA SEQUENCING

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The poultry industry has historically selected for more efficient feed conversion and faster growth. As a result, common practice is to restrict broiler breeder feeding during rearing to prevent negative impacts on reproduction. This practice causes metabolic stress and welfare issues in regard to hunger and feeding distress. Similar stressors have been shown to alter the phenotype of offspring, possibly through epigenetic mechanisms. Prior work in breeder hens identified differences in hepatic gene expression as a result of feeding program, but the impact on subsequent progeny was unknown. In this study, we evaluated the effects of broiler breeder feeding programs on their progeny from a functional genomic perspective. Ross 708 breeders were individually fed using a precision feeding system to reach Ross 708 target growth standards as well as incrementally higher body weights up to 22.5% above recommended BW in steps of 2.5% with an additional group fed ad lib. Hens were naturally mated to similarly fed Ross 708 YP males and eggs were individually collected for traceability. All progeny was fed ab lib via a precision feeding system and randomly assigned to one of four chambers with similar environmental conditions. Liver samples were taken from the progeny (n = 23) at 35d for Control, +20%, and ad lib breeder treatments. RNA was isolated from liver samples and RNA sequencing was performed. CLC genomics workbench was utilized for mapping, annotation and statistical comparison of sequences and Ingenuity Pathway Analysis was used to make pathway connections. Three comparisons were made across the progeny treatments: Control vs. +20%, Control vs. ad lib, and +20% vs ad lib. Using a minimum absolute fold change of 1.5 and p-value of 0.05 as the threshold of significance we saw 431 differentially expressed genes in the Control vs. +20% comparison, 360 in the Control vs. ad lib comparison, and 334 in the +20% vs. ad lib comparison. The pathway analysis highlights sex hormones such as betaestradiol, progesterone and downstream molecules being significantly affected by the feeding program of the prior generation. This is consistent with our previous findings regarding the effect of feeding programs on the breeder generation and stocking density on broilers. This suggests a potential stress induced genomic effect with transgenerational implications that may alter growth and efficiency and should be considered in poultry production systems.

PATHWAY ACTIVATION ANALYSIS OF LIVER GENE EXPRESSION DATA FROM CHICKENS UNDER HEAT STRESS REVEALS SHORT-AND LONG-TERM METABOLIC EFFECTS

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Next-generation RNA sequencing can allow a deeper understanding of physiological responses to nutritional, health, or environmental challenges. In this study, we compiled a gene expression dataset of 116 liver samples from five publicly available BioProjects testing the effect of heat stress in chickens. Data were analysed using a common data pipeline to uncover the pathway activation fingerprint of heat stress. Six breeds of chickens and only young chickens (14-48 d) were included in the analysis. Data were split into 14 blocks to control for various batch, breed, nutritional, and other experiment-related effects. Gene expression abundances were calculated against the reference chicken genome and normalized. Pathway Activation Scores (PAS) were calculated and normalized for each sample using the Biofractal activation algorithm, which accounts for differential gene expression, significance level, and topology importance of the genes in each pathway. The Reactome pathway database was used for annotation. PAS were analysed using a Generalized Linear Model comprised of the main effects of Heat Stress (control or heat-stressed), Type of stress (acute or chronic), their interaction, and the random effect of Block nested within Type. In addition, pathway enrichment was calculated using GSEA and compared to the PAS results. PAS allowed a more sensitive detection and quantification of differences between heat-stressed chickens and controls compared to the pathway enrichment method. The strongest heat-stress triggered activation of pathways occurred in pathways related to gene expression (transcription), cell cycle, and the metabolism of watersoluble vitamins and co-factors. Conversely, the greatest relative inhibition was found in pathways related to lipid metabolism, extracellular matrix organization, peroxisomal protein import, and platelet activation, signalling and aggregation. Compared to chronic heat stress (Heat Stress x Type interaction), acute heat stress greatly reduced the relative activity of pathways related to the adaptive and innate immune system, and the hepatic metabolism of proteins, whereas it greatly activated pathways related to organelle biogenesis and maintenance, GPCR signalling, lipid metabolism, transport of small molecules, and extracellular matrix organization. Pathway activation analysis can enable the design of strategies to ameliorate the consequences of heat stress and other challenges affecting animal welfare and performance.

EFFECT OF CYCLIC HEAT STRESS ON FEEDING-RELATED HYPOTHALAMIC NEUROPEPTIDES OF THREE BROILER POPULATIONS AND THEIR ANCESTOR JUNGLE FOWL

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With global temperatures rising and increased frequency and severity of heat waves predicted over the next decades, heat stress (HS) is a substantial threat to the sustainability of poultry production. Additionally, modern high-performing chickens are far less able to withstand HS than their predecessors due to higher growth and metabolic rates. Decreases in feed consumption significantly contribute to the performance losses caused by HS. Since feed intake is tightly controlled by the hypothalamic centers of hunger and satiety, we sought to determine the effect of chronic cyclic HS on the expression of feeding-related hypothalamic neuropeptides in unselected chickens (ancestral red jungle fowl [JF]) and three broiler populations from diverse stages of genetic selection (the slow growing Athens Canadian Random Bred [ACRB], the moderate growing 1995 Random Bred [95RB], and the fast growing Modern Random Bred [MRB]). From 29 to 56 d, birds (n = 150 birds for each population) were subjected to either thermoneutral (TN, 25°C) or cyclic heat stress (HS, 36°C for 8h) conditions. On d56, birds were humanely euthanized, and hypothalamic tissue were dissected and stored at -80°C. Gene expression was analyzed by two-way ANOVA with environmental temperature and line as main factors. The expression of major neuropeptides, including neuropeptide Y, agouti-related peptide, proopiomelanocortin, and cocaine and amphetamine regulated transcript, were unaffected by line or temperature, which may be due to acclimation to long-lasting HS exposure, or these may be stable hypothalamic pathways unaffected by genetic selection. However, melanocortin receptor 1 exhibited a line-dependent decreasing trend from JF to MRB under both TN and HS (p = 0.09), adiponectin expression was highest in 95RB irrespective of the environmental temperature (p = 0.08), and JF had a greater mRNA abundance of visfatin than ACRB under TN (p < 0.05). In conclusion, this is the first assessment of the impacts of HS on feeding-related hypothalamic neuropeptides of three broiler populations characterized by different growth rates and in their ancestor jungle fowl. The hypothalamic differences seen among these birds may be the result of, or mechanism underlying, the phenotypic differences, and can help quide further research in mitigating the effects of HS in poultry.

ID: 1767 - 2292

NAKED NECK GENE AND INTERMITTENT THERMAL MANIPULATION DURING INCUBATION: EFFECT ON HATCHING PROCESS AND POST HATCH PERFORMANCE OF SASSO BROILERS IN TROPICAL CLIMATE.

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Background: Many studies have shown that thermal manipulations during the embryonic period (TME) and naked neck gene (Na) have positives effects on thermotolerance, hatching process and post-hatch performance of heat-stressed broilers. Their combination could increase the beneficial effect on broilers reared under natural tropical climatic conditions. Objectives: The aim of this study was to investigate the effects of Na gene and TME on hatching and post-hatch performances of Sasso broilers under tropical climate. Methods: 900 hatching eggs from two different crosses (one between Sasso SA51 females and Ruby XL males, both with normal plumage called the nana group, the other between SA51 females and Ruby N males with naked neck called Nana group) were incubated until day 7, when they were divided into 3 sub-group for each cross: the control group(C) was incubated at standard incubation conditions (37.8°C, 60% RH). T1 group was subjected to TME-1 (T°=38.5°C, RH=65%, E10-18, 6h/day) and T2 group to TME-2 (T°=39.5°C, RH=65%, E7-16, 12h/day). Between 450 and 510h of incubation, eggs were checked for hatching event. After the brooding period, chicks from each incubation sub-groups (Nana-C, Nana-T1, Nana-T2, nana-C, nana-T1, nana-T2) were raised for 12 weeks at a natural ambient temperature. Hatchability, hatching time, chick's temperature, final body weight (FBW) and feed conversion ratio (FCR) were determinated. Statistics software GraphPad Prism 8 was used for data analysis which was made using two-way Anova and Tukey's test. Results: Na gene reduced (p<0.05) hatchability, chick quality, and improved (p<0.05) FCR and FBW. T2 group had the lowest chick quality and hatching weight compared to T1 and control group. T1 and T2 had the best FCR and mortality rate (MR). There is an interaction between Na gene and TME on hatching time, FCR and MR (p<0.05). Conclusion: The effects of thermal manipulation are influenced by Na gene. TME-1 combined with Na gene allows for the best productive performances of broilers in tropical climate.

Key words: Thermal manipulation, Na gene, Hatching process, Post-hatch growth, heat-stressed broilers.

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Genetics x nutrition interaction to optimize gut function

Selected short communications

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GENE EXPRESSION ANALYSIS OF LIVER SAMPLES REVEALS METABOLIC AND IMMUNOLOGICAL PATHWAYS ASSOCIATED WITH THE PERFORMANCE OF COMMERCIAL BROILER FLOCKS

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Factors causing sub-optimal performance of broiler flocks are often subtle and difficult to pinpoint. This study used gene expression data from broiler chicken liver samples to identify metabolic and immunological pathways associated with final flock performance. Four flocks of Ross-708 broilers of the same age, raised without antibiotics, were selected from four farms in a commercial broiler integration. Liver samples were randomly collected from 64 healthy birds (16 per flock) at 28 d. RNA was extracted using the Zymo Direct-zol RNA kit and sequenced using the Illumina platform. Gene expression abundances were converted into pathway activation scores using the Biofractal activation algorithm. The algorithm calculates scores based on topological importance, differential expression and associated statistical significance of genes within each pathway. Next, machine learning models were fitted to pathway activation scores to determine metabolic and immunological pathways that best explained the variation in flock feed conversion ratio (FCR), average body weight (BW), and mortality rate. Performance data were available for 3 houses in Farm 1, and by farm for Farms 2, 3 and 4. Final (63 d) FCR by farm were 1.951, 1.901, 1.914, and 1.923 and final BW were 4.188, 4.246, 4.309, 4.096 kg/bird for Farms 1 to 4, respectively. Models for metabolic pathways were able to explain 64%, 66%, and 86% of the variation in final FCR, BW and mortality, respectively. Inositol phosphate metabolism, cytochrome P450 system, sulfur amino acid metabolism, and the pentose phosphate pathways showed a strong positive association with FCR and mortality, and negative association with BW. Conversely, glycogen metabolism had the strongest negative association with FCR and mortality rate, and positive association with BW. Models for immunological pathways explained 49%, 46%, and 42% of the variation in FCR, BW, and mortality, respectively. The FCERI signalling pathway had the strongest negative association with FCR and positive association with BW, whereas interleukin-6 family signalling had the strongest negative association with FCR and mortality. Initial triggering of complement and FCG receptor dependent phagocytosis had the greatest positive association with mortality and FCR. This study showcases the ability of transcriptomics data to provide an in-depth and comprehensive evaluation of biological factors affecting broiler health and performance in a commercial setting.

SUSTAINABILITY OF POULTRY MEAT PRODUCTION: GROWTH PERFORMANCE AND CARCASS TRAITS OF SLOW-GROWING GENOTYPES FED LOW INPUT DIETS

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The development of alternative and sustainable poultry meat production, consistently with the objectives of the European Green Deal and the F2F strategy, requires the use of resilient chicken breeds and low input diets (based on national and local raw materials). Thus, the present study compared performance and carcass traits of a fast-growth genotype (Ross 308) with those of two local breeds (Bionda Piemontese, BP; Robusta Maculata, RM) and their crosses with a moderate-growth genotype (Sasso, Sa). A total of 441 chickens were housed in 40 pens with 2 pens/genotype/sex, i.e. Ross (51 females and 52 males), BP (37 and 38), RM (25 and 47), BP×Sa (49 and 48), and RM×Sa (47 and 47). Within genotype, half of the pens received a standard diet (diet S: ME 3,050 kcal/kg; CP 18.5%) and half a low input diet (diet LI: ME 2,921 kcal/kg; CP 17.5%) from 20 d of age until slaughtering (47 d for Ross and 105 d for other genotypes). Data were submitted to ANOVA with genotype, diet, and sex as main effects with interactions and pen as random effect. At the end of the trial, BP showed the lowest live weight followed by RM and BP×Sa, RM×Sa, and Ross (1620 g vs. 2024 g and 2037 g vs. 2376 g vs. 2643 g, respectively; P<0.001). Daily weight gain and feed intake changed accordingly which affected feed conversion (3.37 and 3.28 for BP and BP×Sa vs. 3.09 and 3.01 for RM and RM×SA vs. 1.85 for Ross; P<0.001). At slaughtering, BP showed the lowest dressing out percentage followed by BP×Sa, RM, RM×Sa and Ross chickens (58.3% vs. 64.7% vs. 70.1%, 68.8% and 71.1%; P<0.001) with corresponding changes of breast proportion (24.3% and 25.5% in BP and BP×Sa vs. 26.7% and 26.9% in RM and RM×Sa vs. 33.6% in Ross; P<0.001). Chickens fed diet S presented a higher growth rate (28.5 g/d vs. 23.8 g/d; P<0.001) and feed intake (69.4 g/d vs. 65.7 g/d; P<0.001) compared to those fed diet LI, and a lower feed conversion ratio (2.80 vs. 3.03; P<0.001). Differences in growth rate and feed conversion between chickens fed the two diets were small in the case of BP and BP×Sa and larger in the case of RM, RM×Sa and, especially, Ross chickens. In conclusion, growth results and carcass traits of local breeds were far lower compared to those of Ross chickens. As for pure breeds, RM performed better than BP especially when crossed with Sa. In addition, most performant chickens (Ross, RM and RM×Sa) suffered the use of a low input diet, whereas BP and BP×Sa chickens were more resilient to dietary changes.

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Genetics x nutrition interaction to optimize gut function

Posters

EFFECT OF AGE, BREED, SEX AND DIETARY FACTORS ON METABOLITE CONCENTRATION AND IMMUNOLOGICAL TRAITS IN THE CAECUM OF BROILERS

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Age and genetic background as well as dietary factors may affect bacterial activity and mucosa-associated immune system in the gut. A total of 2,880 one-day-old male and female broilers from two breeds, Ross and Cobb, were randomly allocated to 72 pens. Broilers fed a wheat-soybean based diet without (CO) or with either a probiotic (PO; 2.4 x 109 CFU/kg diet; Bacillus subtilis and Bacillus amyloliquefaciens) or a phytobiotic (PY; 165 ppm procyanidins and 585 ppm polyphenols). At day 7, 21, and 35 of age, one chicken per pen was sacrificed to dissect the caecum. Data were subjected to ANOVA using GLM procedure with a 3 $(age) \times 2$ (breed) $\times 2$ (sex) $\times 3$ (diet) factorial arrangement. The concentration of acetic, propionic, i- and n-butyric, i- and n-valeric acid increased with age (p < p0.05). L- and D-lactic acid (LA) decreased from day 7 to 21 (p < 0.05) and stayed constant until day 35 of age. Short chain fatty acids and LA were not affected by breed, sex, diet, and interactions among the main factors (p > 0.05), except for D-LA which was higher in Cobb than Ross (p < 0.05). Gene expression (log10) copies number/ng RNA) of interleukin (IL)-2, 4, 6, 8, 10, 12, 17 and 18, as well as IFN- γ and TGF- β increased with age, whereas expression of IL-1 and TNF-a increased from day 7 to 21 and then decreased at day 35 (p < 0.05). The expression of MUC2 decreased from day 7 to 21, and then increased at day 35, while CLDN5 only increased from day 21 to 35 (p < 0.05). Birds fed PY diet showed higher expression of IL-10 than those fed CO diet (p < 0.05). Cobb showed higher expression of IL-1, IL-6, TNF-a and CLND5 than Ross (p < 0.05). Sex and the interactions had no effect on gene expression of the evaluated markers (p > 0.05). Several metabolites, especially propionate, were positively correlated with expression of the evaluated markers (r = 0.160 to 0.548, p < 0.05), except for TNF-a showing a negative correlation with all the metabolites (r = -0.154 to -0.285, p < 0.05) but acetate and n-butyrate. In conclusion, age affected bacterial activity and gene expressions related to epithelial barrier integrity and inflammation in the caecum, while sex, dietary factors and interactions had almost no effect on them. A few impacts of breed on the variables measured did not show any systematic biological pattern. The alterations in the evaluated metabolites by age affected gut immune response in the caecum. However, the mechanisms behind the effects need to be studied further.

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Innovative strategies to answer new expectations from the society

Selected short communications

MICROBIOTA STUDY AT THE FARM LEVEL FOR QUICK DECISION-MAKING

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The growing global population demand for affordable and high-guality animal protein has led to the intensification of the poultry production [1]. With poultry meat reach the market weight as early as 5 to 7 weeks, efficient management requires technological advancements that it make possible for farmers to certified and manage the animals' health status [2]. MinION (Oxford Nanopore Technologies, UK), is a handheld sequencer used in human disease diagnosis that could profile the infectious agents and tailored precise treatments in a short turnaround time [3]. The aim of this study was to perform a MinION and Bento Lab-based platform combination workflow for the composition of microbial communities in faecal samples as powerful microbiota analysis tool at farm level. A total of 42 day-old-chicks were housed (7 birds/pen) under farm production conditions. On days 14 and 42, cecum samples of 3 birds/pen were taken. The mobile genomics setup Bento Lab was used for the DNA extraction with the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). The sequencing was done in a portable MinION sequencing device. Taxonomy was assigned using the RDP classifier [3]. The statistical analyses were performed in R software version 4.1.1 using the gdata [4], vegan [5]. Twelve caecal pools were collected, processed and sequenced. After filtering of sequences (80% of gi), total of 235 unique OTUs were left for taxonomic assignment. The alpha-diversity indexes reveal high values for 42 days-old-chicks (P<0.001). The beta diversity showed strong differences in the microbial community composition among the two moments. Examination of the taxonomic profiles showed that the most predominant identified genera were Bariatricus, Faecalimonas and Faecalibacterium for 14-day old chicks and Paralactobacillus, Faecalibacterium and Bariatricus, for 42 day-old chicks. In this study, a new strategy enabling the cecal microbiota composition at farm level using Nanopore sequencing was explored. Nanopore MinION not only allows an overview of the animal microbiota, but also could provide the identification of the target bacteria, as pathogens, at real time in situ.

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ID: 1932 DEVELOPMENT OF AUTOMATED MEASUREMENTS OF SOUND INDICATORS FOR THE EARLY DETECTION OF HEALTH DISORDERS IN POULTRY: THE CASE OF INFECTIOUS BRONCHITIS

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The vocalisations produced by poultry provide informations on their health and welfare (e.g. stress or comfort vocalizations). This information, complementary to that of the breeder, can allow earlier detection of the appearance of a pathology and to date no acoustic analysis solution is marketed for this use in breeding. This technology would allow the farmer to be warned when alert criteria are exceeded so that appropriate corrective measures can be taken immediately, thereby limiting the aggravation of the problem. The objective of this work is to evaluate the feasibility of automatically detecting symptoms of Infectious Bronchitis (sneezing and rales) in poultry. A second objective is to identify one or more acoustic indicators for early detection of the establishment of this virus. The first step in this study was to collect soundtracks under controlled experimental conditions from groups of ROSS 308 broilers infected (trial; n=30) and uninfected (control; n=30) with infectious bronchitis (IBV, avian coronavirus). A first stage of listening by a group of experts allowed the detection and isolation of rales and sneezes, thus allowing them to be characterised using acoustic descriptors. An algorithm was then developed to automatically and specifically detect and isolate these symptoms on the test group. The second step of this work was to differentiate the healthy group from the sick group based on the temporal evolution of the relative noise level. Three days post-inoculation, a difference of 3 decibels was observed at night between the groups (+3 dB for the infected group; sound intensity multiplied by 2). This work highlights the value of acoustic analysis for the early detection of pathologies in broilers. It should be continued with trials in field conditions.

LEARNING FROM THE DUCK: NEW POSSIBILITIES FOR IMMUNE FITNESS IN TRANSGENIC CHICKENS

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Wild waterfowl represent the natural reservoir for avian influenza viruses (AIVs). The genomic characteristics of the wild duck (Anas plathyrynchos) were suggested to be a key factor in the resistance against AIV-induced disease. One of these characteristics is the presence of the retinoic acid-inducible gene I (RIG-I), a cytoplasmic sensor responsible for the activation of mitochondrial antiviralsignaling proteins (MAVs) and triggering the antiviral response via type I and type III interferons (IFNs) followed by the production of IFN stimulated genes (ISGs). Since the chicken lacks the RIG-I, its possible role in the chicken immune response was exclusively investigated in vitro by overexpression studies in chicken fibroblasts. However, no transgenic chickens expressing the duck RIG-I were generated so far. In this study, we generated fully genetically modified chickens that express the duck RIG-I. We genetically modified chicken primordial germ cells (PGCs) that were re-introduced to chicken embryos which were raised to sexual maturity in order to obtain chimeric roosters. Heterozygous chickens expressing the duck RIG-I were healthy and reached sexual maturity with no apparent abnormalities. Furthermore, we monitored the effect of RIG-I expression on the adaptive immune response including B and T cell populations. Interestingly, RIG-I transgenic chickens had significantly higher number of T cells in comparison to their wild-type siblings (p<0.05), while no significant differences were observed in the plasma levels of biologically active type I IFN. Our data reveal for the first time the effect of the duck RIG-I in genetically modified chickens. The obtained results suggest that the RIG-I, an innate immune sensor, may affect the adaptive immunity which may explain the immune fitness of wild birds in comparison to domestic chickens and promises new preventive strategies for poultry industry.

PRECISION FARMING: A NEW TOOL AND DATABASE TO MONITOR RECOMBINANT TURKEY HERPESVIRUS VACCINE APPLICATIONS IN COMMERCIAL BIRDS.

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Turkey herpesvirus (HVT) is a commonly-adopted vector used in many different commercial chicken vaccines by applying it in ovo around 18-19 days of the embryonic development. Different protein gene inserts from other pathogens could be added into the HVT vector in order to provide broad protection against multiple diseases at the same time. There are many benefits to using HVT as the vaccine vector, such as long-term disease prevention. Due to the limitation of HVT transmission between chickens, it is very critical to ensure vaccine application accuracy in order to achieve optimal protection for commercial flocks. Commercial real-time PCR (rtPCR) kits have been used in the field to detect HVT replication cycles in chickens after in ovo vaccinations. However, there are many limitations with the rtPCR surveillance method. First, users won't be able to tell if the submitted samples contain enough genetic materials for PCR amplification, which could be heavily influenced by sampling technique. Second, as has been well documented, the biological variances between different commercial breeds and the nature of HVT replication could result in negative PCR results through different ages. Here we describe a unique Next Generation Sequencing (NGS) platform developed to overcome these issues. There have been more than two thousand samples from all over the world uploaded into the platform database and used to build the baselines for different commercial breeds. With this unique tool, poultry producers will be able to achieve the goal of precision farming by monitoring and advancing recombinant HVT vaccine applications.

Innovative strategies to answer new expectations from the society

Posters

CARCASS PERFORMANCE OF SIX LOCAL GERMAN CHICKEN BREEDS TOWARDS SUSTAINABLE USE OF REGIONAL ANIMAL GENETIC RESOURCES

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Many local chicken breeds are at risk of extinction worldwide because they have often been replaced by high-performing specialized hybrids in agricultural production. However, given that they contribute to the genetic reservoir and enrich biodiversity, it is crucial to explore their potential for use. Since carcass performance is important for profitability, the slaughter data pertaining to six local German chicken breeds have been studied in this context. The cockerels slaughtered were reared under standardized conditions at three different research locations. In each case, the following pairs were raised at the same location: the Altsteirer (ALT) and the Augsburger (AUG); the Malines (MAL) and the Bielefelder (BIE); the East Frisian Gull (EFG) and the Ramelsloher (RAM). The first half of the cockerels were slaughtered at 14 weeks of age (Time 1) and the other half of the roosters were slaughtered at 18 weeks of age (Time 2). However, some of the AUG and all of the ALT were slaughtered in the 19th week of life for unavoidable experimental reasons. Fasting live weights before slaughter, carcass weights, cut weights as well as organ and abdominal fat weights were recorded for all of the cockerels. The heaviest cockerels at Times 1 and 2 of slaughter were the MAL with 1648.8 ± 243.7 g (mean \pm SD) and 2095.0 \pm 198.4 g, respectively, and the lightest cockerels at Time 1 were the AUG at 852.7 ± 110.4 g and at Time 2 were the EFG at 1205.6 \pm 127.6 g. Carcass yield was lowest for the BIE at both the first and second slaughter: $64.7 \pm 0.12\%$ and $66.6 \pm 2.9\%$, respectively. At Time 1 Carcass yield was greatest for the RAM at 69.8 \pm 1.5%, the ALT at 69.7 \pm 2.1% and the AUG at $69.5 \pm 2.1\%$. At Time 2 for the AUG, the greatest carcass yield was $72.1 \pm 1.3\%$. There was no difference between the carcass yields in Week 18 and 19 for the AUG. The breast meat as the most valuable cut was heaviest in the MAL at both the first and second slaughter dates, weighing 249.4 \pm 43.5 g and 287.1 ± 38.1 g, respectively. As a percentage of the carcass yield, breast meat was greatest at $15.4 \pm 1.3\%$ on the first slaughter date and $16.8 \pm 1.3\%$ on the second slaughter date, respectively, in RAM. The results of the performance tests will be used to evaluate the breeds. In a further course of the project, these breeds will be used for crossbreeding with parents of the laying and broiler hybrids to investigate potential uses.

INFLUENCE OF STRAIN AND STOCKING DENSITY ON PERFORMANCES AND MEAT QUALITY OF BROILERS REARED WITH ENRICHMENTS AND NATURAL LIGHT

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Facing strong societal expectations regarding animal welfare, the conventional chicken production model is in transition. Improving housing conditions of birds is expected and a decrease in growth rate is being considered. The objective of this study was to evaluate animal performance and meat quality for six strains with different growth potential and reared at conventional or reduced density (39 or 30 kg/m²) for a total of 12 experimental treatments. Birds were reared in 48 pens of 18 m² (230 or 300 as-hatched birds/pen) and had access to natural light and environmental enrichments (pecking blocks, straw and alfalfa bales). They were all fed ad libitum with the same diets formulated to meet Ross 308 requirements. To account for difference in their growth rate, birds were slaughtered between 1.8 and 2.2 kg either at D32 (Ross 308), D39 (Redbro, Rustic Gold, Ranger Classic, and JA787), or D46 (JA757). At slaughter, only a significant effect of strain on animal performance was observed. Thus, comparatively to Ross 308 (67 q/d), average daily gain (ADG) from D0 to slaughter was significantly reduced by 24-32% (46 to 51 g/d) and 40% (41 g/d) for strains slaughtered at D39 and D46, respectively (P<0.001). On the same period, comparatively to Ross 308 (1.392), feed conversion ratio was significantly increased by 8-18% (1.510 to 1.646) and 35% (1.882) in strains respectively slaughtered at D39 and D46 (P<0.001). Moreover, 32 birds per treatment were selected and slaughtered in order to measure carcass yields and meat quality criteria. Only strain had a significant effect on those parameters (P<0.001). Ready-to-cook and breast yields were respectively decreased by 1.0-3.4 points and 1.5-5.5 points in strains slaughtered at D39 or D46, comparatively to Ross 308 (73.7 and 23.2%), those yields being highly correlated with ADG (r = 0.60). Finally, for breast meat, the lower ADG was associated with a significant decrease in ultimate pH (-0.14 to -0.27 pH units; Ross 308: 5.89) as well in drip+cooking water losses (-3.5 to -6.5 points; Ross 308: 21.8%). These results, combined with health/behavioral observations made during this trial (Guinebretière et al., WPC 2022), will provide objective data to search for the best trade-off(s) between competitiveness, meat quality and improved animal welfare. In that sense, Redbro or Rustic Gold could represent promising alternative strains with intermediate performances and some improved meat quality criteria.

WHAT IS THE EFFECT OF THE GENETIC BREED ON MICROBIOTA DEVELOPMENT AND ANTIMICROBIAL RESISTANCE DYNAMICS?

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Global health is underpinned by the One Health concept, promoting the implementation of alternative sustainable poultry production systems [1]. Indeed, animal welfare improves resilience and promotes beneficial microbiota and integrity of the intestinal epithelium. Consequently, interactions between environmental and intestinal bacteria are reduced [2]. This way, it could be possible to reduce antibiotic administration at the field level, decreasing the appearance of antimicrobial resistance (AMR) in humans [3]. Hence, the aim of this study was to evaluate the effect of the genetic breed on microbiota development and AMR dynamics. To this end, 576 broilers of fast-growing (FG, Ross®) and slow-growing (SG, Hubbard®) breeds, were located in two identical poultry houses (144/breed/room), without antibiotic administration during the growing period. Animals from each group were sampled on arrival day, at midperiod (21 days of age) and at the slaughter day (42 and 63 days of age for FG and SG, respectively), and caecum samples were taken. To evaluate microbiota composition, 16S rRNA sequencing analysis was performed. Results showed that Firmicutes represented the dominant phylum for both breeds. At the outset, Proteobacteria was the second prevalent phylum. However, during the rest of the production cycle, Bacteroidetes was more abundant. Finally, regardless of the breed, the predominant genera identified were Oscillospira spp., Ruminococcus spp., Coprococcus spp., Lactobacillus spp. and Bacteroides SDD. To study AMR dynamics, Escherichia coli was selected as indicator bacterium, antibiotic susceptibility testing was assessed according to Decision 652/2013, and a generalized linear model was used to perform the statistical analysis. Isolates from FG day-old-chicks showed significantly higher AMR rates. However, at the slaughter day, no significant differences were found between breeds and the presence of AMR (above 95%) or multidrug resistance (MDR) (above 80%). In conclusion, FG and SG broiler microbiota are in constant development throughout rearing, being relatively stable at 21 days of age. Moreover, high levels of AMR and MDR demonstrate that although it is crucial to control both antibiotic use and animal welfare during the growing period, measures should be taken at all levels of the production chain.

1. Mackenzie et al., 2019. DOI 10.3390/tropicalmed4020088. 2. Hancock et al., 2021. DOI 10.1038/nrmicro2745. 3. Sharma et al., 2018. DOI 10.3389/fvets.2017.00237.

LIVE INSECT LARVAE AS ENVIRONMENTAL ENRICHMENT IN MUSCOVY DUCK: EFFECTS ON WELFARE AND BLOOD TRAITS

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Introduction. Interdisciplinary approach is necessary to evaluate animal welfare, since it comprehends animals' good health, comfort, and expression of their natural behaviors [1]. Aim of the present work is the evaluation of the effects of Hermetia illucens (HI) and Tenebrio molitor (TM) live larvae as environmental enrichment in Muscovy ducks on behavioral patterns and blood parameters. Material and methods. Three-day-old females Muscovy ducklings were allotted in 18 pens (6 replicate/treatment, 7 birds/pen) and assigned to 3 experimental treatments. The C group (control) was fed with commercial feed, while HI and TM groups where fed with commercial feed supplemented with HI and TM live larvae (provided as 5% of expected daily feed intake), respectively. Video recordings were made on 3 replicate/treatment every week during the trial and were performed in 3 periods during the day: the hour before insects' larvae provision (T1, 9.00-10.00 am), the hour during the larvae provision (T2, 10.00-11.00 am), and the hour after larvae provision (T3, 11.00-12.00am). At the end of the trial, blood samples were collected from 12 birds/treatments in EDTA tubes and in serum-separating tubes. The total red and white blood cell counts, serum protein, lipid, minerals, liver and renal function serum enzymes were evaluated. One-way ANCOVA was used to compare the observed behaviors in the experimental treatments using the week as a covariate, while one-way ANOVA was used to the collected data for blood traits analyze (P<0.05). Results. During T2 and T3 the birds of the C group showed higher time spent in stand position compared to the HI and TM groups (P<0.05). Moreover, during T3 the HI group showed lower time spent in walking activity compared to the C group (P<0.05). The overall blood traits were not affected by the experimental treatments (P>0.05) except for the H/L ratio that resulted lower in the insects fed groups compared to the control (P<0.05). Conclusion. The behavioral patterns observed were only slightly affected by the daily provision of live HI and TM larvae. However, the observed reduction of the H/L ratio results to be promising in terms of the improvement of animal welfare due to the dietary administration of live insect larvae.

BLACK SOLDIER FLY LIVE LARVAE AS ENVIRONMENTAL ENRICHMENT IN MEDIUM-GROWING CHICKEN DIET

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Introduction. Few studies on the effects of live larvae provision in poultry have been previously conducted [1,2,3]. However, trials on the long-term provision of live larvae in chicken reared for meat consumption have never been performed before. This study evaluated the impact of Black Soldier Fly (BSF) live larvae provision on growth performance and larvae consumption behavior of intermediate-growing strains. A total of 240 Label naked neck birds were reared from 21 to 82 days of age, and four experimental groups (10 birds/pen, 6 replicates/treatment) were considered according to the birds' gender and larvae provision. Experimental groups were fed 10% supplementation of BSF live larvae based on the daily feed intake. The live weight (LW), feed conversion ratio (FCR), average daily feed intake (ADFI) and average daily gain (ADG) were evaluated considering two periods: 21-35d and 35-82d. The larvae were provided daily and consumption times were analyzed considering periods of 10 days (5-time frame-T1, T2, T3, T4, T5). Data were analyzed by means of a GLMM (SPSS software, P < 0.05). The larvae groups displayed a lower ADFI than the control groups regardless the birds' gender at 21-35d (P=0.01). This could be explained by the larvae nutritional contribution that led to a lower feed consumption in the experimental groups. Moreover, treated birds revealed a lower FCR than control groups (21-35d; P<0.001). Otherwise, only treated males performed a better FCR than control groups during the second period (P < 0.01). Overall, time of larvae consumption at T1 and T5was respectively higher and lower than the other considered periods in both sexes (P < 0.05). Such differences could be related to a progressive birds' adaptation to larvae consumption. Significant differences between sexes were recorded only at T5, when females employed much time than males in larvae consumption (P < 0.05). Live larvae provision ameliorated both the ADFI and FCR. Furthermore, the time of larvae consumption shrinked as birds became older.

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CROSSBREEDING OF THE OLD DUAL-PURPOSE BREED DEUTSCHES LACHSHUHN WITH THE LAYER BREED WHITE ROCK AS A WAY TO PRESERVE GENETIC RESOURCES

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The breed Deutsches Lachshuhn was established in 1912 and is the German breeding type of the French Faverolles chicken. The slow-growing Deutsches Lachshuhn is a dual-purpose breed with a high meat quality and moderate laying performance. The German Society for the Conservation of Old and Endangered Livestock Breeds lists it as 'under observation, not endangered at the moment', but the breed will only survive if it continues to be used. To improve the economic viability of the breed while preserving its genetic ressources, Deutsches Lachshuhn males were crossed with females of the laver breed White Rock. From 2019 to 2020, the trial was conducted on the organic farm Wendland Geflügel, a breeder of Deutsches Lachshuhn (LA). The White Rock hens for the crosses (LA*WR) were supplied by Ökologische Tierzucht gGmbH (ÖTZ). The dualpurpose crosses ÖTZ Coffee (CO) and ÖTZ Cream (CR) were kept as controls, because they are gaining popularity due to the ban of the culling of male layer chicks in Germany (since January 1, 2022). Of each genotype, around 200 chicks were reared in two mixed-sex groups until the slaughter of the cockerels at the age of 18 weeks. The hens remained for laying until the age of 72 weeks. Data collection included feed consumption, live weight, slaughter and laying performance. Feed consumption during mixed-sex rearing ranged from 78 (LA*WR) to 85 g day-1 (CO) and did not differ between genotypes. The final live weight of the cockerels was significantly lower for LA*WR and LA (2.9 kg) than for CO and CR (3.1 kg). Percentage of breast filet in the cockerels was 15.5, 15.7, 19.1 and 17.4 for LA*WR, LA, CO and CR, respectively. At the end of the laying period, LA hens were heaviest with 3.0 kg, while LA*WR, CO and CR weighed 2.6, 2.7 and 2.5 kg. The average number of total eggs per hen alive was 250, 126, 249 and 242, and average laying performance was 69, 35, 69 and 66 % for LA*WR, LA, CO and CR, respectively. To summarize, crossing Deutsches Lachshuhn with White Rock did not impair the fattening performance of the cockerels, but improved the laying performance of the hens to the level of other German dual-purpose crosses.

Acknowledgement: Funding for the project was provided by the Lower Saxony Ministry for Nutrition, Agriculture and Consumer Protection. I would like to thank the project partners Wendland Geflügel for hosting the experiment and ÖTZ for providing White Rock hens and chicks of ÖTZ Coffee and ÖTZ Cream. 26th World's Poultry Congress, abstracts selected in 2022

Mananagement of broiler breeders

Selected short communications

DATA ANALYTICS OF GROWTH DYNAMICS DURING REARING ON THE REPRODUCTIVE PERFORMANCE OF COMMERCIAL BROILER BREEDERS

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Growth control and nutrition during rearing have been demonstrated to affect the reproductive results of broiler breeders. Data analytics is useful to determine optimal growth patterns of pullets to achieve the best reproductive performance. A data analysis study was conducted to determine the effects of Ross 308AP pullets' growth dynamics on egg production (EP) and hen housed egg production (HHEP). A database containing weekly BW, feed intake (FI), cumulative FI (CFI), and reproductive performance records from pullets and hens reared under commercial tropical conditions was assessed. This study explored data of 45 broiler breeder flocks that represented 1,400,769 hens housed between 2017 and 2019. Partition trees were used to classify low (LP) or high reproductive performance (HP) groups according to the HHEP at 55 wk. Data were analyzed in JMP 15 Pro, using a one-way ANOVA with performance as the main effect and mean separation by students' t-test. Linear, quadratic, and multiple linear regressions were fit to establish relationships among HHEP at 31, 40, 50, 53, and 55 wk with BW and CFI at 4, 8, 17, and 22 wk. Seven flocks were classified as LP with an average of 149 eggs/hen-housed, and five flocks into the HP with 160 eggs/hen-housed up to 55 wk. Significant differences on BW indicated that LP pullets were heavier (P<0.05) in the first 2 wk and lighter between 7 and 13 wk of age. Broiler breeders in the HP group had greater (P < 0.05) FI at 7 and 8 wk, and CFI (P < 0.05) from 5 to 13 wk. The LP breeders had consistently lower EP (P<0.05) from 25 to 45 wk of age. Despite no significant differences on EP (P>0.05) between groups onwards, HP hens laid 11.3 eggs more than LP at 55 wk. The LP hens reached the peak of EP 2 wk later than the HP group (32 vs. 30 wk) with a difference of 4.9% on EP (83.5 vs. 88.4%) and seven eggs more of HHEP at 32 wk (35 vs. 42 eggs). HHEP at 40, 50, and 53 wk increased linearly as CFI increased (P<0.05) at 8 wk of age. Similarly, a positive linear effect (P<0.05) of CFI at 17 wk was observed at 40 wk. An optimal average BW of 1,810 at 17 wk (P<0.05), demonstrated to produce the highest HHEP at 31 (R2=0.42) and 40 wk (R2=0.36) while accounting for linear effects of CFI at 4 and 8 wk. In conclusion, heavier pullets in the early rearing resulted in greater feed restriction afterward to control BW reducing the whole egg production. Data analytics aid in identifying critical growth periods during rearing that affected HHEP.

EFFECTS OF A DIVERGENT SELECTION FOR BREAST MUSCLE ULTIMATE PH, A PROXY FOR GLYCOGEN RESERVES, ON REPRODUCTION AND EGG QUALITY TRAITS IN BROILER BREEDERS

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Two divergently selected broiler lines were created by selection for low (pHu-) or high (pHu+) breast meat ultimate pH (pHu) in order to better understand the genetics of meat quality traits in broilers and are also unique genetic resources reflecting low and high glycogen levels in chicken muscle. This study aimed to reveal the effects of divergent pHu selection in broilers on reproduction and egg quality traits in their breeders. Regarding reproduction traits, age at first egg (AFE), egg production (EP), broken egg percentage (BRKEP), clutch length (CL) from AFE to 38 weeks and 6 weeks-old body weight (BREBW) in the females of pHu breeders were individually determined from generation 2 to 14. For egg quality traits, external (egg weight: EW and shape index: SI), internal (albumen height: AH, Haugh unit: HU, yolk index: YI and color: YC) and shell (shell percentage: ESP, thickness: EST and strength: ESS) characteristics in pHu- and pHu+ lines were measured in all eggs for four consecutive days at 26, 27, 28, 30, 31, 32, 41 and 42 weeks in generation 14. Analyses of variance were performed using XLSTAT statistical software to test the line effect. Results are expressed as least square means. The pHu- hens started to lay at an earlier age than in pHu+ (183.5 and 186.5 days, respectively) (P < 0.001). The pHu+ line showed a significantly lower EP (55.3 vs 60.5 eggs), CL (3.04 vs 3.44 days) (P < 0.005), higher BRKEP (6.11 vs 3.52%) (P < 0.05) and lower BREBW (754.6 vs 803.8 g) (P < 0.001) compared to pHu- line. The mean EW in pHu- line (57.2 g) was significantly lower than in pHu+ (59.0 g) and increased with age in both lines (P < 0.001). ESP, EST and ESS were lower in the pHu+ eggs compared to the pHuline (P < 0.001). AH and YI were lower in pHu- eggs than in pHu+ (P < 0.001). YC was more intense (P < 0.001) and HU higher (P = 0.001) in pHu+ eggs than pHu- in pre-peak and peak laying period. In conclusion, in this genetic model, the selection for higher glycogen reserves was more favorable for egg production (due to earlier sexual maturity) and quality (smaller but thicker and less fragile eqq shell), while lower metabolic reserve status impaired egg production and quality possibly because feed quantity or concentration is no more adapted to these birds. The results of this study also provide an interesting perspective for both selection and better breeder management according to the metabolic status of birds.

EFFECTS OF DIET DENSITY AND FEEDING FREQUENCY ON BROILER BREEDER BEHAVIOUR DURING REARING AND LAYING PHASES

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Feeding broiler breeders (BB) ad libitum during rearing will result in high adult body weight, which negatively affects mortality, reproduction, health and welfare. Therefore, BB are fed restricted, especially during rearing. Numerous studies have shown that this restriction causes hunger and stress, resulting in stereotypic behaviour such as object pecking. Nutritional strategies can be used to alleviate hunger and stress and thus, improve BB welfare. Therefore, an experiment was conducted to evaluate the effects of diet density and feeding frequency on BB behaviour during rearing and laying. In total, 960 female pullets (Ross 308) were randomly allotted to 24 floor pens and to 4 different treatments within a 2×2 factorial design. From 3 weeks onwards, pullets received either control (CON) or diluted diets (DIL; containing oat hulls). Besides the diluted diets, pullets were fed once (FO) or twice (FT) a day. During rearing, behaviour was observed at 5, 10, 15 and 20 weeks of age (WOA) and during laying at 30 and 39 WOA. Observations were done by live scan sampling throughout the light period. Observed behaviours included drinking, standing, sitting, walking, foraging, object pecking and comfort. Data were analysed using generalized linear mixed model, using binomial distribution, where dispersion parameter phi was also estimated. During rearing, at 5 and 10 WOA (resp. Δ =-5.7% and Δ =-5.3%) less FT pullets were sitting as compared to FO pullets, but this difference was absent at 15 and 20 WOA (p=0.005). At 5 and 10 weeks of age, more CON pullets showed foraging as compared to DIL chickens (p=0.009; resp. Δ =+1.2% and Δ =+2.0%), whereas the opposite was found at 15 and 20 WOA (resp. Δ =-5.0% and Δ =-5.5%), and, FT foraged more at 5 and 10 WOA (resp. $\Delta = +1.4\%$ and $\Delta = +1.0\%$) whereas FO foraged more at 15 and 20 WOA (resp. Δ =+2.8% and Δ =+6.9%). FT pullets tended to perform more standing behaviour than the FO pullets (Δ =+1.2%; p=0.085). In the laying phase, FT hens showed more foraging behaviour compared to FO hens (Δ =+2.4%; p=0.028). Furthermore, dilution of the diet in rearing tended to result in more drinking behaviour in laying as compared to the CON diet in rearing (Δ =+1.8%; p= 0.083). In conclusion, this diet dilution and feeding frequency or the combination in this study had minor impact on BB behaviour and welfare. No consistent effects of the nutritional strategies on behaviours that are indicative for the degree of frustration and/or hunger were found.

OMEGA-3 FATTY ACIDS SOURCES FED TO BROILER BREEDERS AND PROGENY: IMPACT ON INTESTINAL LESION SCORES AND LYMPHOID ORGAN WEIGHT IN PROGENY CHALLENGED WITH EIMERIA

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Enriching broiler breeders (BB) and progeny diets with functional omega-3 fatty acids (n3FA) such as a-linoleic acid (ALA) and docosahexaenoic acid (DHA) may improve chick response to immune challenge. The objective of this study was to examine the effect of maternal age and dietary n3FA on progeny response to Eimeria challenge. A total of 588 pullets and 60 cockerels (Ross 708) d-old chicks were placed in separate rearing pens (28° and 20_{\circ} /pen) and fed three diets: corn and soybean meal control (CON) or CON + either 1.2% algae product as source of DHA (DMA) or ~2.5% flaxseed-based product as source of ALA (FFF). The DMA and FFF diets were formulated to have same total n3FA and n6FA: n3FA ratio. Birds were fed (\circ pens; n=9 for CON & n=6 for DMA/FFF) to sexual maturity (22 wk of age, woa), moved to laying house and grouped based on rearing diets (60 and 10σ /pen) and fed 7 diets. Birds reared on CON were split into 3 groups: CON-CON; fed CON, CON-DMA; fed DMA and CON-FFF; fed FFF. Birds reared on DMA and FFF either continued these diets (DMA-DMA and FFF-FFF) or switched to CON (DMA-CON and FFF-CON). Fertile eggs (210/diet) were collected at 34, 44 and 54 woa, incubated, sexed and hatchlings placed in cages (8 chicks/cage) based on rearing-laying diet, sex, and BW. On d10, birds were orally challenged with E. acervulina and E. maxima. Intestinal lesion scores were recorded on d 15 and bursa, liver and spleen weight on d15 and 42. Data was analyzed in nested design of rearing, laying and progeny diets, BB age and progeny sex, and interactions. Jejunal lesion score was affected by a combination of BB rearing and laying diets, BB age, progeny diet and progeny sex (P>0.001). Female progeny fed DMA diet, from CON fed 54 woa BB and female progeny fed CON diet, from 34 and 44 woa BB fed DMA during rearing and CON during laying had the highest lesion jejunal lesion scores. However, female progeny fed DMA from 34 and 44 woa BB fed CON during rearing and lay, had the lowest jejunal lesion score. Progeny from week 54 BB had significantly lower duodenal lesion scores than progeny from week 34 or 44 old BB (P < 0.001). BB age influenced immune organ weights at d42 such that relative liver and spleen weights decreased as BB aged (P<.001). In conclusion, the combination of BB rearing, laying diet, age and progeny sex and diet affected progeny response to Eimeria challenge such that DMA diets had higher lesion scores.

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Mananagement of broiler breeders

Posters

COMPARATIVE EFFICACY OF TYLVALOSIN PREMIX VS. A TILMICOSIN WATER MEDICATION FOR THE CONTROL OF MYCOPLASMA SPP. IN SOUTH-EAST ASIAN BROILER BREEDERS

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To make an informed decision about a Mycoplasma disease control program best aligned with antimicrobial stewardship, a broiler breeder integration had compiled accurate data sets from its hatchery and corresponding production in differently treated flocks. Valosin® 425 premix and Tilmicosin 25% local water-soluble product were used and analysed in Mycoplasma infected flocks. We describe the intervention yielding best results by analysing key performance indicators. Experimental design and material and methods. Prior to treatment, ELISA and PCR investigations were carried out to quantify the Mycoplasma infection. Four medicated feed treatment time points in the production period per flock were selected in 3 Valosin® premix (170 ppm, 7 days) as compared to 3 Tilmicosin drinking water (20 mg/kg BW/day, 5 days) treated houses. Mixed models controlling for the random effect of flock, were used to evaluate the performance of those 6 investigated flocks during the 3 weeks after initiating treatment. Hatchery data and pipped embryo data analysis (PEA) (20 embryos/flock) were analysed accordingly. The statistical analysis of hatchery and production data revealed a 2 % (P-value = 0.02) advantage of eggs/hen/day for the Valosin® treatment. Other parameters were being significantly influenced as well and will be given in detail. PEA revealed significant improvement of the TYL group in the % of culled chicks and % dead in shell embryos. Non-significant tendencies for higher % hatch per egg, higher female body weights and higher egg weights in Valosin treated birds compared to Tilmicosin treated birds. The Valosin® 425 premix medicated feed treatments improved laying %, a significantly lower PEA score and better overall chick quality. In addition, the Valosin® treated groups generated more and healthier eggs. This study showed superior efficacy results for the Valosin® 425 premix versus the Tilmicosin water medicated flocks in broiler breeders, showing a clear advantage for Valosin® 425 Premix. This aligns nicely with antimicrobial stewardship to administer a licensed treatment reducing disease burden most efficiently when needed.

EFFECTS OF PHYTOMOLECULES ON BODY COMPOSITION, GUT HEALTH, AND REPRODUCTIVE PERFORMANCE OF BROILER BREEDERS

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In this experiment two phytogenic gut health additives were combined from dayold till 62 w of age. The effects on gut health, hatching egg production, bodyweight and fertility in broiler breeders were assessed. A total of 1.560 Ross 308 parent stock day-old-chicks were randomly allocated in rearing pens. Treatment birds were provided a microencapsulated phytogenic formulation (MPF) (EW Nutrition, Visbek, Germany) in feed at 100 ppm continuously and a liquid phytogenic formulation (LPF) (EW Nutrition, Visbek, Germany) at 500 ml/1000L water from 1-4 d, and at 250 ml/1000L water from 8-11 d, 18-21 d, and 4 days weekly from 24-29 weeks of age to support gut health during challenging episodes. Both MFP and LPF contain carvacrol and cinnamaldehyde as bioactive phytogenic compounds. Control birds were not supplemented. Males and females were transferred to a production house at 19 and 20 weeks of age, respectively. Per treatment, 10 replicate pens were randomly populated, each with 55 hens and 7 males. Treatment birds reached 5% production 6.4 days (P<0.01) earlier than control birds, around 24 w of age. Hatching egg production / hen housed was significantly higher in treatment birds (180.8 vs. 169.5, P<0.05), partly due to a better laying persistency after 40 wk of age. Fertility & hatch of fertile eggs, assessed in 13 hatchery experiments with 90 eggs / pen, were not affected by treatment, nor was hen mortality (w 62 cumulative mortality: 5.2%). The resulting chick production / hen housed was increased by 10 chicks in treatment birds (155 vs. 145, P=0.07). Hen BW of treatment birds was higher at 22 w of age (P<0.001), but the difference disappeared, with treatment birds being 50g lighter at 54 weeks of age (4032g vs. 4082 g, P=0.08). Average hen feed intake was unaffected by treatment, at 164 g/d. At 22, 26, 31, 44 and 54 w of age a random female per pen was sacrificed, and breast muscle and abdominal fat pad were weighed, indicating heavier fat pads in treatment than control at 22 w (P<0.01). At 44 w of age, villi length and crypt depth were assessed, showing a borderline significant increase in villi length for treatment birds (0.840 vs. 0.735 mm, P=0.08).

This study showed that phytogenic gut health additives resulted in an earlier onset of lay and improved persistency and, consequently, a significantly increased chick production. This effect is likely related with the improved gut health demonstrated in the second semester of the production period.
EVALUATION OF BROILER FATTENING TREATMENTS ACCORDING TO FEED EFFICIENCY

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Improving the production traits of modern broiler hybrids requires adequate feed in order to fully exploit the genetic potential. Growth efficiency is affected by: body weight (BW), average daily gain (ADG) and feed conversion ratio (FCR). Feed costs represent the highest percentage in variable costs and directly affect productivity and profitability of production. The aim of this research was to evaluate the impact of various feeding treatments in broiler fattening on the efficiency of applied feed. Ross 308 chickens (N = 120), which were divided into 4 groups, were used in the research. Feeding treatments differed in finisher mixtures which, in addition to other feeds, also contained 5% oil, namely: P1 sunflower, P2 soybean, P3 rapeseed and P4 flaxseed oil. The mixtures were balanced at the level of 20% crude protein and 15.7 MJ kg/Me. All groups of chickens were housed in the same poultry pen with water and feed provided ad libitum. The paper examines production and economic indicators: Productive efficiency index (PEI), European broiler index (EBI), Economic efficiency index (EEI) and Cost index (CI). The efficiency of production indices was as follows (PEI and EBI): P1 363 and 351, P2 347 and 335, P3 362 and 351 and P4 367 and 359. PEI indicators depended largely on broiler final weight and feed conversion. EBI values followed PEI values. Feed costs (kg/BW) were as follows: P1 0.85, P2 0.90, P3 0.88 and P4 0.89 euros. The economic efficiency index and the feed cost index differed among groups of broilers (except P1 group) as follows: P1 100 and 100, P2 94.8 and 105.4, P3 96.7 and 103.4 and P4 95.4 and 104.6. The cost prices of the feed mixtures depended on the type of oil used, since the other feeds in the mixtures were the same. Dividing the cost price of the feed consumed (euro) by the actual final weight (kg), the cost price of a kilogram of live weight yield of broilers was determined. The value of the EEI indicator was the highest in the P1 group and the lowest in the P2 group. The highest CI value was found in the P2 group, followed by the P4, P3 and P1 groups of broilers. PI and EBI indicators were used to compare the productivity of the performance of several treatments (groups) of broilers. Economic efficiency and feed costs can be presented by the values of EEI and CI indicators.

Keywords: broilers, trait efficiency, conversion ratio, cost index

GENETIC SELECTION FOR BROILER GROWTH OVER 6 DECADES AND THE EFFECTS ON SEXUAL MATURITY THRESHOLDS FOR BROILER BREEDERS

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Broiler BW has increased by over 400% as a result of quantitative genetic selection between the years of 1950 and 2005 (Zuidhof et al., 2014). However, the gap between growth potential of broilers and broiler breeder target BW has increased over the last 60 years due to feed restriction of breeders, which is required to promote reproductive performance. There are multiple thresholds (age, BW, and body fat) for sexual maturation, which can be influenced by the level of feed restriction. The objective was to investigate the effect of commercial selection on the body conformation and sexual maturity of broiler breeders using two University of Alberta Meat Control strains unselected since 1957 and 1978 and two strains originating from the University of Arkansas; 1995 Random-bred and 2015 Random-bred. The study was conducted with a 4×2 factorial arrangement with the 4 strains fed either ad libitum or restricted to achieve a 2015 commercial breeder target BW profile. Age at sexual maturity and carcass components were measured. Total body fat was estimated using the following equation: Total carcass fat=4.4922+4.1181 × abdominal fatpad (Zuidhof, 2018). Huxley's allometric model was used to run a nonlinear covariance analysis on carcass components curves and predict carcass component weights for each strain and treatment (Huxley and Teissier, 1936). Under ad libitum feeding conditions there were no strain differences in the age of sexual maturity, suggesting that age was the last threshold to be reached under ad libitum feeding conditions. Achieving the target BW and concomitant feed restriction level had the greatest impact on reducing BW relative to its genetic potential in the 2015 strain. There was a positive relationship between the degree of feed restriction and the age of sexual maturity. Minimum BW at sexual maturity increased from 1,241 g for the 1957 strain to 2,768 g for the 2015 strain (P < 0.001). Minimum body fat (% of BW) at sexual maturity decreased from 12% for the 1995 and 1978 strains to 10% in 2015 strain (P <0.001). Relative to 1957 and 1978 strains, breast muscle and fatpad yield curves in the 1995 and 2015 strains were shifted upward and downward, respectively. Genetic selection programs have resulted in broiler breeders that prioritize nutrient allocation to growth and breast development rather than adipose sites. As a result, feed restricted modern broiler breeders may have marginally sufficient fat resources to support reproduction.

HEMATOLOGICAL PARAMETERS IN BROILER BREEDER FLOCK FED DIET SUPPLEMENTED WITH NATURAL 1,25(OH)2D3-GLYCOSIDES

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Vitamin D is an important factor in calcium metabolism, bone development and eggshell formation in laying hens. However, it also has an important function in improving immune response and lowering inflammatory reaction acting on several signaling pathways. In this field trial we monitored the effect of natural 1,25(OH)2D3-glycosides from waxy-leaf night shade (Solanum glaucophyllum) on hematological parameters of broiler breeder hens from 55 till 61 weeks of age. Two separate houses with 5000 animals of Ross 308 breeders each received standard feed (CON) or supplemented with 1 µg of 1,25(OH)2D3-equivalents (SG), with total vitamin D content in both treatments at 3200 IU. Blood samples were taken at 14, 25 and 35 days after the beginning of trial and tested for number of red and white blood cells, hematocrit and hemoglobin concentration. Gained data were compared between treatments per time point and within treatment over time using LSD-model with significance set on $p \le 0.05$. Level of red blood cells move between 2,32 \pm 0,21 and 2,7 \pm 0,42 x1012/L, with rise till 25 days after start of treatment in both groups, with slight drop till 35 days in both groups, without significant differences between groups. Number of white blood cells in SG group significantly dropped at 35 days of age from $12,64 \pm 5,42$ to $3,74 \pm 1,68 \times 109/L$, and was significantly lower compared to all sampling days within the group, as well as compared to CON group which rose to $16,04 \pm 4,37 \times 109/L$ at 35 days. Hematocrit values in group SG rose significantly at 35 days within the group to $0,35 \pm 0,033$ L/L, without significant difference between treatments. Hemoglobin concentration rose continuously in SG group until the end of the trial, but with significantly higher values in CON group 25 days after start, at $90,94 \pm 11,47$ g/L, both between the treatments and among sampling times. Results showed continuous rise and persistence of red blood cell number, hematocrit and hemoglobin concentration parameters in the group with added natural vitamin D. However, number of white blood cells until the end of trial significantly dropped what could indicate modulation of immune and inflammatory reactions in the organism.

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WHOLE GENOME SEQUENCING AS A TOOL TO UNDERSTAND THE EPIDEMIOLOGY OF SALMONELLA IN INTEGRATED POULTRY COMPANIES.

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Introduction: Salmonella contamination is a major challenge for poultry companies since this foodborne pathogen can thrive at any step of the food production chain. This presents challenges regarding the intervention strategies that must be employed to reduce the risk of Salmonella transmission to consumers. Our aim was to study the dynamics of Salmonella in the broiler production chain of two integrated poultry companies using a whole genome sequencing (WGS) approach. Methodology: Isolates from integrations A (n=68) and B (n=176) were sequenced according the harmonized FDA GenomeTrakr/CDC PulseNet WGS protocol. Raw reads were submitted to Enterobase for serotyping, MLST designation, and SNP tree analysis. Identification of genetic determinants of antimicrobial resistance (GDAMR), virulence genes and genes conferring resistance to disinfectants were performed. The pESI-like p-F219 (CP038508) was screened among this genome collection. Results: 82% of isolates belonged to the serovar Infantis. Over 98% of S. Infantis strains contained GDAMR to more than 3 antimicrobial classes, disinfectants and heavy metals. All S. Infantis strains belonged to ST-32 and contained virulence genes associated to enhanced pathogenesis. Furthermore, the pESI-like plasmid was observed in 87% of S. Infantis strains. These plasmids contained insertion sequences in class 1 and 2 integrons, along with AMR genes that confer resistance to first-line antibiotics. SNP tree analysis showed that isolates that contaminate poultry carcasses could be trace back to the farm level. It was also observed that some isolates were either able to colonize multiple farms, or were restricted to specific farms within each integration. Moreover, some isolates were only detected in slaughterhouses, suggesting the possibility of carcass cross-contamination events at this level. Interestingly, isolates originating from the feed mill plant were observed to be the most genetically unique, when compared to the remaining collection. Conclusion: This investigation demonstrates the use of WGS analysis to gain insights into the dynamic epidemiology of Salmonella in integrated poultry companies. The results can be used to implement interventions that target the control of Salmonella during the early stages of broiler production. Moreover, we showed that S. Infantis is a major player in the epidemiology of Salmonella and that its genome can possess multiple genetic determinants of resistance and virulence.

SULFUR AMINO ACID REQUIREMENTS OF GROWING TURKEYS FED WITH HYDROXY METHIONINE OR DL-METHIONINE

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Little data has been generated in turkeys relative to the digestible sulfur amino acid (SAA) requirements. This trial aims to determine the digestible SAA requirements of Nicholas Tom & Hybrid Tom turkeys fed increasing doses of either DL-Methionine (DL-Met) or OH-Methionine (OH-Met) from 0 to 28 days. Birds were fed a basal diet based on corn and soybean meal formulated to be low in SAA or 14 treatments supplemented with seven increments of DL-Met or OH-Met in equimolar levels. Additionally, a positive control diet sufficient in SAA was fed as a positive control to ascertain that maximal growth has occurred i.e., 16 treatments in total. All other nutrients, including dLys were offered in adequate levels. Male turkeys were placed 20 birds/pen with 10 replicates/treatment. Ten birds/pen of the basal diet were euthanized and ileal digesta collected for amino acid digestibility determination using TiO2 at 0.5% as a marker. The mortality, body weight, body weight gain, feed/gain were collected. Data were analyzed by regression and breakpoint analysis to determine a requirement. Mortality was low for the trial and showed no differences between treatments. The 14-d growth rate and feed efficiency improved from the basal until the 0.94% SAA treatment with no difference seen after this level for both DL-Met and OH-Met. Feed efficiency appeared to be significant at 0.94% SAA than at lower levels. With the SAA levels as explanatory variable, the estimated requirement using a 2-slopes model for gain was 0.982% for both OH-Met and DL-Met. It was estimated respectively at 1.003% and 1.000% for the 14-d feed: gain. At 28-d, the feed efficiency was also significantly more efficient at 1.10% SAA than lower levels. The requirement in SAA was estimated at 1.070 and 1.007% for the 28-d gain respectively for OH-Met and DL-Met and it was estimated at 1.009 and 1.002% respectively for the 28-d feed:gain ratio. Other statistical models (quadratic, one slope ratio on both calculated, analytical or intake of SAA) were also tested in this study and resulted in a requirement from 0.98% to 1.07% for the two Met sources. The estimated requirements matched the positive control's which offered 1.02% of SAA. While a variety of analytical methods would result in variable values for requirements, it appears that the SAA requirements are similar for both OH-Met and DL-Met.

HOW MONITORING SYSTEMS ARE USED BY FARMERS AND ADVISORS FOR A BETTER CONTROL OF ANIMAL HEALTH MANAGEMENT?

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Different approaches to manage health exist in all animal production sectors, but they are not often widely shared between them. «UniFilAnim Santé» is a collaborative project concerning different animal production sectors (poultry, pigs and ruminants) that aims to help farmers to improve animal health management by drawing inspiration from different monitoring systems applied in other sectors. First, a questionnaire was sent to farmer's advisors (veterinarians, technicians, dairy controllers....) to detail any tools or steps used on farms to improve animal health management. From the 178 responses collected, a typology of all these tools/steps allowed us to distinguish five groups: mandatory tools, tools related to the creation of benchmarks, tools based on slaughterhouse data and analytical laboratory results, tools related to farmer's discussion groups, and finally tools to be specifically set up on farm by the farmer, alone or with an advisor. Then, 12 farmers and/or advisors focus-groups for the different production fields were conducted (i) to test this typology, (ii) to identify tools they are using or that they could use to monitor the health of their animals and (iii) to classify them in one of the predefined groups. After a gualitative analysis, the results for the different animal production sectors show a discrepancy between the tools used by farmers and those used by advisors. Advisors are using rather tracking tools that are forward-looking, expensive and guite sophisticated. Farmers are mainly using "sensitive" indicators (smell, sight, etc.) on a daily basis. DIfferent actors (farmers and/or advisors) regret, whatever the production field, the lack of inter-operability sometimes between their tools and the lack of data valorization. There is a distribution of roles and tools between farmers and advisors opposing a daily health management operated by farmers to a medium-term surveillance of livestock health provided by advisors. Each sector is distinguished by the partnership scheme that the farmer builds around the farm health surveillance, and by the nature of the contract which binds him/her to economic operators: cattle and pig farmers are more involved in the management of their farm and their animals, whereas in the poultry sector (that is more integrated), it is mainly coordinated by the advisors of the poultry industry organization.

THE EFFECT OF DIETARY AND LIGHTING SOURCES OF VITAMIN D3 ON FLOCK UNIFORMITY, FEATHER SCORE AND GASTROINTESTINAL ORGAN WEIGHTS

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Introduction Broiler growth rates have increased due to genetic selection, superior nutrition and better management. However, increased growth rates have resulted in reduced size and capacity of the gastrointestinal tract (GIT), increased time sitting on a wet litter, which contributes to poorer flock uniformity (FU) and feather score (FS), leading to poor performance and economic losses. Dietary vitamin D3 and UVB light have been reported vital to the health and wellbeing of broilers. This study evaluated the effect of dietary vitamin D3 and UVB light on FU, FS and GIT components weights of broiler chickens. One day (d) old Ross 308 broiler chicks were weighed and allocated to dietary vitamin D3 in IU/kg at 4000 only (HD), 1000 + UVB (LDU), and UVB wavelength only (UVB) to determine FU % on 38 d, FS on 35 d and GIT wts relative to body weight (BW) g/100 on 42 d. FU (n=300) was based on pen BW; for FS (n=150), feathers were assessed for different body parts using the RSPCA scoring scheme and the percentage of overall cover scores (%CS). GIT organ parts (n=60) were collected, weighed and averaged. UVB lamps with wavelength: 280-315 nm, intensity; 28.12 µW/cm2 hung 50 cm above the substrate were used with the lighting schedule of 23L:1D (1-7 d) and 18L:6D (8 d to end) in all the treatment groups but were filtered to remove UVB in HD. Data were analysed using one-way analysis of variance of SAS 9.4 software. Overall, UVB birds had better FS (P<0.05), heavier (P<0.05) pr+Gizz and heart weights with similar (P>0.05) FU with other treatments. In conclusion, UVB light has the potential to support the production and welfare of commercial broilers.

26th World's Poultry Congress, abstracts selected in 2022

Pluridisciplinary approaches to reach the One health objectives

Posters

GROWTH PERFORMANCE AND GAIT SCORE OF BROILER CHICKENS TREATED WITH DIETARY AND LIGHTING SOURCES OF VITAMIN D3

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Introduction of Vitamin D (vitD3) is required for optimal performance and can be produced in the skin or absorbed from the diet. The use of controlled, windowless, indoor houses in conventional broiler farms does not allow the skin to produce endogenous vitD3, while raw materials in broiler diets contain little or no vitD3. As a result, requirements could be met by dietary supplementation or UVB irradiation. This study aimed to evaluate the effect of dietary vitamin D3 and UVB light on growth performance (GP) and (GS) of broiler chickens. Materials and methods. One day (d) old Ross 308 broiler chicks (n=300) were weighed and allocated to dietary vitamin D3 in IU/kg at 4000 only (HD), 1000 + UVB (LDU), and UVB light only (UVB) to evaluate body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) on 38 d and GS on 37 d. For GS, birds were individually assessed using the gait scoring system described by Garner et al. (2002). UVB lamps with wavelength: 280-315 nm, intensity; 28.12 µW/cm2 hung 50 cm above the substrate were treated as the lighting schedule of 23L:1D (1-7 days) and 18L:6D (8 days to end) in all the treatment groups but were filtered in HD group. Data were analysed using a one-way analysis of variance of SAS 9.4 software. Results. Treatments differed (P<0.05) for their effect on BWG and GS (Table) of broilers. FI and FCR of broiler chickens were the same (P>0.05) for all treatments; UVB birds had lower and better GS (P<0.05) compared to their counterparts Conclusion. Although none of the treatment strategies improved growth and walking ability of broilers, results suggest some beneficial effects of UVB light on the welfare of broilers. Further research on the optimum exposure time and level of UVB light that could be applied in poultry housing for providing vit D3 to maximize growth and welfare benefits is needed.

Acknowledgments The authors thank Tertiary Education TrustFund of Nigeria and UKRI, BBSRCfunded project (BB/T001747/1) for funding

INFLUENZA PATHOGENS: INNOVATIVE ASSAYS FOR DIAGNOSIS AND VACCINATION MONITORING OF H5, H7 AND H9 AVIAN INFLUENZA A VIRUS.

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Influenza viruses belong to the family Orthomyxoviridae and infect a variety of human and animal hosts. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further divided into subtypes based on their Hemagglutinin (H) and Neuraminidase (N) antigens. Eighteen H antigens (H1 to H18) and eleven N antigens (N1 to N11) have been isolated. Most avian influenza viruses (H1 to 18 subtypes) are low pathogenic, such as H9. Co-infection between avian respiratory diseases and low pathogenic virus can lead to important losses in poultry flocks. Additionally, some subtypes containing H5 and H7 are associated with highly pathogenic forms of the disease, with high rate of mortality.

For disease diagnosis, competitive ELISAs are used for screening (e.g. ID Screen® Influenza A Antibody Competition Multi-species) and serotyping (e.g. ID Screen® Influenza Hx Antibody Competition). Their advantage lies in that competitive ELISAs are multi-species and highly specific due to the monoclonal antibody conjugate. Moreover, real time-PCR may be also used for disease diagnosis (e.g. ID Gene[™] Influenza A qPCR, the ID Gene® Influenza H5/H7 Triplex and ID Gene® Influenza H9 lineage G1-like Duplex).

However, due to vaccination diffusion in many countries, and given that competitive ELISAs are not suitable for monitoring of antibody titers, IDvet has developed unique indirect ELISAs to monitor vaccination uptake for H5, H7 or H9 AI. Vaccination monitoring with indirect quantitative ELISAs has the advantage to be highly correlated with HI test, and could be used to monitor both conventional and recombinant vaccines (with DIVA strategies application for vector vaccines).

The following presentation summarizes the preliminary validation data obtained for Avian Influenza diagnostic tools developed by IDvet.

FEEDING BROILER CHICKENS WITH A DIETARY MURAMIDASE: ENLIGHTENING THE PERFORMANCE IMPROVEMENT THROUGH A MULTI-OMICS APPROACH

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Muramidases, also known as lysozymes, have been shown to catalyse the degradation of intestinal lumen bacterial cell debris, like peptidoglycans (PGNs). The muramidase-mediated hydrolysis of PGNs can optimize digestive and absorptive functions and modulate the intestinal inflammatory response, thereby improving gut health and performance of broilers. However, muramidase mode of action still needs to be elucidated (Goodarzi Boroojeni et al. 2019; Sais et al. 2020; Wang et al. 2020). Thus, we attempted to address this problem by integrating performance, plasma metabolomics, and microbiome data of muramidase-fed broilers. 2,340 one-d-old male Ross308 chicks were randomly assigned to 3 groups (12 replicates each) receiving a basal diet supplemented with 0 (CON), 25,000 (MUL), or 45,000 LSU(F)/kg feed (MUH) of a commercial fungal muramidase for the entire rearing cycle (42 d). On a replicate basis, chicks were weighted at housing, while body weight (BW) and feed intake (FI) were recorded at 9, 21, 28, and 42 d to calculate feed conversion ratio (FCR). Performance data was subjected to one-way ANOVA and post hoc test. From 1 bird/replicate, selected at 42 d according to a similar BW, plasma and caecal content were collected and analysed via 1H-NMR and metagenome shotgun sequencing, respectively. Plasma molecule concentrations were analysed through one-way ANOVA and post hoc test, whereas microbiome analysis is still in progress. Compared to CON, MUH reached higher BW (2.775 vs 2.906 g; p < 0.05) and lower FCR (1.729 vs 1.686; p < 0.05) from 0 to 42 d. MUL did not significantly outperform CON from 0 to 42 d. MUH showed lower levels of circulating pro-oxidant nucleotide- and amino acid-catabolism endproducts (histidine, uracil, hypoxanthine) and higher levels of energy metabolismrelated molecules (pyruvate, 2-oxoglutarate, glucose) than CON and MUL (p < 0.05). Overall, the muramidase supplementation considerably improved BW and FCR in a dose-dependent fashion. MUH plasma metabolome potentially reveals better oxidative state and bioenergetics, which may be related to the performance improvement. Preliminary microbiome results suggest that MUH may have diverged from its untreated and low-dose-supplemented counterparts, revealing an intriguing eubiotic effect of muramidase. Hence, based on these observations, muramidase could be considered as a metabolism- and gut-health-enhancer for broiler chickens, partly clarifying the mechanism of its growth promoting effect. References

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ID: 1720 MATERNAL FLAXSEED DIET DID NOT AFFECT GROWTH RATE OF BROILER CHICKENS DIAGNOSED WITH NOVEL AVIAN REOVIRUS AND INFECTIOUS BRONCHITIS

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Maternal feeding of omega 3 fatty acids (n-3 FA) can modulate offspring immune responses by directly affecting immune cells and mediators. During a research trial, broiler chickens were diagnosed with novel avian reovirus (runting and stunting syndrome) and infectious bronchitis. The objective was to assess whether maternal fed n-3 FAs affected the growth rate or uniformity of immune challenged broiler chickens. Ross 708 broiler breeders obtained at 1 day of age were fed control or "LinPro" flaxseed enriched Maternal Rearing Diet (MRD) or Maternal Laying Diet (MLD) in a 2x2 factorial design. The breeders were housed in 8 parental pens. Broiler chicks were hatched in two trials, in the same hatchery at the University of Guelph poultry research station. Trials overlapped, with trial 2 starting 3.5 weeks after trial 1. Broiler chicks were housed in mixed-sex groups of 20 chicks (from the same parental pen), in 2 rooms per trial, each containing 12 floor pens following a randomised complete block design (N=960; n=12 pens of 20 birds per treatment combination). Chickens were weighed weekly up until 6 weeks of age (WoA); low body weights were recorded from the onset of the trials and infectious bronchitis symptoms were recorded from 1.5-4 WoA. Post-mortem diagnostics were conducted by a specialist poultry veterinarian. A linear mixed effect model in R was used to analyse the weight data and a 2-way repeated measures ANOVA was used to analyse differences in coefficient of variation (uniformity). The mean weight (g) of broiler chickens with Ross 708 target weights in parentheses for week 1-6 respectively; 125.85 (198), 318.06 (490), 534.85 (928), 810.34 (1486), 1117.10 (2120), 1642.27 (2782). The variation in the data is explained by a 3-way interaction of MRD x MLD x WoA (P<0.001). A post-hoc analysis showed that there were no effects of MRD (P=0.829), MLD (P=0.911) or their interaction (P=0.988) on body weight. MRD tended to effect uniformity (P=0.098), control MRD broilers were more uniform than LinPro MRD broilers. There was no effect of MLD on uniformity (P=0.948). However, there was a significant interaction between MRD and MLD (P=0.012) with control MRD - control MLD offspring being most uniform. Mortality rates were not analysed due to low incidences of mortality. In conclusion, a maternal diet enriched with flaxseed did not affect the growth rate, but affected the uniformity of broilers diagnosed with novel reovirus and infectious bronchitis.

EFFECTS OF MANNANASE ON INTESTINAL HEALTH IN BROILERS ANALYZED IN 24 EXPERIENCES IN ASIA

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This project was conducted to investigate the effect of β -1,4-mannanse on intestinal health by statistical evaluation of lesion scoring data from Asia. A total of 1216 apparently healthy broilers in 24 trials including China (18), Japan (5) and Vietnam (1) were collected for the necropsies. Birds are humanely euthanized by cervical dislocation. Clinical diagnosis of intestinal conditions is recorded for each bird individually as described in broiler disease reference quide (2019) by trained veterinarians. At each necropsy, 5-30 birds were collected per house in the field trials. Data from all genetics and posting ages were included in the analysis. Uneven numbers of posted birds on each treatment were deemed acceptable for the field trials. Lesion scores from 23 conditions related to intestinal health were used to calculate an intestinal integrity index. Chi-square analysis of categorical score proportions was used to compare treatment means of posting data, and analysis of variance and Tukey-Kramer HSD were used to compare treatment means of the index by JMP15 (SAS). Overall, the intestinal integrity index was increased by 1.38 with administration of β -1,4-mannanse (P<0.01). Six different conditions related to intestinal integrity were significantly affected, notably by excessive mucus content (-5.85%), excessive watery content (-13.03%), intestine tone (-6.7%), hyperemia (-8.28%), thin intestine (-3.01%), and cellular sloughing (+4.08%) (P<0.01). Additionally, reduction in the incidences of pododermatitis was observed (P<0.05). This study suggested that the use of β -1,4-mannanse to hydrolyze β -mannans improved intestinal health.

Keywords: broiler, mannanase, gut health, meta-analysis

NOVEL BLOOD BIOMARKERS TO PREDICT BETTER RESPONSES TO VACCINATION IN CHICKEN

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Because of the multiple factors controlling host responses to vaccines or to infections, more effective disease control strategies should be developed in an integrated animal health management approach, including prevention, cure, environment control and breeding for improved immunocompetence. The impact of host genetic variation in shaping innate and adaptive immune response is an emerging lever to consider in new vaccination strategies. Indeed, in the same flock, not all birds even vaccinated simultaneously and with the same dose, will respond equally to a vaccine. This study aimed at, 1- utilizing the host genetic variability of the response to Eimeria maxima vaccination of chickens to search for blood biomarkers predictive of vaccine response intensities; then 2- to test the candidate biomarkers in validation populations for another vaccine response, to Newcastle Disease Virus Vaccine (NDV).

For the search of biomarkers to E. maxima vaccine, 90 male commercial broilers (Cobb 500) were used. Nine days after vaccination with 100 E. maxima Weybridge strain oocysts at 16d, a subset of 79 birds were challenged with 50,000 parasites of the homologous species/strain. Body weight gain, lesion scores, parasite load and IL-10 serum levels were used to assess birds' responses. Birds were ranked according to their vaccine responses and RNAseq data were produced from blood sampled prior vaccination for 63 birds representing the diversity of responses. For each response phenotype, high and low responders were selected (upper and lower quartiles) and sparse Partial Least Squares-Discriminant Analyses (sPLS-DA) were carried out to identify the best predictive blood biomarkers classifying birds into each group. Finally, 108 candidate genes, predictive with accuracy higher than 97% for all vaccine responses, were selected. A custom OpenArray for high-throughput RTqPCR assays was then design to test the candidate biomarkers in validation populations. First test was performed on a population of White Leghorn selected birds displaying a large variability of response to NDV vaccine.

This is a proof of concept that blood before vaccination can be used as a relevant source of biomarkers predictive of vaccine responses. Future researches, combining genetic variability of response to vaccine and disease and novel vaccines, pave the way for better and more efficient vaccination through "precision vaccination" and "personalized health management".

EXPERIMENTAL PRODUCTION OF GUT INFLAMMATION MODEL IN BROILER CHICKENS

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A two-week cage study was carried out for the experimental production of gut inflammation model in broiler chickens.

One hundred and ninety-two 8-day-old male Ross 308 chicks were used and experimental duration was from days 8 to 22. Before the start of the trial, the chicks were housed in cages in pre-trial room, where they received a common starter diet based on corn and soybean meal. On day 8, the chicks were weighed individually and allocated to two treatment groups. Each group comprised of eight replicate cages and twelve birds in each replicate cage. Birds in the challenge group were given a challenge diet high in wheat and fish meal content while the birds in the control group were kept on the common starter diet until the end of the trial. At 9 days of age, birds in the challenge group were orally gavaged with a coccidial vaccine at 20x dose (Coccivac B®, Schering-Plough Animal Health) in a 0.5 ml solution (sterile water). Birds in the control group were given a placebo gavage with equal amount of sterile water. Data were subjected to twoindependent t-test using SPSS (version 22th). Each cage served as an experimental unit. The effect of treatment groups was considered significant at P < 0.05.A total of ten birds died in the challenge group and there was no death in the control group throughout the trial. Results revealed that the birds in the challenge group had significantly impaired performance and feed conversion ratio when compared with birds in the control group during each week and cumulatively. Birds in the challenge group had higher jejunal viscosity (P < 0.001) and dysbacteriosis score (P = 0.095) but less efficient energy digestibility (P = 0.059) at ileal level compared with those in the control group. mRNA expression of IFN-y and IL-1 β in the jejunal mucosal was significantly increased in the challenge group than in the control group. There was no difference (P > 0.05) in the abundance of Cl. perfringens in cecal content between the two treatment groups. In conclusion, using a specific diet formulation combined an overdose of live coccidial vaccine were successful in inducing gut inflammation as evidenced by growth depression, decrease in energy digestibility and upregulation of mRNA expression of IFN-y and IL-1β in jejunal mucosal.

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NOVEL PLANT EXTRACTS IN BROILER DIETS: A PROTEOMIC INVESTIGATION FOLLOWING AN LPS CHALLENGE

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Two novel plant extracts, citrus and cucumber, were identified as possible alternatives to antibiotic growth promoters in broiler diets. The presence of a range of active compounds in citrus and cucumber makes them potential candidates for modulation of the immune system and proteome of broilers (1). A dietary trial (12birds x4replicates x3diets) was conducted to test the hypothesis that a baseline diet (CTL) supplemented with citrus (CTS) or cucumber extract (CMB) can modulate the proteome of broilers under challenge conditions. Each diet was fed from day old and all birds were challenged at 15 days old with E. Coli lipopolysaccharide (LPS), a bacterial endotoxin known to stimulate the host immune system. Liver and breast muscle samples (n=12xtimepointxdiet) were chosen for this investigation and collected at T0h pre-challenge and T12h post challenge. Samples were extracted, guantified by BCA assay, and proceed using the FASP protocol and TMT labelling. High resolution LC-MS/MS analysis was performed, and proteins identified/quantified using Proteome Discoverer. T-tests comparing CTL vs CTS and CTL vs CMB at T12h were performed in Rstudio and gene ontology explored. Significant proteins were validated by Western Blot (WB). In the liver, several proteins were found to be modulated by the CTS diet compared to CTL at T12h. Among these, heat shock protein 90 (HSP90) was discovered to be lower in abundance in CTS diet while lactate dehydrogenase (LDHA) was found to be higher in abundance. Protein validation WB studies demonstrated subtle differences, but these were not significant. In breast muscle at T12h, many proteins were again found to be modulated by the CTS diet; myosin had a lower abundance while Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was higher in abundance compared to CTL diet. The CMB diet in contrast to the CTS diet did not appear to modulate the proteome of the liver or muscle at T12h post LPS challenge. In conclusion, the CTS diet had an impact on the liver and muscle proteome of broilers at T12h post LPS challenge while the CMB diet had no effect. This is the first investigation looking at the effect of novel plant extracts on the liver and muscle proteome following an LPS challenge in broilers. Additional proteins that could have a beneficial role on the immune system of broilers are currently under investigation.

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YEAST MANNAN RICH FRACTION HAS THE POTENTIAL TO MODULATE ANTIBIOTIC EFFICACY AGAINST SENSITIVE AND RESISTANT E. COLI

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Antibiotic resistance has the potential to become one of the greatest problems of our generation, given the ever-increasing rise in bacterial strains that are less and less sensitive to existing treatments. As antibiotic use in the agricultural industry is continually discouraged in a bid to slow down the rise of antibiotic resistance, there has never been such an urgency to find novel ways in which to combat pathogenic microbes such as Escherichia coli (E. coli). Nutrition and its impact on the gut microbiota is an area of interest in this fight and has led to the development of numerous yeast cell wall products that assist the move to antibiotic-free production status. Some of these products, such as yeast mannan rich fraction (MRF), are designed to regulate and support the gut environment and its microbiota. Research has shown that MRF has the capability of enhancing the antibiotic sensitivity of resistant strains (Smith et al., 2020). This study investigates the effects of MRF on the metabolic energy state of susceptible and resistant Escherichia coli in the presence and absence of ampicillin. It has been previously demonstrated that the activity of bactericidal antibiotics is associated with accelerated bacterial respiration (Lobritz et al., 2015). Experiments were carried out in triplicate using a Seahorse XFe96 Analyser, where oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured for both the Basal and End timepoints and then plotted together to form energy maps. The maps indicated a change in the metabolic state of E. coli caused by the addition of 0.01 mg/ml ampicillin, 0.1% v/w MRF, or a combination of both. When treated with the combination of 0.01 mg/ml ampicillin and 0.1% v/w MRF, the resultant energy map demonstrated a metabolic shift from a quiescent to glycolytic state in the case of resistant E. coli. This was also evident in the susceptible strain but to a lesser extent. This study indicated that MRF imparted a beneficial effect on the efficacy of antibiotics through modulating respiration and metabolic energy state of antibiotic susceptible and resistant bacteria. The changes in metabolic energy observed are indicative of the antibiotic killing effect and the beneficial potentiating impact of MRF on resistant E. coli. These findings could have positive implications for diet formulations focusing on antibiotic-reduction or antibiotic-free farming objectives.

BACILLUS LIPOPEPTIDE FENGYCIN ATTENUATES CLOSTRIDIAL VIRULENCE EXPRESSION VIA SIGNALLING INTERFERENCE

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The accessory gene regulator (Agr)-based guorum sensing (OS) signalling is instrumental to Clostridial and Staphylococcal virulence expression for intestinal colonization and infection1,2. Fengycin, a lipopeptide produced by many probiotic Bacilli, including Bacillus subtilis, was shown to inhibit the Staphylococcal Agr-QS, resulting in complete elimination of the pathogen from the host2. The QS system in Clostridium perfringens (i.e., agrBD and virSR) shares a homology with that in Staphylococcus aureus (i.e., agrBDCA), but the impact of fengycin on C. perfringens remains unexplored. Using a farm strain 4.6 (toxinotype G) as a C. perfringens model, we show that fengycin, a lipopeptide produced by many probiotic Bacilli, including Bacillus subtilis PB6, inhibited not only transcription of major toxins such as Perfringolysin O (pfoA), Alpha toxin (plc or cpa) and NetB toxin (netB), but also the QS regulatory elements like autoinducer peptide (AIP) synthase (agrD) and two-component system (virR and virS), in a concentrationreponsive and time-dependent fashion. Corroborating with the RT-qPCR results, an in vitro hemolytic assay confirmed the reduction of hemolysin activities, likely mediated by Perfringolysin O and Alpha toxin, in C. perfringens culture supernatant. While fengycin could suppress early biofilm formation (~ 3 h), such QS blocking effect was revoked at later stages (i.e, \geq 6 h) presumably due to accumulation of endogenous AIP in the culture that subdue the binding of fengycin to the QS receptors, tally with the hypothesis that fengycin being an Agr QS antagonist. In conclusion, our study demonstrate that the anti-virulence is an effective control of Clostridial pathogenicity, and this can be achieved through interupting QS-regulated gene expression and/or direct virulence factor inactivation. Specially, we show for the first time that fengycin can interfere with the Clostridial QS system and attenuate virulence expression at concentrations typically produced by B. subtilis culture. These outcomes re-define the mode of action and a new role of probiotic nutrition in governing animal health from pathogens.

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COULD PHAGE THERAPY ADMINISTERED ORALLY BE SPREAD SYSTEMICALLY TO THE BRAIN?

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INTRODUCTION. Bacteriophages (BP) are viruses that can specifically infect and kill targeted bacteria [1]. In large production as poultry, the BP administration in feed makes it suited to mass treatment [2]. Orally applied BP could cross the gut barrier and be disseminated by blood, reaching internal organs [2]. This ability remains of great interest as it enables penetration into even very inaccessible areas of the body [3]. However, gastrointestinal environmental conditions could compromise the effectiveness of the BP therapy [4]. Thus, protection of BP by methods such as polymer microencapsulation could avoid the problems [4]. In this context, the objective of this study was to access the ability of orally microencapsulated BP to reach the brain. MATERIAL AND METHODS. The Salmonella BP FGS011 was microencapsulated using the anionic polymer Eudragit® L100 using the spray drying technique [5]. A total of 60 1-day-old chicks were randomly housed in pens simulating the real production conditions. Once per week 10 birds were moved to another house and were randomly divided into 2 groups. Group L100 received L100 (1010 UFP/g) at 1% in feed and control group did not receive any BP. After 24 h, serum and brain samples of each animal were taken and processed for BP detection [5]. A GLM was used to evaluate the presence in serum and brain of the BP at different ages of BP application (weeks 1 to 6). A P < 0.05 was considered for statistically significant difference. SPSS 27.0 software. RESULTS. L100 was present in all the weeks in both type of samples. In the first two weeks of rearing, the L100 phage was detected in all serum samples, however, a decreased was observed since the third week onwards (P < 0.05). Concerning brain samples, L100 phage was detected without differences across the weeks (P < 0.05). The control group was negative for phage detection. CONCLUSION. The detection of BP in the brain is of special significance due to their complex accessibility to for delivery of drugs [1]. The results of this study demonstrate that the orally administered BP could cross the gastrointestinal barrier and be disseminated by blood, reaching the brain in chickens.

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DIETARY SUPPLEMENTATION WITH GRAPE SEED EXTRACT: EFFECTS ON GROWTH PERFORMANCE AND GUT RESPONSE OF BROILER CHICKENS

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In recent years, dietary additives coming from plants are becoming attractive for keeping and improving gut health in broiler chickens. To this purpose, tannins can be supplemented in diets due to their anti-microbial, anti-oxidant, and antiinflammatory activities, but their effects can differ according to their origin. Thus, the present study evaluated the effects of a grape seeds extract (GSE) containing tannins on growth performance, and gut morphology and immune response of broiler chickens. A total of 800 chickens (half females and half males) were housed in collective pens (25 birds/pen, 8 pens/group) and fed a control diet (C) or the same diet added with 0.1% (diet GSE01) or 0.2% (diet GSE02) or 0.4% GSE (diet GSE04) from 0 (hatching) to 41 d of age (commercial slaughter). At 14 d and 28 d of age, 8 chickens per dietary treatment were slaughtered to sample jejunum mucosa. Serial sections were stained with hematoxylin/eosin for morphometric evaluation and with antibodies against intraepithelial CD3+ T-cells and CD45+ leukocytes to evaluate the anti-inflammatory activity. Data were submitted to ANOVA using a mixed model with diet as the main effect and pen (growth data) or animal (qut mucosa data) as a random effect. Final live weight averaged 3,179 kg, which corresponded to a daily growth rate of 76.1 g/d and a feed intake of 113 g/d, for a feed conversion ratio at 1.49, without any significant difference due to the dietary GSE supplementation or level. As for the GSE supplementation, villi height tended to decrease when chickens were fed diet GSE02 compared to those fed diets C, GSE01 and GSE04 (965 µm vs. 1,046 µm, 1,059 µm and 1,058 µm, respectively; P=0.07), the density of CD45+ increased (2497 vs. 1867, 2067, and 1858 cells/10,000 µm2; P<0.05). As for slaughtering age, villi height (968 to 1096 μ m; P<0.01), goblet cells density (18.4 to 20.1 cells/300 μ m; P<0.05) and CD3+ (1.812 to 2.193 cells/10.000 µm2; P<0.05) increased from 14 to 28 d of age. In conclusion, under the condition of the present study, GSE dietary supplementation did not affect chicken performance, but somewhat impaired gut mucosa status (as for reduced villi height) which was associated to a pro-inflammatory gut response (as for the higher density of inflammation cells) when using intermediate supplementation level (0.2%).

EFFECT OF YEAST CELL WALL ON MODULATION OF IMMUNE RESPONSES IN AFLATOXIN-CHALLENGED BROILERS

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The objective of this study was to determine the effect of a yeast cell wall (source of β -glucans and MOS) to modulate the immune responses in aflatoxin-challenged broilers. The birds were housed in pens at 1 day old and divided into 4 treatments: Negative Control [NC] (basal diet without contamination); Positive Control with 2.5 ppm of aflatoxin [AFB]; Yeast cell wall supplemented group [YCW] from Saccharomyces cerevisiae (ImmunoWall®, 0.5 kg/MT); YCW with 2.5 ppm of aflatoxin [YCW+AFB]. The birds received the diets until 35 days old, and the aflatoxin was administrated through the diet. The liquid chromatography method coupled to sequential mass spectrometry (LC-MS/MS) was performed to verify the presence and level of aflatoxin contamination in the ingredients used. The New Castle vaccine was administered on day 14. On days 1 and 14, blood samples were collected with anticoagulant for ELISA antibodies against Newcastle Disease (NDV) to determine maternal titer (10 birds/treat). ELISA for antibodies against NDV was assessed in blood samples at days 28 and 35 (8 birds/treat). The blood collection samples with anticoagulant for determination of cell profile by flow cytometry, the phagocytic capacity of peripheral blood lymphocytes, and the assessment of intestinal permeability (using Dextran-FITC, 3-5 kD) were performed at days 7, 28, and 35 (8 birds/treat). The data from each analysis were submitted to the D'Agostino-Pearson omnibus K2 normality test. However, the data that failed this test were submitted to non-parametric analyses, Kruskal-Wallis test (GraphPad Software). The challenge with aflatoxin was verified and resulted in 2.24±0.28 ppm in the challenged groups. The Aflatoxin challenge promoted the depletion of cytotoxic T lymphocytes on day 14. The YCW+AFB promoted an increase (P<0.05) in diverse populations of immune cells, including total lymphocytes, T and B lymphocytes, in addition to phagocytic macrophages and heterophils (at day 28). Even in the absence of aflatoxin challenge, animals treated with YCW showed higher production of NDV antibodies (P<0.05) at day 35. No differences between the treatments were found in the intestinal permeability. The YCW supplementation in broilers challenged with 2.5 ppm of aflatoxin showed an immunobiological modulation effect; and, in non-challenged birds, it was observed higher production of antibodies thought cytotoxic T lymphocytes.

THE APPLICATION OF PROTEOMICS TO EXPLORE BLOOD-BASED BIOMARKERS FOR GUT HEALTH IN BROILER CHICKENS

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Intestinal health is of vital importance to the performance and wellbeing of poultry. Therefore, there is a clear need for easy to measure generic biomarkers for evaluating gut health. The aim of this study was to explore a proteomics approach to investigate potential blood-based biomarkers representative for the intestinal health status in broiler chickens. An experimental model of necrotic enteritis (NE) induced by Clostridium perfringens was used in this study to promote different degrees of intestinal damage in broilers (Ross 308). Birds were divided into two groups: C. perfringens-infected (n=80, challenged group) and non-challenged (n=20, control group). Blood was withdrawn immediately prior to euthanasia and aggravation of enteritis was evaluated according to a well-established macroscopic lesion scoring system (0 to 6). Following bottom-up proteomics analysis was performed using blood plasma, thus, samples were prepared according to endorsed S-trap mini-protocol with overnight trypsinization. To identify and compare acquired peptide signals, two available mass spectrometry data acquisitions methods were compared: data-dependent acquisition (DDA), and data-independent acquisition (DIA). A significantly different (adj. p-value<0.005) regulation of 84 (DDA) and 79 (DIA) plasma proteins at minimum one NE stage was revealed. Interestingly, both workflows shared only 23 proteins in common, which belong to classes of cell adhesion, intercellular signaling, carrier function, protein binding and enzyme modification. Other discovered proteins play a role in catalytic, transducer, transporter, and structural molecule activity, or are engaged in molecular function regulation. A positive correlation (FDR<0.05) between the severity of gut damage (lesion score>4) and protein regulation in blood has been established. The results confirm that blood plasma, like other described biological matters, is a valuable matrix for the search of gut health biomarkers in poultry research. Furthermore, both acquisition methods, although with some divergence, provide conclusive results, and the use of one/both is advisable for obtaining a complete snapshot of intestinal health status. Undoubtedly, a cluster of further analyses must be conducted to examine and validate a solid causal link between biomarkers of interest and the gut health.

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Selected short communications

IMPROVING THE LIVELIHOODS OF FAMILY POULTRY PRODUCERS THROUGH POULTRY FARMER FIELD SCHOOLS

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Over the past two decades, farmer field schools (FFS) have helped family poultry (FP) producers across developing regions to achieve sustainable food production and to improve their livelihoods. The FFS approach overcomes constraints associated with past top-down extension activities by enabling adult participatory hands-on learning and encouraging local innovation, particularly by women and youth. In Poultry FFS, groups of producers meet regularly throughout the production cycle to test, validate and adapt good poultry husbandry and marketing practices fitted to local agroecosystems and socio-economic realities. FFS allow farmers to make conscious decisions and critical analysis regarding management practices in poultry and crop production, including planning, marketing, consumption and savings. Producers acquire new skills and knowledge in poultry feeding, disease prevention and entrepreneurship, among others, and establish linkages with service and input providers, researchers, extension agents and private operators to improve their enterprises, increase access to markets and generate incomes, while promoting gender equity. FFS often result in the formation or strengthening of associations and cooperatives. Recognizing the potentials of the approach, FAO and IFAD, along with partners, are building FFS capacity by developing guidelines and a Poultry FFS manual and are integrating FFS into several projects in support of FP producers' livelihoods in the context of rising global risks, e.g.(re-)emerging infectious diseases, climate change and antimicrobial resistance. For instance, FAO has used the approach to introduce semi-intensive egg production in vulnerable communities hosting Syrian refugees in Lebanon and pastoral communities affected by recurrent droughts in Kenya. In both countries, FFS helped farmers to address complex local challenges and to improve farm and breeding management; apply biosecurity measures; adopt good feed storage and feeding practices; improve bookkeeping; increase sales; and add value to products by developing marketing strategies. As a result, farmers' income increased and household nutrition, resilience and food security improved. The many positive experiences registered and increased interest in Poultry FFS from development organizations and governments call for united action by all poultry development stakeholders to ensure successful learning and sustainable implementation and scaling-up of this lifelong learning approach.

FEED THE FUTURE INNOVATION LAB FOR GENOMICS TO IMPROVE POULTRY: INCREASING FOOD SECURITY IN AFRICA BY ENHANCING RESISTANCE TO NEWCASTLE DISEASE AND HEAT STRESS IN CHICKENS

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Homestead and small-scale poultry production have tremendous potential for alleviation of malnutrition and poverty in climate-stressed rural communities in Africa. Newcastle disease (ND) is the number one constraint of raising poultry in Africa, causing high mortality among village flocks. This research and development program seeks to improve poultry production by households and smallholder farmers, and thereby improve food security, nutrition, and livelihoods in Africa -key goals of the USAID Feed the Future Initiative. Our program is applying advanced genetics and genomics to sustainably enhance innate resistance to ND and heat stress in chickens adapted to the African environment. Over the past eight years, we have identified many genes for genetic resistance through an integrated analysis of Newcastle disease virus (NDV) challenge experiments and genomic analyses of well-characterized chicken lines under strictly controlled environmental conditions by RNA-seq and genome-wide association analysis (GWAS) using chicken 600K SNP panel, and of six African indigenous chicken ecotypes by GWAS. In addition, natural exposure field trials of approximately 3,000 birds of African ecotypes with velogenic NDV strains revealed a significant favorable negative correlation between survival time and NDV shedding. Our results confirmed the polygenic control of resistance to NDV. We estimated heritabilities for disease resistance and growth traits in African chickens to be moderate to high (0.14 - 0.55), which indicates that selection to improve these ecotypes for resistance to ND is feasible. Based on our results, a low-density 5K SNP panel that can be imputed to 600K SNP was developed to select and breed local ecotypes with enhanced resistance to ND, primarily focusing on survival time upon infection with velogenic NDV. We are currently assessing correlations of disease resistance traits with production traits, such as egg production and growth rate. To improve our understanding of the epidemiology of NDV in Africa, we are characterizing strains of NDV circulating in chickens in these countries. Finally, to assess the potential demand for genetically improved indigenous poultry lines, we are also conducting value chain assessment by implementing choice experiment surveys and developing a business plan to enable distribution of improved chicken lines to rural farmers with a focus on women who would benefit the most from an increase in resistance and productivity.

HEAT TOLERANCE FEATURES OF SASSO COLORED BROILER BREEDERS IN OPEN SIDED CAGE HOUSES EXPOSED TO EXTREME SUMMER CONDITIONS IN TROPICAL SOUTH INDIA AND SUGGESTIONS FOR FURTHER IMPROVEMENT IN PERFORMANCE IN VIEW OF FUTURE CLIMATE CHANGES

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A study was conducted in April 2021 to find our heat tolerance features of the existing batch of Sasso colored broiler breeders SA 51A strain at Coimbatore in tropical South India. Birds were housed 3 birds per cage in open sided naturally ventilated elevated houses. Performance of the batch of 8874 F and corresponding Rainbow males aged 36 weeks was studied with reference to Temperature Humidity Heat (THI) and Temperature-Humidity Heat Index THW corrected for existing wind velocity. The wind velocity was generally higher in this area due to a particular geo climatic condition. 24 randomly selected birds were measured for internal body temperature(cloacal), panting incidence and intensity of Panting per minute for 13 days of April, 3 times per day at 10:00, 14:00 and 18:00 hrs. Wind velocity tended to moderate the heat Index values to the extent of 10%. THI values were 81,82 and 81, THW values 74.8, 74.4 and 70.9, wind velocity values 5.5,6.8 and 15.2 miles/ hour respectively. The bird performances were not negatively affected by tropical summer heat stress for production, reproduction and bird welfare traits. Weekly hen day production values were 71 to 73 %, hatchability 89-91 %, egg weight 53-54 g, female body weight 1930-1950 g, internal body temperature averaged 105 F, incidence of panting 40, 57 and 26% and intensity of panting 27, 52 and 16 per minute for morning, afternoon, and evening respectively, all within physiological limits showing good heat tolerance features to prevailing climatic conditions at Coimbatore. In anticipation of expected future climate change with rise in summer temperatures, it is suggested to install and judiciously use circulating fans in the morning and noon hours to improve inhouse ventilation to combat heat stress and improve the performance further. Key words: Heat tolerance, Temp-Humidity Heat Index THI, Temp-Humidity-Wind velocity THW Heat Index, Sasso colored broiler breeders in tropical south India.

BREEDING PROGRAM TO ESTABLISH THE CROSSBRED SLOW – GROWING KORAT CHICKEN FOR SUSTAINABLE OCCUPATION OF SMALLHOLDER FARMERS

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Regarding food security, sustainable occupation of smallholder farmer, and for conservation of indigenous chickens, are the main goals of the establishment of slow – growing Korat chicken (KR). The establishment is under the collaboration of Suranaree University of Technology, Thailand Research Fund, and Department of Livestock Development. Breeding goal of KR are 1. acceptance from niche market in term of texture, and flavor of their meat, 2. competitiveness cost of one - old day KR chick, and 3. overall performances, bodyweight at 10 weeks, feed efficiency can acceptable by farmers, and they can gain some profits. Leung Hang Khoa (LK), Thai indigenous chicken, and SUT (the abbreviation of Suranaree University of Technology) chicken are used as sire, and dam line, respectively, of KR. Three hundred females, and 60 males of each LK, and SUT were used as a foundation herds, and were expanded to be 800 females and 200 males of each line in the later generations. In term of overall performances, recurrent selection was used to select both of LK, and SUT line to produce heterosis of growth performance of KR. From the year 2011 – 2019, 8 generations passed, in each generation, 1,200 – 2,300 KR were produced by AI, pedigrees, individually bodyweight at 10 weeks of age were collected, the accumulated data from all generations were used to estimate the estimated breeding value (EBV), and dominance of bodyweight at the 10th week. Animal model with dominance effect was used, sex, hatch, month – year, were used as fixed effects. Selection intensity is approximately 70%. Heterosis was calculated in each generation. The bodyweight at the 10th week of age of KR were varied from the 1st (1357 grams + SE 0.06) to the 8th (1260 grams + SE 0.03) generation, heterosis of the trait were 50% at the 1st generation, and 37% at the 8th generation. Genetic parameters composed with heritability were 0.01 and the 1st generation and 0.2 at the 8th generation. While the ratio of variance dominance and total variance were 0.008, and 0.04 at the 1st, and 8th generation, respectively. Average of feed conversion ratio of KR from the 1st to the 8th generation were 2.3 - 2.34. The stable of genetic progress was found in this breeding program because of the selection intensity was low since we intended to monitor the effect of growth on meat qualities, and the other negative consequences. Nowadays, KR are used for occupation of more than 200 smallholder farmers in Thailand.

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Posters

AVIAN INFECTIOUS LARYNGOTRACHEITIS: INNOVATIVE SEROLOGICAL FOR DIAGNOSIS, VACCINATION MONITORING AND DIVA TESTING.

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Avian infectious laryngotracheitis (ILT) is a respiratory disease of chickens caused by the infectious laryngotracheitis virus called Gallid herpesvirus 1. ILT leads to major losses as a result of mortality and/or decreased egg production. Vaccination is an essential tool for ILT disease control.

Different types of vaccines are commercially available. Conventional live attenuated vaccines (TCO and CEO) based on native virus (partially or totally inactivated) offer good protection but can produce latent infections and reactivation of the virus in the field. Vector vaccines are created by genetic modification(s) of vector microorganisms and the integration into their genomes of exogenous gene(s) encoding for immunogenic protein(s) from viruses responsible of diseases of interest. In the case of poultry vector vaccines, the Fowl Pox Virus (FPV) or the Herpes Virus of Turkey (HVT) are commonly used as vector virus. One or more exogenous genes may be expressed to ensure stronger protection or to widen the spectrum of protection to more diseases. Benefits associated with vector vaccines include biosecurity, protection efficiency, ability to breakthrough passive immunity, and long-lasting immunity. Additionally, the use of vector vaccines allows to implement DIVA strategies (Differentiation between Infected and Vaccinated Animals). In the case of ILT, two types of vaccines exist, one based on the gI protein, and the other on the gB protein.

Given that the conventional serological kits do not efficiently detect seroconversion to vector vaccines, the ID Screen® ILT gB Indirect and the ID Screen® ILT gI Indirect innovative ELISA's were developed to monitor respectively FP-ILTgB and HVT-ILTgI vaccines.

EFFICACY OF BACTERIOPHAGE COCKTAIL ON SALMONELLA INFECTION AND PERFORMANCE IN BROILER CHICKENS.

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Salmonella is one of the most frequent cause of foodborne outbreaks worldwide. Poultry, particularly chickens, are known to be the main animal reservoir for this bacterium. Due to limitations of using antibiotics there is a growing need for preparations that could prevent Salmonella infection during meet and egg production. An alternative solution may be using bacteriophages - the natural enemies of bacteria.

The aim of the study was to determine the efficacy of a cocktail of four virulent bacteriophages on Salmonella infection and to compare performance parameters of Salmonella spp. challenged broilers treated or not with phages. The efficacy of phage cocktail was tested in four in vivo trials. In each study, there were three treatment groups: negative control group (broilers received plain drinking water), positive control group (broilers infected with Salmonella spp.), and treatment group (broilers infected with Salmonella spp. and receiving bacteriophage cocktail at 2x106 PFU/bird/day). The following parameters were investigated: Salmonella on boot covers at Day 21, caecal Salmonella at Day 35, average daily weight gain (ADG), average daily feed intake (ADFI), water intake (WI) and feed conversion ratio (FCR; feed: gain).

The results of individual studies were subjected to statistical analysis (Statistica version 13.1). For ADG, ADFI, FCR and WI 1-way ANOVA followed by Tukey's post hoc test was performed. To determine Log10MPN Salmonella in boot swabs and caeca t-test was conducted. Meta-analysis was performed using Review Manager (RevMan; version 5.3).

The results of four independent studies revealed a significant reduction in number of Salmonella spp. both in boot swabs at 21st day of each study and in caeca at the 35th day (1.1 log reduction, P=0.001 and 0.78 log reduction, P=0.003, respectively). Feed intake was significantly decreased after bacteriophages treatment while weight gain and FCR were improved but level of statistical significance was not reached.

In conclusion, the results of efficacy studies revealed that bacteriophage cocktail administered via the drinking water to broilers at 2x106 PFU/bird/day significantly reduced environmental and caecal Salmonella in chickens and can improved their zootechnical performance.

COULD CAMPYLOBACTER AND SALMONELLA INTERACT IN AEROBIC CONDITIONS?

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Campylobacter and Salmonella are both responsible of the two major foodborne zoonotic diseases in Europe with more than 246 000 and 91 000 cases reported in 2018 and poultry is generally recognized as the main source of contamination. A European regulation enforced the monitoring of these two bacteria on broiler carcasses post chilling at the slaughterhouse. The only monitoring study in France involving Salmonella and Campylobacter simultaneously has been conducted in 2008 and showed prevalence on broiler carcasses of 87.5 % for Campylobacter spp. and 7.5 % for Salmonella spp. Few previous works reported that Campylobacter can interact with other microorganisms to cope with adverse environmental conditions but interaction between them has not been studied. The aim of this study is to investigate possible interactions between Salmonella and Campylobacter grown simultaneously in aerobic laboratory conditions to better understand their behavior in natural environment such as live animals or poultry meat products. Several concentrations of Campylobacter jejuni C97Anses640 (2 to 6 log CFU/ml) were placed in 250 mL buffered peptone water with or without Salmonella Blegdam (around 10 CFU) and incubated in aerobic conditions at 37 °C for 16 h. Trials were repeated 4 times for 2 and 3 log CFU/ ml Campylobacter or 10 times for 4 to 6 log CFU/ml. Enumerations of Campylobacter and Salmonella were performed by serial dilutions and plating on mCCDA and Rapid'Salmonella plates respectively before and after incubation.

C. jejuni is a microaerophilic bacterium and as expected, grown alone, enumerations were reduced after incubation in aerobic conditions. Depending on its initial concentration, a 3-log reduction was observed. However, when 3 to 5 log CFU/ml of C. jejuni were incubated in the presence of S. Blegdam, results showed that enumerations were significantly reduced, but to a lesser extent (< 2 log). These results suggest that survival of C. jejuni C97Anses60 could be improved in the presence of S. Blegdam. On the contrary, enumerations of Salmonella were not impacted by the presence of Campylobacter. This study showed encouraging results about possible interactions between Salmonella and Campylobacter in aerobic conditions. It will be completed with other strains of C. jejuni and other serotypes of Salmonella to confirm this interaction among the most circulating strains in poultry production of both pathogens.

SALMONELLA ENTERITIDIS COLONIZATION IN BROILER CHICKENS CHALLENGED ON DAY 14 WITH EITHER A CLOACAL OR INTRA-TRACHEAL CHALLENGE

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Salmonella Enteritidis (SE) is an important food borne pathogen that is typically associated with poultry. This study was conducted to determine which organs are colonized by this bacterium when challenged with these different routes. For this study, 250 straight run broiler chicks were obtained from a commercial hatchery and were randomly placed into 10 pens (25 birds/pen). Standard management and feed practices were followed for the duration of the trial. On day 14, half of the pens were challenged in the cloaca (CC) by administering 100ul of 10x4 cfu of SE via cloacal drinking while the other 4 pens were challenged with 100ul of 10x4 cfu of SE into the trachea (TC) utilizing a 20-gauge animal feeding needle.

The SE utilized in this study was nalidixic acid and novobiocin resistant. On days 35-38 a total of twenty birds were removed from each pen and necropsied. Samples collected include bursa and thymus (pooled), breast, ceca, crop, kidney, liver and spleen (pooled), skin, spinal cord, thigh, and trachea. A sterile swab was utilized to take samples from the abdominal cavity, bone marrow, cloaca and lung. After harvesting, samples were immediately placed into BPW and stored at 4C until all the birds were necropsied after which, samples were incubated at 37C for 24 hours. From each BPW sample a subsample was taken and used to inoculate TTB tubes. The TTB tubes were incubated for 24 hours at 41C. After that time samples were plated onto XLT4 agar that contained nalidixic acid and novobiocin. These plates were incubated at 37C for 48 hours after which the plates were removed, and Salmonella colonies noted. Data was analyzed using a t test at a p > 0.05. Results of this trial had shown at least one sample was positive for SE in 89% of the CC and 85% of the TC birds. There were significant differences between the CC (30.00%) and TC (48.00%) routes of challenge for the cloaca swab, lung swab (CC=1.01%, TC=3.00%), pooled bursa and thymus (CC=75.76%, TC=28.00%), pooled liver and spleen (CC=30.30%, TC=7.00%), ceca (CC=31.0%, TC=51.0%), crop (CC=7.00%, TC=32.00%), kidney (CC=19.00%, TC=5.00%), trachea (CC=2.02%, TC=33.00%). Differences in colonization may be due to the ability of this bacteria to more readily go systemic when administered via the cloaca compared to an intra-tracheal challenge.

This study shows that the route of inoculation may influence where this strain of Salmonella colonizes.

MONITORING OF AVIAN GENETIC RESOURCES WORLDWIDE: TRENDS AND CHALLENGES

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Understanding the current status and trends of each country's poultry genetic resources is essential for their sustainable use, especially in the light of current and future challenges such as climate change. The Domestic Animal Diversity Information System (DAD-IS) is the internationally recognized clearing-house for monitoring livestock agrobiodiversity, with 15222 national breed populations (breed subpopulations at country level) reported as of December 2021. When considering avian genetic resources, there are currently 3670 national breed populations (corresponding to 2525 breeds) from 17 species groups reported in DAD-IS. Chicken, duck and goose breeds accounts for 68%, 11% and 7% of those national breed populations. A specific challenge in avian species relates to the very definition of "breed" as management unit, as avian national breed populations include a large number of experimental and commercial strains as well as hobby breeds. The adaptedness classification, which differentiates native, locally adapted and exotic national breed populations, is unknown for a majority of those avian populations (69%), and 22% are reported as either native or locally adapted. Similarly, demographic risk status remains unknown for a large proportion of avian national breed populations (66%). This is especially the case for developing regions (89%) compared to Europe and Caucasus (48%) and North America (15%). Among the 1224 avian national breed populations with known risk status, 64% are considered to be at risk, 21% not at risk and 14% extinct. Out of 768 avian national breed populations with sufficient population data to estimate a demographic trend between 2000 and 2021, 56% showed a positive trend, with a significantly lower proportion in Europe and the Caucasus compared to other regions (53 versus 76%, P<0.0001). Other factors, such as species, or geographical classification (local, regional and international) did not show significant impact on these proportions. Those results revealed substantial data gaps to be filled in terms of population data and adaptnedness classification, especially for developing regions. Efforts are needed to harmonize breed definition across countries, while implementing inventory and monitoring systems to develop adaptation and mitigation strategies considering fully avian genetic resources.

DIETARY MANNAN OLIGOSACCHARIDES EFFECTS OVER CYTOKINE AND HOST DEFENSE PEPTIDE EXPRESSION IN CAMPYLOBACTER JEJUNI INOCULATED BROILERS

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A study was conducted to assess the effects of dietary mannan oligosaccharides (MO) with(out) a Campylobacter jejuni challenge on broiler growth, innate immune response, cecal colonization, and carcass prevalence. Two thousand two hundredand forty-day-old Ross 708 males were randomly assigned within 8 experimental groups with a 4×2 factorial design, with 4 diets (negative control, positive control) (bacitracin, 50 g/ton), MO constant dose (400 g/ton), and MO step-down dose (800, 400, and 200 g/ton in the starter, grower, and finisher periods, respectively) and with(out) day 21 Campylobacter jejuni oral gavage challenge using a 107 CFU/mL dose. At day 0, 14, 28, and 41 body weights and feed consumption were measured to determine broiler performance. At day 22 and 28 (1- and 7-days post inoculation), 24 birds per treatment were euthanized for ileum tissue collection for gene expression analysis. At day 42, 24 birds per treatment were euthanized for cecal content collection for C. jejuni enumeration and prevalence estimation. Finally, at day 44, 96 birds per treatment were processed for C. jejuni prevalence estimation on the broiler's carcasses. The diet did not influence growth performance however the C. jejuni inoculated birds had higher body weight and lower feed conversion ratio (FCR) (P<0.05). Birds fed with the MO diets had lower FCR during the finisher period (P<0.05). At day 1 post inoculation, significant differences (P<0.05) between treatments inoculated with C. jejuni and phosphate buffered saline (PBS) were observed in the relative expression of avian beta defensin 10 (AvBD10), interleukin 1B (IL-1B), and interleukin 10 (IL-10). At day 7 post inoculation, the expression of AvBD10, IL-1B, and IL-10 was similar among all treatments (P>0.05). At day 42, all birds, regardless of the type of inoculation, had similar levels of C. jejuni (8 log10 CFU/ml) recovered from cecal contents and its prevalence was similar for all treatments (P>0.05). After processing, C. jejuni prevalence was similar among all treatments (P>0.05). Overall, the addition of MO during a C. jejuni challenge did not have an impact on growth performance, innate immune response, cecal colonization or prevalence after processing. Further experiments should evaluate the effects of MO supplemented diets over intestinal morphology, as well as in broilers inoculated with different doses of C. jejuni.

THE PROPHYLACTIC AND THERAPEUTICAL EFFECT OF INDUCTORS PRODUCED BY ENTEROCOCCUS SP AND ITS INFLUENCE ON SALMONELLA HEIDELBERG CONTROL IN BROILERS.

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Diseases and infectious agents' control are the most frequent challenge in poultry production, such as salmonellosis. Salmonella Heidelberg is a common serovar in broilers' digestive and reproductive tract (1). Gram-positive bacteria interaction mechanisms to produce auto inductors peptides capable of inhibiting pathogens via quorum sensing have been described (2). Thus, the present research aimed to study the production of these autoinducers by the lactic acid bacterium Enterococcus sp and its ability to perform bacterial communication with the poultry's microbiota to control Salmonella Heidelberg in vivo.

The Salmonella Heidelberg sample was collected from adult individuals with salmonellosis. Poultry swabs were obtained for the Enterococcus sp isolation. The bacteria were submitted to gram staining and Polymerase Chain Reaction (PCR) to identify its genus. It was obtained 32 broiler chicks for the present study with one year old, which were bought from the farming house. The avians were separated randomly into four groups in wire cages, receiving specific challenges with Salmonella Heidelberg and treatment. The evaluation of the groups was performed through Salmonella Heidelberg counts in the ceca of birds at 21 days of age. It was possible to verify positive filtrate use repercussion to inhibit Salmonella Heidelberg since a lower positive birds' percentage was obtained after prophylactic treatment. Also, there were fewer colonies forming units per milliliter for prophylactic- and therapeutically treated birds. The research evidenced the existence of bacterial communication as part of the positive biological effect of the Enterococcus sp autoinducers in vivo. These results reinforce the importance of pre-financing research involving the auto inductors to develop products to reduce carcasses contamination and improve poultry performance.

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26th World's Poultry Congress, abstracts selected in 2022

Reducing the environmental impacts of poultry production

Selected short communications

CONTROLLING DIETARY AMINO ACIDS CONTENT AT THE LEVEL OF REQUIREMENT REDUCES THE NEGATIVE INFLUENCE OF LOW PROTEIN DIETS ON GROWTH PERFORMANCE OF BROILER CHICKENS

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In broilers, the effects of low protein diets on growth performance are contrasted and slow down the implementation of this strategy, despite a large potential to improve the sustainability of the production. The objective of this meta-analysis was therefore to study the effect of lowering dietary crude protein (CP) content in fast-growing chickens. A database was built from 30 articles published after 2016, for a total of 108 trials. To be included, trials had to be iso-energetic and isodigestible lysine. Trials that showed growth performance below 90% of the genetic potential of broilers were not included. The average age was 21 days, with minimum starting age at 1 day, and maximum ending at 42 days. Nitrogen (N)retention was recalculated considering an average value of 29 g N/kg gain. The effect of dietary CP reduction was analysed by multiple linear regression with the trial as random effect. Dietary CP content was strongly correlated with soybean meal content ($R^2 = 95\%$). For a 10 g/kg decrease in CP, soybean meal content decreased by 19% (P<0.001), while the use of cereals and feed-grade amino acids (AA) was increased by 5% (P<0.001) and 37% (P<0.001), respectively. Decreasing dietary CP content by 10 g/kg resulted in a decrease in average daily feed intake (ADFI) and average daily gain (ADG) by 0.7% (P<0.01) and 1.9%(P<0.001), respectively. With the same dietary CP reduction, feed conversion ratio (FCR) was decreased by 1.9% (P<0.001), while abdominal fat was increased by 8.7% (P<0.001). In this database, only 33 trials met the requirements for Met+Cys, Thr, Trp, Arg, Ile, Val, His, Phe+Tyr, Gly+Ser. In this sub-database. ADG (P=0.89) and ADFI (P=0.18) were maintained when CP content was decreased by 10 g/kg. The FCR was increased by 1.3% (P<0.001) and the abdominal fat tended to be increased, not significantly (P=0,152), for a CP decrease by 10 g/kg. Measured nitrogen retention (Nret) data from 9 papers validated the recalculation of N retention (measured Nret = $0.23 + 1.01 \square$ calculated Nret; RMSPE = 6.9%; R2 =97%, RMSE =2.9). When AA requirements are respected, the decrease of CP content by 10g/kg allows to decrease nitrogen excretion by 10% (P<0.001), without impacting Nret (P=0.88). Reducing CP without impairing growth performance is possible with controlled AA levels. This strategy allows to simultaneously decrease nitrogen excretion and the use of soybean meal imported, therefore reducing the impact of broiler production on the environment.

PREDICTION OF THE INFLUENCE OF LOW PROTEIN DIET STRATEGIES ON LITTER CHARACTERISTICS AND NITROGEN VOLATILIZATION IN BROILER CHICKENS

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Poultry production contributes to a significant portion of nitrogen (N) emissions related to agriculture. About half of N intake by birds is excreted and a large proportion is then lost into groundwaters (i.e. nitrates) or the atmosphere (i.e. N20 volatilization) causing negative environmental NH3 and impacts (eutrophication, acidification, climate change). In three previously published trials conducted at INRAE in fast-growing broilers, it was demonstrated that decreasing dietary crude protein content (CP) significantly influenced litter characteristics and N volatilization. The goal of our study was thus to quantify the influence of low CP strategies on these parameters. Data associated to 11 dietary treatments differing in CP & soybean meal content (3344 broilers; 21 to 35d of age) were to fit general linear models with trial as random effect. Effects of dietary CP (%) and N intake (Nint, g/bird) were tested against litter weight (Wlitter, g/kg BW gain), litter nitrogen content (Nlitter, g/kg fresh mass), litter dry matter content (DMlitter, %) and nitrogen volatilization (Nvolat, in % of N excretion). In addition, Nvolat was also tested against the other response variables. Reductions in dietary CP and Nint significantly (p < 0.001) decreased Wlitter and Nvolat and increased DMlitter, but did not influence Nlitter (p = 0.45 and p = 0.71, respectively). Based on the adjusted R², the quality of the predictions was considered similar with either CP or Nint as explanatory variable. Thus, per % point of dietary CP reduction, Wlitter was decreased by 33 g/kg broiler BW gain), DMlitter was increased by 1.8% point and Nvolat was reduced by 5.8% point. Even though volatilization is a multifactorial phenomenon, the effect of dietary CP on Nvolat was largely explained by DMlitter (p < 0.001; $R^2 = 48\%$). Consequently, reducing Nvolat also significantly increased Nlitter (p < 0.001), meaning reducing dietary CP could help better close N cycle in farms. This could also partly explain why the effect of reducing CP on Nlitter was not obvious. Our study confirms that implementing low CP strategies in broilers strongly reduces N emissions and litter amount, and provides quantitative data on these parameters. Using our equations, the quantification of NH3 and N2O emissions with EMEP and IPCC methodologies could therefore be refined (Tier 3 approach). Yet, the influence of low CP diets on the proportion of the different gases emitted requires further investigations.

INFLUENCE OF DIFFERENT LITTER TYPES ON HARMFUL GASES EMISSION IN BROILER PRODUCTION

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One of the key factors for the success of broiler production is the selection of an adequate bedding material. In an intensive broiler production system, broilers are raised under a deep litter system that has high absorption potential. Poultry production has been developing rapidly in recent years and with it the growing concern for the waste disposal and harmful gases emission. In poultry rearing bedding material plays a very important role. Emission of harmful gases affects both birds and workers. The aim of this study was to estimate the emission levels of harmful gases, such as ammonia (NH3), carbon dioxide (CO2) and hydrogen sulfide (H2S) using six different litter types. Measurement of harmful gas concentrations were performed weekly from each treatment. The concentration of ammonia was first recorded above the permitted value of 20 ppm at day 28. Treatment with the lowest ammonia concentration at the end of the experiment was observed in chickens reared on the mixture of 30% chopped wheat straw, 30% wood shavings, 30% peat, 10% zeolite, nonpelletized form with significant difference (P > 0.05). During the fattening period the concentrations of measured carbon dioxide did not exceed the permitted level of 3000 ppm, while hydrogen sulfide was not detected. The lowest carbon dioxide emission at the end of the experiment was measured in the treatment with pellets consisted of 80% wheat straw, 10% zeolite, 10% charcoal. The mentioned research indicates the influence of different materials and formulations used in broiler production. Due to the fact that broilers are in constant contact with the litter, careful selection, adequate management, proper storage and proper use contributes to better and higher quality air inside the broiler house, which leads to lower gases emission.

Key words: broilers, bedding materials, ammonia, carbon dioxide, hydrogen sulfide

OPTIMIZATION OF PRODUCTION OF BLACK SOLDIER FLY LARVAE ON BROILER CHICKEN MANURE

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Insects have been proposed as high quality, efficient and sustainable alternative protein source for livestock animals. Besides, due to the increasing quantities of poultry manure, there is no sufficient agricultural land for the application of poultry manure to be used as fertilizer. Therefore, the aim of this study is to optimize the production of black soldier fly (BSF) larvae on broiler chicken manure, more specifically densitv larval and dietary nutrient concentration. BSF larvae were cultured in isolated chambers at a constant temperature (27°C) and humidity (70%) in plastic containers (52x36x10 cm) filled with 8-10 kg of broiler manure. In a first experiment of 10 containers, the optimal larval density was defined by seeding BSF larvae at a density of 200 – 3,000 larvae / kg broiler manure with a dry matter content (DM) of 33%. In a second experiment, the optimal layer thickness of the substrate was defined by seeding larvae in 12 containers ranging from 5.21 - 25.67 kg DM /m². With a constant density of 2,200 larvae / kg substrate (DM content of 33%) corresponding to 7,000 – 34,500 larvae / container. Finally, the impact of adding additional water to compensate the consumed water after 6 days of seeding was defined. Therefore, 12 containers of larvae were seeded at a density of 2,200 larvae/kg substrate (DM content of 33%). Six days after seeding, the 0-100% of the decreased moisture level was compensated by adding water. The composition of nutritional values of the broiler manure before and after rearing and the produced BSF larvae themselves were determined with the Weende analysis. The most optimal conditions to grow BSF larvae on broiler manure were a density of 2,200 larvae /kg substrate (DM content of 33%), a layer thickness of 12.65 kg DM broiler manure $/m^2$ and a maximum compensation of 27.3% of the decreased moisture level by adding water 6 days after seeding. BSF larvae reduced broiler manure with $52.5 \pm 12.5\%$ (DM). A final average bodyweight of the BSF larvae was 64±12 mg achieved with a feed conversion ratio of 2.76±0.63, based on DM. BSF larvae reared on broiler manure contain $46.8 \pm 2.0\%$ of protein, $10.9 \pm 0.24\%$ of fat and $7.58 \pm 1.07\%$ of chitin (DM). In conclusion, this optimized production of BSF larvae on broiler chickens manure has a great potential to produce a high level of animal proteins.

DIVERGENT SELECTION FOR WATER CONVERSION RATIO IN COMMERCIAL BROILERS

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With a growing global population comes a growing demand for freshwater supply. As many countries are already experiencing freshwater scarcity, finding ways to reduce its usage is of utmost importance, particularly in agriculture. A current challenge with freshwater management is that the ability to measure it accurately on a small-scale basis. This technology is especially important when considering the use of water efficiency related traits in broiler selection programs where water intake measurements need to be done on a per-bird basis. To address this, a lowflow water monitoring system was developed for use in a broiler selection program that is capable of measuring water intake on an individual bird basis and has been evaluated for its accuracy and repeatability. From there a divergent selection program was implemented to assess the effect of selection for both water efficiency and water inefficiency. Two lines were developed from a modern random bred population (MRB) and were selected for a single trait of water conversion ratio (WCR=water consumed/body weight gain). The low WCR (LWCR) and high WCR (HWCR) lines have been selected for 3 generations. Each generation, traits such as body weight, growth rate and feed conversion ratio (FCR) are also measured to fully understand the impact of WCR on production related traits. After generation 3, the LWCR line had an overall WCR of 2.90, the HWCR line a 3.31 and the MRB line included as a control had a WCR of 3.05. FCR was also improved in the LWCR line, compared to the HWCR line by a total of 4 points (1.35 vs 1.39). Body weights did not differ between the LWCR and HWCR lines to 6 weeks of age. Current results indicate that selection for improved water efficiency as exhibited by the LWCR line are possible in a selection program and may also have a positive effect on feed efficiency. Continued selection for WCR will provide further insight to the heritability of the trait as well as the response to selection on growth, processing yields and heat tolerance.

PERSISTENCE OF SULFADIAZINE AND TRIMETHOPRIM IN BROILER CHICKEN FEATHERS

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The regulation of the use of animal by-products is constantly evolving. Today, feathers from poultry farms are valorized and transformed into feather meal. These meals can be incorporated into fertilizers or into animal feed for pets, aquaculture and, since 2021, for pigs. However, some studies have already shown that residues from veterinary treatments administered to control infectious diseases in poultry can persist in feathers for several days after the treatment is completed. The risk associated with the use of feather meal in the animal feed chain is that feathers and feather meal could be reservoirs of antimicrobial residues and thus may increase the emergence of antibiotic resistance. The objective of our study was to investigate the behavior of sulfadiazine and trimethoprim in broiler feathers. The sulfadiazine, known for its persistence in plasma (t1/2 = 7 hours), is commonly used in French poultry farms associated with trimethoprim to treat respiratory and digestive infections. To date, no studies have been performed for these antibiotics in feathers and our study aimed to provide some knowledge about the persistence of sulfadiazine associated with trimethoprim in feathers. A first study was performed on some (n=2) broiler chickens (Ross 308). The animals were placed in cages composed an elevated grid floor and droppings collection trays to avoid contamination of faeces on feathers. They were treated once a day by gavage for five consecutive days with an oral solution of sulfadiazine at 25 mg.kg-1 bodyweight and trimethoprim at 5 mg.kg-1 body weight. Feathers were collected at different times over a period of two months. The samples were analyzed by a LC-MS/MS validated method for ranges from 5 to 4000 µg.kg-1. The first results obtained show a persistence of sulfadiazine residues in feathers up to 55 days after the end of the treatment has a concentration of 60 μ g.kg-1. A second animal experiment will be carried out with same treatment on six animals to confirm the first results obtained. During this second study, feathers will be collected from different areas of the broiler (wings, breast, legs and back) in order to map the distribution of sulfadiazine and trimethoprim residues. The results of this second part of the study will also be presented.

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Reducing the environmental impacts of poultry production

Posters

INFLUENCE OF PHYTASE LEVEL AND FORM ON RESIDUAL PHYTATE PHOSPHORUS IN BROILER MANURE

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Beyond its role in the gastrointestinal tract to liberate bound dietary phosphorus, supplementing phytase in broiler diets can offset potential environmental impacts (e.g., oxygen depletion of waterways, eutrophication, and loss of aquatic life) associated with poultry production by reducing phytate phosphorus in manure. This research evaluated the individual and combined effects of coated and uncoated (heat stable) phytase on residual manure phytate phosphorus. Two replicated studies were conducted using 240 day-old Cobb 500 male broilers per trial. For each trial, birds were assigned to 4 treatments with 4 replicate battery cage units per treatment (60 birds/treatment) and grown for 21 d. Treatments included: (1) negative control; no phytase added (NC), (2) NC + 1000 phytase units (FTU) coated phytase (C), (3) NC + 1000 FTU uncoated phytase (U), and (4) NC + 500 FTU coated + 500 FTU uncoated phytase (CU). Representative fresh manure samples from each pen were collected on d 21 and frozen for phytate phosphorus level determination using a standard extraction-colorimetric procedure. Data were analyzed in the SAS statistical package using ANOVA and means were separated using Tukey's HSD. As anticipated, phytase supplementation decreased residual phytate phosphorus when compared to NC (C=630.1 mg/kg, U=509.7 mg/kg, CU=551.7 mg/kg, NC=2422.9 mg/kg; P<0.001). No differences were observed between single form phytase sources and CU (P>0.05). Results indicate that all phytase supplementation forms used in this research substantially reduced phytate phosphorus in manure, with combining forms showing no advantage

FEEDING BROILER CHICKENS MOIST BY-PRODUCTS: EFFECTS ON PERFORMANCE AND INTESTINAL HEALTH

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One of the major developments is the gastrointestinal tract (GIT) of broiler chickens post hatch. Previous studies on moist feeding strategies in broilers show positive results on several production parameters, morphology of the GIT and immune parameters. However, there is a large gap of knowledge on providing broiler chickens a moist diet based on non-human edible by-products. Increasing the usage of by-products in broiler feed can reduce the pressure on the competition over agricultural land for either feed or food production. This project is aimed to develop a moist feeding strategy based on moist by-products (fresh brewers spent grain and wheat yeast concentrate) for broilers. This moist by-product-based feed will be used as a novel and sustainable way to enhance feed intake and potentially modulate the GIT in early life. A total of 382 male Ross 308 broilers were divided over 4 different treatments (T1 to T4), with 8 repetitions. T1 birds were fed a commercial dry pelleted feed (control), T2 and T3 birds were fed a moist diet, including 20% brewers spent grain (T2) or 20% wheat yeast concentrate (T3) and water, and T4 birds were fed a moist non-pelletized commercial feed moisturized by adding water. All moist diets were designed in a 1:1 water to feed ratio. Body weight gain of birds during the 1st week on the moist diets (T2, T3 and T4) were significantly (P< 0.001) higher (6.03, 6.03, and 6.90% for T2, T3, and T4 respectively) as compared to those of the dry fed birds (T1). Body weight gain of the broilers who were fed the brewers spent grain diet (T2) was significantly higher than those fed the wheat yeast concentrate (+12.1%; P < 0.0001) or the moist mash diet (+7.43%; P= 0.013). The diet had no significant effect on the blood levels of NK-cells, B-cells and T-cells on day 7, 14 and 35. The villi length was found to be significantly longer for T2 compared to T1 on day 7 (P=0.023). On day 35 all treatment groups were found to significantly differ in villi length except between T1 and T3, both representing the shortest villi (P<0.001). The crypt depth on day 14, was found to be significant (P<0.001) different for all groups except between T1 and T2, from small to large; T3, T1 and T2, T4. At day 35 only the crypt depth of T1 is significantly smaller (P<0.038) compared to T4. Pending analysis on IL6, IL22 and microbiota will be presented at the congress.

Keywords: moist feeding, by-product, intestinal health, gut development, broiler, sustainability.

EVALUATION OF TWO DIFFERENT FLOORING SYSTEMS CONCERNING THEIR DUST EMISSIONS IN BROILER PRODUCTION

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Since the use of alterative flooring designs gains more and more interest in poultry research and practice, the aim of this case control study was to investigate a Partially Perforated Flooring system (PPF) with regard to its dust emissions. This flooring system was used in the experimental barn, where 50% of the floor was equipped with a perforated plastic area underneath the feeders and water lines. The chicken could reach them by perforated plastic ramps. The other half of the floor of the experimental barn as well as the complete floor of the control barn was covered with pelleted straw. The two equal barns had a housing capacity of 500 fast-growing Ross 308 broilers with a final density of 39 kg m⁻² each and were ventilated by negative pressure ventilation. Dust emissions were measured in parallel in the exhaust chimneys of both barns with two portable aerosol spectrometers 1.109 by Grimm Aerosol Technologies (Ainring, Germany). Particle counts in different size fractions $(0.25 - 0.32 \,\mu\text{m})$ and particle mass were recorded. Additionally, temperature and relative humidity were tracked using Tinytag Plus loggers (2 – 230 TGP-4,500 loggers, Gemini Data Loggers Ltd, Chichester, West Sussex, UK). In total, eight 24h-measurements were conducted on two consecutive days in each of the four weeks of the fattening periods (Nov to Dec, 2019). Comparing particle counts from the conventional floor ($M = 178.43 \times 103$ m-3, SE = 3.34×103) versus the PPF (M = $172.67 \times 103 \text{ m-3}$, SE = 1.79×103), there was no influence on the particle counts (p = 0.129), but a significantly lower particle mass in terms of dust concentration when using the PPF (M = $153.32 \mu g$ m-3, SE = 3.03 vs. M = 265.4 μ g m-3, SE = 32.98 on conventional floor, p = 0.001). Correlations between the temperature and particle counts (r = 0.763) could also be shown. Relative humidity and particle counts (r = -0.648) as well as relative humidity and particle mass (r = -0.590) were also correlated. The PPF offers improvements for welfare and emissions. Chickens are still able to show exploratory and foraging behaviour in the remaining litter, and make use of the elevated PPF for resting. At the same time, particle mass emissions are lowered and chickens are separated from at least 50 % of their faeces. In conclusion, the PPF influences the dust emissions from poultry production systems positively by reducing the captured particle mass, thereby improving animal health and welfare. Further research is ongoing.

BENEFITS OF LOW PROTEIN DIETS TO DECREASE GREENHOUSE GAS AND NITROGEN EMISSIONS OF EUROPEAN BROILER PRODUCTION

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Reducing the environmental impacts associated to feed production and manure management is a major challenge. This study aimed at quantifying to which extent low crude protein (CP) diets affect broiler performance, greenhouse gas (GHG) emissions of feed production, and nitrogen (N) flows from barn to field, including (NH3), nitrous oxide (N2O) and nitrates (NO3-) ammonia emissions. Literature data, collected in December 2019, was selected according to four criteria: 1) published after 2017, 2) performance and diet composition available, 3) constant dietary energy and digestible lysine, 4) indispensable amino acids (AA) supplied at the requirement. GHG emissions associated to feed were calculated considering feed intake (FI) and GHG of feedstuffs from GFLI (2019) database and of micro-ingredients from ECOALIM (2016) database. Soybean meal and oil were considered as average used in Europe in GFLI. N excretion was calculated as difference between N intake and N body retention. EMEP and IPCC models were used to estimate N manure emissions (NH3, N2O, NO3-) at the barn, storage and after spreading. The impact of dietary CP on indoor N volatilization was also considered using data from Belloir et al. (2017). Results were expressed by ton of body weight (BW), assuming growth to be linear from 0 to 35 days with a final weight of 2.2kg. Data was analyzed using a general linear model, with "trial" as random factor and "CP" as covariable. The database included 30 treatments (6 papers; 10 trials). All CP reductions were performed by gradually replacing soybean meal and oil by cereals and feed-grade AA. Lowering CP significantly increased broiler FI (P=0.020) but did not affect feed conversion ratio (P=0.847). Lowering dietary CP by 10 q/kq led to a significant (P<0.001) average saving of 226 kgCO2-eg/t BW (-7%) associated to feed production (P<0.001). In the same time, N flows (/t BW), were found to be significantly decreased (P<0.001): N excretion (-3.6kgN; -11%), barn NH3 emission (-0.9kgN; -23%), total NH3 manure emission (-1.0kgN; -13%), N2O manure emission (-0.03kgN; -11%) and NO3- leaching (-0.3kgN; -11%). In control treatments, 61% of N intake was retained by animals and 28% was volatilized or leached. With a 20 g/kg CP reduction, 68% of N intake was retained and 23% lost. This study indicated that low CP diets in broiler are an efficient tool to reduce GHG emissions from feed and manure as well as NH3 and NO3- emissions from manure, without impairing performance.

CIRCULAR POULTRY PRODUCTION: CLEANING WATER AS A MEDIUM TO GROW MICROALGAE AS ALTERNATIVE PROTEIN SOURCE FOR BROILERS

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In the scope of circular agriculture, this project aimed to reuse the cleaning water from broiler houses as a medium to grow microalgae, and harvest these algae as a feed source for broilers. A prior laboratory study revealed Chlorella sorokiniana as the best microalgae species to be grown on cleaning water. Dehydrated C. sorokiniana powder was used in this study as an on-top feed additive for broilers, and the production, welfare and health parameters of the birds were evaluated. Sexed Ross308 broilers were used in this trial. The chicks were divided per sex in 12 pens of 20 animals. Three different feed treatments were defined: control mash feed; control+5% algae; control+10% algae. There were two repetitions for each Sex and AlgaeContent pair. Control feed was available in three phases, with phase transition at day 11 and day 28. Feed and water were ad lib available for the birds in suspended feed and water buckets, and manually weighed on a daily basis. After each feed phase transition and the day before slaughter, all birds were weighed manually. For data analysis, a two-way anova test was used with Sex and AlgaeContent as fixed model effects. A Tukey posthoc test was used to reveal the differences between these fixed model effects. Overall growth of the birds was poor, probably due to mash feeding and feed bridging in the feed buckets, resulting in periods of restricted feed access and very low slaughter weights. The study revealed that AlgaeContent had a statistically significant negative effect on average daily growth (p=0.002), feed use (p=0.007) and slaughter weight (p=0.011). Both water use (p=0.081) and water-feed ratio (p=0.053) tended to differ across the different AlgaeContent. No difference was found in feed conversion rate. Litter quality (dry content and looseness) and bacteriological samples of the litter were not different between treatments. There were no visible effects of algae feeding on organ and muscle development. However, a discoloration probably due to carotenoids in the algae, of the abdominal fat and feet was observed. In conclusion, the C. sorokiniana algae added to the feed did not improve the production, welfare and health of the broilers. There was however a significant discolouring effect on the feet and abdominal fat of the birds. Future studies should reveal if inclusion of algae in the feed matrix can replace soy protein, and if the processed wastewater can be reused for cleaning or safely discharged to the environment.

LITTER AMMONIA CONCENTRATIONS, LITTER QUALITY, AND PERFORMANCE OF BROILERS FED 3- AND 5-PHASE FEEDING PROGRAMMES

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Broilers' amino acid (AA) requirements decrease with age. An oversupply of AA leads to excretion of excess nitrogen (N) in the litter, which is converted to ammonia (NH3). One strategy to limit the supply of excess nutrients is by feeding more phases throughout the growth trajectory. By meeting the nutritional needs of the birds more closely, the excretion of excess N may be reduced. The aim of this trial was to study the effect of a 3- and 5-phase feeding program on NH3 concentrations at litter level, litter quality, broiler performance, and meat yield and quality. A total of 744 1-d-old Ross 308 male broilers were assigned to 12 pens and offered a 3- or 5-phase treatment, each with 6 replications. The 3-phase program (3P) had CP contents of 20.5 (0-9 d), 19.5 (9-24d), and 18.33% (24-39d) and the 5-phase program (5P) had CP contents of 20.5 (0-9d), 19.5 (9-17d), 18.78 (17-24d), 18.33 (24-32d), and 17.7% (32-39d). The diets were supplemented with synthetic AA to meet certain digestible AA:Lys ratios. Data were analyzed with linear mixed models (R Core Team, 2019). The NH3 concentrations at litter level were lower for the 5P treatment than the 3P treatment at 23 and 37d of age (P < 0.05 for both) and numerically lower at 31d of age (P =0.144). The lower NH3 concentrations coincided with numerically lower total ammonia nitrogen (TAN) concentrations in the litter at 24, 32, and 39d of age (P = 0.096, 0.309, and 0.075, respectively). In addition, the average litter pH measured at 24 and 32d was lower for the 5P treatment than the 3P treatment (P = 0.037 and 0.032, respectively). The total N content of the litter tended to be lower for the 5P treatment than the 3P treatment (P = 0.063) at 39d. For the overall period (0-39d), dietary treatments had no effect on average daily feed intake, body weight, and average daily gain (P > 0.05 for all). The feed conversion ratio was higher for the 5P (1.46) treatment than the 3P treatment (1.44) for the overall period (P = 0.001). The dietary treatments had no effect on the slaughter yield and meat quality parameters (P > 0.05 for all). The present study demonstrates that, except for feed conversion, a 5-phase feeding program is effective in maintaining equivalent performance, slaughter yield and meat quality compared to a 3-phase feeding program. Furthermore, by reducing the excess N excreted, TAN formed in the litter as well as NH3 volatilized from the litter can be reduced, which may have environmental benefits.

EFFECTS OF DIETARY CRUDE PROTEIN REDUCTION ON PERFORMANCE, WELFARE AND NITROGEN EXCRETION OF TURKEYS, A QUANTIFICATION VIA META-ANALYSIS

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The study objectives were to quantify to which extent low crude protein (CP) diets affect growth performance, welfare indicators like footpad dermatitis score (FPD) and nitrogen (N) excretion (Nex) of turkey. A database compiling 12 literature and internal trials with 27 treatments has been created. Trials were fulfilling the following criteria: 1) evaluating low CP feeding strategies, 2) formulated with constant dietary energy and digestible lysine, 3) indispensable amino acids (AA) adequately supplied and 4) trial duration above 40 days. Reducing dietary CP was performed by replacing protein contributors, namely soybean meal, by cereals and commercially available feed-grade AA. Weighted average of CP for the full experimental period was calculated using animal feed intake in the respective phases. The different FPD scoring systems were harmonized and differences were expressed in relative value in comparison to the control treatment (high dietary CP treatment). Nex was estimated by the difference between N intake and N retention (Nbody = 29 gN/kg gain; ITAVI, 2013). Analyses of variance (ANOVA) were performed for each criteria of interest with GLM. Following results are expressed per percent point of dietary CP reduction. Dependent variables were average daily feed intake (ADFI), final body weight (BWf), average daily gain (ADG), breast meat vield (BMY), feed conversion ratio (FCR), FPD and Nex. According to GLM model, reducing dietary CP had no significant effect on performance indicators: ADFI, BWf, ADG, FCR and BMY (p>0.05) but significantly reduced turkey FPD score (P<0.05) and Nex (P<0.001). Overall a 1%-point reduction of dietary CP reduced FPD score and Nex, by 11% and 8% respectively. This study highlighted that implementing low dietary CP strategies in turkey has the ability to improve turkey welfare indicators like FPD and to reduce N excretion in the environment. This approach can be implemented without negatively impairing animal performance as long as indispensable AAs are adequately supplied via the feed. A proper evaluation of raw materials nutrient composition is essential to implement a safe and reliable low CP feeding strategy.

Institut Technique de l'Aviculture (ITAVI) 2013. Estimation des rejets d'azote, phosphore, potassium, calcium, cuivre, zinc par les élevages avicoles. Comité d'Orientation pour des Pratiques Agricoles Respectueuses de l'Environnement, Paris, France, 63pp.

GROWTH AND ENVIRONMENTAL PERFORMANCE OF BROILERS FED A LOW PROTEIN DIET WITH FULL OR PARTIAL SOYBEAN MEAL REPLACEMENT

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The study objective was to evaluate the effects of full or partial replacement of soybean meal (SBM) in reduced crude protein (CP) grower diets on the sustainability chickens. performance and indicators of broiler From 14 to 36 days of age, 324 Ross 308 male broilers were randomly allocated into 6 dietary treatments with 6 pens per treatment. Experimental diets were A: control at 20.7% CP with 21%-SBM; B: 18.7% CP & 0%-SBM, C: 19.7% CP & 0%-SBM; D: 19.7% CP & 5%-SBM; E: 19.7% & 10%-SBM; F: 19.7% & 15%-SBM. All diets were isoenergetic and iso-digestible lysine and all the essential amino acids (AA) were at least at the level of the requirements. In the low CP diets, inclusion of feed-grade L-Val, L-Arg & L-Ile was used to balance the diets and extra L-Thr to compensate low Gly+Ser. SBM was replaced by mainly sunflower, rapeseed and some corn gluten meals. Daily feed intake (DFI), weight gain (WG), feed conversion ratio (FCR) and final body weight (BWf) were evaluated. For each diet, global warming potential from feed production (GWPf) was calculated using feed composition and raw materials origin via SIMAPRO (EF Method 2.0) and then multiplied by broiler FCR. In the assessment, SBM originated from Brazil, other ingredients from France (Agribalyse, 2020) and AA had two possible origins: Europe or China as part of a sensitivity analysis (METEX NOOVISTAGO, 2021). Nitrogen excretion (Nex) was estimated by difference between intake and deposition (Nbody = 29 gN/kg gain; ITAVI, 2013). Ammonia volatilization (NH3v) was estimated using Nex and modulated according to CP level using equations from Belloir et al. (2017). There were no significant (P>0.05)differences in DFI, WG, FCR, or BWf among treatments. Significant Nex and NH3v reductions were achieved when dietary CP was reduced (P<0.001). The GWPf was ranging between 998 and 2125 kgCO2eg/T BWG. In addition, the interaction between treatment & AA origin was tested significant (P<0.001) for GWPf. The switch of AA origin from CN to EU reduced only numerically GWPf in the control treatment (-1.8%; 40 kgCO2eg/T BWG) while it significantly reduced GWPf in all the other treatments (between -6.8% to -22%, equivalent to between -131 to -306 kgCO2eg/T of BWG). The study demonstrated that replacing SBM and lowering CP in broiler feed is efficient to reduce GWPf, Nex and NH3v without negatively affecting performance. It also indicated that the lower the diets in SBM and/or in CP are, the more the origin of AA matters for GWPf.

EFFECTS OF DIETARY CRUDE PROTEIN REDUCTION ON PERFORMANCE, WELFARE AND GLOBAL WARMING POTENTIAL OF BROILER CHICKENS: QUANTIFICATION VIA A META-ANALYSIS OF COMMERCIAL TRIALS

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Development of feeding strategies favouring optimal usage of protein resources represent a major lever to ensure sustainability of the poultry sector. A meta-analysis was performed to evaluate the effects of dietary crude protein (CP) reduction on performance and global warming potential of broiler chickens. Trials (29 in total) had to be 1) run by poultry sector companies, 2) iso-energetic and iso-digestible lysine within the same trial, 3) at least at the assumed requirements for all dietary indispensable amino acids (AA). In these trials, reducing CP led to the partial or total replacement of soybean meal (SBM) by cereals (corn and wheat), meals (rapeseed and sunflower), other plant byproducts or legume seeds, and feed-grade AA. Average daily gain (ADG), average daily feed intake (ADFI), feed conversation ratio (FCR) and breast meat yield (BMY) were reported. Litter moisture and foot pad lesion score (FPS) were also reported in most of the trials. Global warming potential (GWP) from feed production was calculated using feed composition and raw materials origin from GFLI (2019) and then multiplied by broiler FCR to obtain GWP per ton of live weight (GWPf, in kg eq.CO2 / T live weight). SBM was an average value for European imports (RER) and AA had two possible origins: Europe or non-Europe (METEX NOOVISTAGO, 2021). Analyses of variance (ANOVA) were performed for dependent variables of interest with general linear models including experiment as a fixed effect and dietary CP level as a covariate. All the following results are then expressed for one point of dietary CP reduction. In this meta-analysis, reducing dietary CP led to a significant increase of ADFI and ADG (+0.62 g/d; P = 0.026and +0.34 g/d; P = 0.023, respectively), while FCR and BWY were not impacted (P = 0.249 and P = 0.159, respectively). Reducing dietary CP also led to a significant reduction in litter moisture (-3.35% pt; P < 0.001) and thus FPS (-18.6)points on a [0-200] scale, P < 0.001). In addition, GWPf was also significantly reduced but the beneficial reduction was stronger when the AA used were coming from Europe (-145 kg eq.CO2 / T live weight; -7.3%; P <0.001) than when the AA were sourced from outside Europe (-35 kg eq. CO2 / T live weight; -1.6%; P <0.001). This meta-analysis allows to accurately quantify and predict the performance, welfare and environmental benefits when applying a reduction of dietary CP for broiler chickens raised in field conditions.

PREVENTION AND CONTROL OF AVIAN INFULENZA IN POULTRY FARMS IN SERBIA

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Avian influenza is a disease which have a great importance for animal and human health. It is on the list of the World Organization for Animal Health (OIE). The epidemiology, control and prevention of avian influenza are complex. Wild birds are virus reservoir in nature, and poultry farms can be infected by direct contact or indirectly. Virus transmission may occur through movement of infected poultry, movement of contaminated equipment, fomites, vehicles and people. The key is to keep migratory wild birds away from poultry and commercial bird breeding operations. Airborne transmission over long distances between farms has not yet been demonstrated. The Highly Pathogenic Avian Influenza H5N1 virus was detected in poultry and goose found dead in zoo in the end of November 2021, in South Bačka district, in Serbia. We have used depopulation as measure to control and prevent the spread of disease in poultry tested positive for H5N1 virus, as well as other birds and poultry in zoo which were in direct contact with diseased poultry. By the end of December 2021, the virus was diagnosed in birds and poultry in the territory of 4 municipalities, in 2 districts of Serbia, with a total of 5 incidents reported. In our case biosecurity measures are represent the first line of defense against avian influenza, but strict hygienic measures sometimes appear to be inapplicable for every farm. Strict biosecurity practices that prevent exposure to any animals or other items potentially contaminated with avian influenza are vital for preventing and controlling the spread of disease. If biosecurity measures of a high standard are implemented and maintained, they create a firewall against infection penetration. Protective equipment must be used on daily farm work in order to maintain high biosecurity and prevent infection.

Keywords: Avian influenza, poultry, wild birds, prevention, biosecurity.

Robotics and big data for precision farming

Selected short communications

GROWTH DYNAMICS THROUGH DATA ANALYTICS OF COMMERCIAL BROILER CHICKENS

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Data collection is common in commercial broiler production; however, growth modeling is still a challenge since this data lacks an inflection point (IP). This study evaluated BW dynamics, feed intake (FI), BW gain, FCR, and mortality of broiler flocks reared under commercial tropical conditions with control feeding. The data analyzed included performance records of 1,347 male and 1,353 female Ross AP broiler flocks with a total of 95.4 million chickens obtained from 2018 to 2020. Decision trees determined high (HE) and low (LE) feed efficiency groups using FCR at 35d. Logistic, Laird-Gompertz, and Von Bertalanffy growth models were fit with week BW data in R software. All models resulted in similar R2, AIC, BIC, and RMSE. The logistic model indicated more accurate estimates. Initial growth rate (IGR), age at the IP, weight at IP (Wi), and maximum daily increment (MDI) were determined for individual flocks. Data were analyzed using a one-way ANOVA with efficiency as the main effect. Linear and guadratic broken-line models were fit for FCR as a function of BW and FI. Fifty HE male flocks had 1.37 g:g FCR and 105 LE flocks had 1.52 g:g, while 31 HE female flocks had 1.42 g:g and 127 LE flocks had 1.52 g:g FCR. LE males had an IP at 30d (P<0.001) with IGR of 0.122 (P<0.001) and Wi (P<0.001) of 1,467 g. IP in HE males was 4d later, with an IGR of 0.114 and Wi of 1,828 g. MDI in HE males was 14 g higher (P<0.001) than the LE rate. On females, despite no differences were observed on the IGR (P>0.05), the HE group had an IP 2d later (32 vs. 30d), with greater Wi (1,510 vs. 1,329 g), and MDI. HE males were heavier (P < 0.001) at hatch and 35d, but lighter at 14, 21, and 28d. LE females were only heavier at 14d (P=0.040), but lighter at 35d (P<0.001). LE groups ate more (P<0.001) along the production cycle, gained more BW at 14d (P<0.001) but less at 35d (P<0.001), and were consistently less efficient (P<0.001) from 7d until slaughter age. No differences in mortality (P>0.05) were observed. Cumulative FI lower than 477, 995, and 1,837g at 14, 21, and 28d were estimated to produce FCR (P<0.05) better than 1.50 in male chickens at 35d, according to guadratic broken-line and guadratic regressions. On females, less than 960 and 1,720 g of FI at 21 (P<0.001) and 28d (P<0.001) resulted in FCR below 1.56. In conclusion, greater control of feeding in the stater and early grower phases was associated with changes in growth dynamics and more efficient chickens at 35d.

FROM MEASURING AVERAGE BODY WEIGHT OF THE FLOCK TO PRECISION FEEDING IN BROILERS: A MODELLING APPROACH TO ADJUST DAILY FEED COMPOSITION

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Precision feeding in broiler production could reduce feed cost and nitrogen and phosphorus excretion. However, in order to adjust daily feed composition, daily requirements of birds according to their actual growth performance must be evaluated. In commercial farms, body weight (BW) is now frequently measured with automatic weighing scales. We propose here to use the data provided by these devices to 1) forecast BW gain to come, 2) estimate associated metabolizable energy (ME), digestible lysine (dLys) and available phosphorus (avP) requirements, and 3) recompose a full diet using two types of pellets with different nutritional characteristics. Firstly, BW data collected during the previous days are used to fit a quadratic function (BW=f(age)). Using this function, BW(d) and BW(d+1) are predicted, and "most probable" BWG for the next day (BWG(d+1)) is then calculated as BW(d+1) - BW(d). This first model was validated by comparing BW and BWG predictions (Y) to measured data (X, n = 814). BW is very well predicted (Y = 1.04 X - 0.03, R² > 99%), while BWG prediction quality is lower but still acceptable (Y = 0.76 X + 0.02, R² = 56%). Secondly, lipid and protein depositions according to actual BW(d) and predicted BWG(d+1) are estimated with allometric relationships developed using literature data. Requirements for ME and dLys are then estimated from these daily depositions. For avP, we considered an equation from the literature, directly using BW(d) and BWG(d+1) values. The requirements sub-model was validated by comparing predictions (Y) to measurements of ME, dLys and avP daily intake (X, n = 325). ME predictions are very good (Y = 1.04 X, R² = 96%) while the predictive quality for dLys and avP is slightly lower but still acceptable (Y = 0.93 X + 139, R² = 75% and Y = 0.99 X + 31, $R^2 = 71\%$ respectively). Thirdly, 1001 blend of A/B pellets are computed (increase of %A in the blend from 0 to 100% with a step of 0.1%), with A being poorer in ME and richer in dLys and avP than B, respectively. For each blend, assuming that feed intake is regulated on an energy basis, daily feed intake is estimated using ME requirement and blend content. dLys intake is then calculated and compared to dLys requirement. The best blend is the first one where dLys intake is above daily requirement. In practice, this blend could be easily prepared and distributed with a commercial weighing/mixing hopper.

VALIDATION OF AN INDIVIDUAL VIDEO TRACKING SYSTEM TO DETECT BROILER BEHAVIORS ON FARM

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Behaviors expressed by individuals are very often used as indicators for assessing the welfare of broilers. Many studies are currently looking into the possibility of using image analysis to build quantitative "proxy" variables for animal behavior. Using the results of an individual image tracking system, this work aims to verify the consistency between the behaviors expressed by broilers, quantified by an observer and the data calculated from the tracking system. 238 male broilers (Ross 308) were filmed in a commercial farm, and the videos were then analyzed, on the one hand, directly by an observer and, on the other, automatically by image processing. During the 10mns observations, more than half of the broilers expressed at least once the stretching (57.1%), foraging (76.9%) and preening (82.8%) behaviors. However, these behaviors are expressed on very short durations (< 8 seconds among the 10mns). Less than 15% of the broilers expressed social behaviors or dustbathing. Automatic measures of drinking behaviors are positively correlated to the drinking behavior observed by videos (p<0.001; r=0.62). The same results are obtained about automatic measures of feeding behaviours and feeding behaviours observed by videos (p < 0.001; r = 0.73). The total travelled distance calculated automatically is positively correlated to the standing behaviour observed by videos (p<0.001; r=0.62). Indeed, the data from tracking are strongly correlated with those obtained from behavioral observation on these three behaviors. These results underline the interest in continuing the development of an automatic detection system for broiler behavior, through the elaboration of proxy indicators. The ultimate goal is to integrate this system into a complete tool for automatic recording of welfare indicators in commercial broilers.

ID: 1775 A FARMER ASSISTANT SYSTEM (FAS) TO CREATE MORE BROILER WELFARE, HEALTH AND PRODUCTIVITY

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With the advent of modern production systems since the 70 ies the numbers of broilers per farm increased considerably and worldwide production of poultry meat rose by a factor of 12 following the demand of a fast-growing world population for food of animal origin. Citizens and consumers are increasingly concerned about the living conditions of intensely farmed chickens, and the health and welfare problems in broiler houses such as diarrhoea, leg and foot diseases, pododermatitis, poor air quality, respiratory diseases and other health and behavioural issues. Most of these problems are recognized rather late because of the low surveillance frequency in large broiler herds and the impossibility to monitor chickens permanently under current production conditions. This paper reports about a new mobile Farmer Assistant System (FAS) which runs permanently on ceiling-based rails and monitors continuously and in real time bird distribution, faeces and litter conditions, by visual and infrared cameras, temperature, relative humidity, carbon dioxide (CO2) and ammonia (NH3) close above the heads of the animals. The system can identify anomalous faeces structures, wet litter areas and immobile and dead birds. All data are stored in a protected, cloud-based system, mapped across the barn floor and presented to the farmer via PC or smart phone at any time. This enables him to survey animals continuously, and he can take early action in case of animal disorders or indisposition and the data mapping leads him directly to the points of technical failure or animal health or welfare problem in the barn. Experiences in broiler farms in Germany and Spain show the great potential of the FAS to improve the guality of life of the broilers. The value of this new intelligent Precision Livestock Farming (PLF) technology is that it enhances the confidence of the farmer in his production: "I sleep much better knowing the system warns me, when there is a problem in the barn". It increases understanding of animals, improves close surveillance of health and welfare of the animals, stabilizes the productivity and may create more confidence of consumers by the transparency in the so surveyed broiler herds. Estimates show that the system pays off within about 1 year. The savings result from lower labour and health costs, lower mortality and improved productivity.

26th World's Poultry Congress, abstracts selected in 2022

Towards longer carriers in layers

Posters

FEEDING MODERN LAYER GENOTYPES FOR EXTENDED PRODUCTION CYCLES: HAVE NUTRIENT REQUIREMENTS CHANGED?

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The genetic potential of laying hens for egg output (EO) has improved substantially. Also, the productive life of hens has been extended, but few comparisons exist as to how the nutrient requirements of modern genotypes and long-life hens might have changed. Data from three flocks of individually housed laying hens were compared, namely a legacy flock of Hisex Brown hens (aged 37 to 40-week post-hatch), a modern strain of Hy-Line Brown (aged 27 to 30-week post-hatch), and a flock of long-life Hy-Line Silver Brown (aged 87 to 90-week post-hatch). The birds were offered diets ranging in dietary metabolizable energy, corrected for zero nitrogen retention (AMEn) from 10.0 to 13.0 MJ/kg. Balanced protein, measured as standard ileal digestible lysine (SID Lys), ranged from 5.0 to 9.0 g/kg. Daily calcium intake was calculated for each hen. A single regression model ($r_2 = 0.807$) for feed intake (FI) was determined, suggesting that a comparison between the flocks was justified. Modern strains were lighter than the legacy strain and egg size was reduced. All genotypes adjusted FI to maintain constant daily energy intakes. Energy utilisation was identical in the two younger flocks (legacy and modern). However, in the late-lay flock, some hens with a reduced EO, used energy more efficiently, suggesting that body reserves were utilised as an energy source. There were no significant differences in SID Lys response and utilisation in the younger flocks, but the late-lay flock the hens utilised SID Lys with reduced efficiency. In conclusion, modern layer genotypes utilise energy and balanced protein with the same efficiency as legacy flocks, but as hens age, they utilise balanced protein less efficiently. The impact of protein intake on egg size would appear to have diminished in modern strains. Ca utilisation differed with both age and genotype, and was used less efficiently by the long-life hens. Practically, the intake of nutrients, be they protein components or minerals, can be manipulated. Hens regulate their energy intake to match requirements, making it challenging to manipulate energy or feed consumption.

INTEREST OF A BLEND BASED ON SODIUM BUTYRATE IN IMPROVING EGGSHELL STRENGTH

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Of all short chain fatty acid (SCFA), butyric acid is the primary energy source for epithelial cells and is considered an important modulator of intestinal microflora. Herein it improves intestinal health and can be beneficial for laying hens' performance and egg quality. A trial was conducted to evaluate the interest of sodium butyrate in association with calcium lactate and trace elements on improving eggshell guality. Sixty-four ISA Brown laying hens between 54 and 60 weeks of age were randomly assigned to two groups: a control group (C) and the control group supplemented (S) with sodium butyrate as Butirex C4 (520ppm), calcium lactate and trace elements. Laying hens had free access to their mash diet and water. Each group contained 16 cages of 2 laying hens. Laying performances were recorded weekly for 6 weeks and eggshell quality was measured at the beginning and at the end of the 6-week trial. Data were analysed using R version 4.0.2: one-way analysis of variance for eggshell quality data, mixed models for laying performances data and logistic regression for egg qualification (discarded eggs). A p value of less than 0.05 was considered significant. The supplementation had no impact on laving performances. Laving rate was 95.2% in C and 95.5% in S (p=0.779), mean egg weights were 65.9 and 65.9 (p=0.885) and feed conversion ratios 2.04 and 2.07 (p=0.395) in C and S groups respectively. Eggshell quality was significantly improved in S group: static stiffness was 202 N/m compared to 194 N/m in the C group (p<0.05). This positive effect was mostly seen on eggs heavier than 65g; for these eggs static stiffnesses were 189 and 203 N/m in the C and S groups. Breaking strength was also significantly improved: 43.5N compared to 42.1 N in C (p<0.05). This effect was also concentrated on eggs heavier than 65q. No differences were seen regarding the number of discarded eggs: 8.7% in C and 9.7% in S group, nor for dirty (7.1 and 7.7%), broken (1.4 and 1.3%), soft (0 and 0.2%) or pecked eggs 0.5% in each group. A supplementation with a combination of sodium butyrate, calcium lactate and trace elements improved eggshell quality with maintained laying performances.

TOWARDS RESILIENT LAYING HENS: TAKING THE GENETICS' COURSE WITH HIGH-THROUGHPUT RECORDING

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Resilience is the capacity of an animal to be minimally affected by disturbances or to rapidly return to the state pertained before exposure to a disturbance. Commercial laying hens can be exposed to a large variety of disturbances (e.g. pathogens). There is growing interest to measure and select for improved resilience in farm animals. We aimed at studying the genetic background of resilience indicator traits, their genetic relationship with health- and productionrelated traits in purebreds and crossbreds. The populations consisted of two egglaying commercial lines (W and B), with records on purebreds and crossbreds. The purebreds (33,825 W and 34,397 B birds) were individually housed, while the crossbreds were housed in cages of 6 to 8 paternal half-sibs (12,852 W and 3,898 B cages). Recorded traits were egg production (EP), date of death (SURV), and, in purebreds only, natural antibodies titers (NAb). Deviations between weekly EP and their flock's average were calculated. The resilience indicator traits were the natural logarithm of the variance (LNVAR) and the lag-one autocorrelation (AUTO-R) of these deviations. Genetic parameters of LNVAR and AUTO-R, and their genetic relationships with EP, SURV, and were estimated. NAb In both purebreds, EP was lowly heritable (mean h^2 , \(\overline { h^2 }\) = 0.12), SURV was almost not heritable (\(\overline $\{h^2\}\) = 0.008$, P-value ≤ 0.05), NAb were lowly to moderately heritable (\(\overline $\{h^2\}$ \)= 0.25), and resilience indicators were lowly heritable (LNVAR: $(\langle h^2 \rangle) = 0.11$, AUTO-R: $(\langle h^2 \rangle) = 0.05)$. Compared to purebreds, crossbred traits were less heritable for EP (\(\overline $\{h^2\}$)= 0.05) and AUTO-R (\(\overline $\{h^2\}$)= 0.02), SURV remained lowly heritable (W: $(\langle verline \{h^2\} \rangle) = 0.02$, B: $(\langle verline \{h^2\} \rangle) = 0.00$, P-value = 0.12), and LNVAR was slightly more heritable than in purebreds (\(\overline $\{h^2\}$ \)= 0.16). The genetic correlations between purebreds and crossbreds were lower than 0.80 in both lines ((r_{pc})) from -0.55 to 0.63), meaning crossbreds' traits were different from purebreds' traits. In both purebreds and crossbreds, LNVAR and AUTO-R traits displayed favorable genetic correlations among themselves, and with EP, SURV, and NAb. These results suggest that selection for resilience based on big data can be implemented in breeding programs to improve health and production of laying hens. Cross-validation is now required to investigate if selection for improved resilience in purebreds results in improved resilience in crossbreds.

SUPPLEMENTATION OF LIGNOCELLULOSE IMPROVED LAYING PERFORMANCE AND HEALTH STATUS OF COMMERCIAL LAYING HENS IN A LATE PRODUCTION PHASE

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Objective

A prolongation of the hens' laying cycle whilst stabilizing laying persistency is a major aim in egg producing industry. One crucial challenge is the negative correlation of equ size and bird's age. A thinner equipment in bigger equipment in compared by the second s the risk of breakage and thus, represents a limiting factor of the duration of a laying cycle. The objective of the present study was to determine the effects of a supplementation of lignocellulose (OptiCell®, agromed Austria GmbH) on the performance and oxidative status of commercial laying hens during the late stage of their production cycle. Due to the specific composition of the standard lignocellulose (LC), a beneficial impact on performance, eggshell quality as well as on health status of layers in a critical phase of their production cycle was hypothesized. 48 laying hens (Lohmann LSL) in the age of 60 weeks were housed for a trial duration of 112 days under controlled conditions and fed a standard diet based on maize, soybean meal and limestone. The hens were allocated to either a control group - standard diet without supplementation of LC, or an experimental group - with 8 g LC/kg feed on top. Diets were formulated to be isoenergetic. For statistical analysis a two-tailed t-test was applied using SigmaStat 4.0 software (Systat Software Inc.). Although daily feed intake was significantly improved in the experimental group, the feed to egg mass ratio was significantly improved by 7 points. Both laying rate and egg weight were enhanced by 4% and 2.5%, respectively, which resulted in an improvement of total egg mass by 7%. Although egg weight was clearly increased, LC supplementation did not negatively affect eggshell stability: with an age-dependent increase in egg size, the frequency of broken eggs was slightly reduced. These findings are in concordance with the observation of an improved oxidative status of birds of the experimental group: LC supplementation significantly reduced malondialdehyde concentration whilst improving enzyme activity of superoxide dismutase. The trial data give proof that the supplementation of LC in layer feed has a clear beneficial impact on the laying performance of commercial layers in a late phase of the production cycle. Moreover, the blood profile of 75-week-old layers demonstrated an improved oxidative status in birds supplemented with LC, suggesting that LC contributes efficiently to maintain health status of birds.

EXTENDING PRODUCTIVITY IN LAYER HENS

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Extending layer hen production beyond 80 weeks of age (woa) requires processes to sustain egg production, egg quality and bone integrity to very late lay. Australian pullets tend to be heavier than breed standard weight for age and may be less suitable for longer laying cycles than smaller hens (Parkinson et al 2015). But smaller hens have innately lower feed intake (FI) (Harms et al 1982) and a more nutrient dense diet may benefit them. Therefore, a 2x2 factorial study was designed to compare the effect of hen size and the nutrient density of the early lay diet on hen egg production, feed conversion ratio (FCR), eggshell guality and bone integrity to very late lay. When 18 woa, 120 heavier (HW) and 120 lighter weight (LW) ISA Brown hens housed in individual cages were evenly assigned to either a lower nutrient density (LND - formulated on 90g FI/day; 2900 kcal/kg,) or HND (formulated to 110g FI/day; 2725 kcal/kg) diet each fed from 18-24 woa. From 25-39 woa, all birds received the LND, from 40-78 woa a mid-lay and from 79-90 woa a late lay diet. Cumulative (cum.) egg production, feed intake (FI), egg mass (EM) and FCR were calculated between 18-89 woa. During 86-90 woa, egg weight (EW), Haugh units and eggshell quality were assessed on 12 hens /treatment. Keel curvature and femur breaking strength were determined on 10 hens/treatment at 90 woa. Heavier birds at 18 woa remained significantly heavier than LW birds to 90 woa. No significant differences existed in rate of lay at 90 woa nor in cum. number of eggs laid/hen from 18-89 woa. Compared to LW hens HW hens had significantly higher cum. FI and cum. EM but poorer cum. FCR from 18-89 woa. Lighter hens fed the HND diet during early lay had the lowest 18-89 woa cum. FCR. The HND diet generated significantly thicker shell and higher shell breaking strength at 86-90 woa, when EW was highest for HW hens fed LND and LW hens fed HND. Concurrently Haugh unit was highest in hens fed early lay LND diet. Femur breaking strength and keel curvature at 90 woa did not differ. Both HW and LW hens sustained egg production across 18-90 woa. The HND diet

Both HW and LW hens sustained egg production across 18-90 woa. The HND diet benefited eggshell thickness and strength during very late lay and, LW hen cum. FCR. An assessment of bird size and diet density in cage-free systems is warranted.

Funded by Australian Eggs

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IS THERE AN OPPORTUNITY TO REDUCE THE ENERGY AND OR PROTEIN LEVEL IN THE FEED OF AGED LAYING HENS?

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Limited information is available on the nutritional requirements of aged laying hens (over 70 wks). Due to a tendency towards extended production cycles, it is important to gain insight in these nutritional demands. In order to fully exploit the genetic potential of laying hens, it is essential to meet their nutritional needs without compromising the birds' health. In order to determine whether there is a possibility to reduce the energy and/or protein level in the feed of aged laying hens, an experiment was conducted. A total of 288 ISA Brown laying hens (75-91 wks) were distributed over 4 dietary treatments with 8 replications and 9 birds per experimental unit (enriched cage). All groups were fed a corn- soy based diet. The control group was fed a standard laying hen diet containing 2760 kcal/kg MElh and 15.5% CP. Dietary treatment 2 (T2) consisted of a 10% reduction of the CP-level and limiting amino acid levels (MElh 2760 kcal/kg; CP 13,95%). %). In the third treatment the energy level was reduced by 5% based on a reduction of starch and also a 10% reduction of the CP-level (+limiting AA's) (MElh 2614 kcal/kg; CP 13.95%). Dietary treatment 4 (T4) consisted of an energy reduction of 5%. (MElh 2604 kcal/kg; CP 15.5%). A generalized linear mixed model was used to investigate the effects of these treatments. Both reductions in energy and protein level did not significantly influence egg production, egg weight, feed intake or feed conversion ratio measured at pen level (75-91 weeks of age). Egg quality (egg weight, Haugh Units, breaking strength, shell thickness) was not affected by dietary energy or dietary protein levels. Liver health associated parameters such as serum lactate dehydrogenase and aspartate transaminase measured at the start of the experiment (75w) and at 85 weeks of age did not significantly differ among treatments. Liver weight, abdominal fat content, liver haemorrhagic and colour scores measured at 87 weeks of age, were not affected by any treatment. It can be concluded that the protein and energy content in the feed of aged laying hens can be reduced by 10%, and 5% respectively without compromising performance traits, egg quality or birds' overall health.

ID: 2293 THE EFFECT OF GENOTYPE AND AGE INTERACTION ON PERFORMANCE OF LAYING HENS

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The objective of this study was to evaluate hen performance in response to different hen genotype in different laying periods. For this purpose, an experiment was conducted on 240 laying hens of Bovans Brown (brown-egg layers), Dekalb White (white-egg layers), and traditional Czech hybrid Moravia BSL (tinted-egg layers) (80 birds / hybrid). The laying cycle was split into three experimental periods: the first between 19 and 27 weeks of age, the middle was between 38 and 42 weeks of age, and the end of lay was between 66 and 70 weeks of age. Laving hens were kept in enriched cages and fed identical feed mixture. In terms of hen performance, Bovans Brown had the highest laying rate (88.3%) compared to Dekalb white (83.5%) and Moravia BSL (76.2%). Daily feed intake was also affected by hen genotype. Moravia BSL had the highest daily intake (129 g), while Dekalb White showed the lowest (112 g). Heavier eggs were laid by Moravia BSL (64.1 g) compared to Bovans Brown (62.5 g) and Dekalb White (56.9 g). Genotype significantly affected the non-standard eggs percentage in our study, where Bovans Brown showed higher percentage of non-standard eggs (1.75%) in comparison with Dekalb White (1.23%) and Moravia BSL (0.96%). Non-significant effect of age on laying rate and daily feed intake was recorded, while egg weight significantly increased with advancing age. The number of non-standard eggs was the highest in the first period (1.92%), and the lowest was detected in the second period (0.67%). Except non-standard eggs, all evaluated performance parameters were not affected by the two-way interaction between genotype and age. Boyans Brown in the third period showed the highest percentage of non-standard eggs (3.39%) compared to Moravia BSL in the second period (0.42%).

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Understanding the microbiome for an improved management of Health and Welfare

Selected short communications

LIMITED MICROBIOTA OVERLAP BETWEEN HEAVY BREEDERS AND THEIR SPECIFIC PROGENY

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Published gut microbiota comparisons show similarity between hens and their progeny, implying the possible vertical transmission of commensal bacteria from one generation to the next through the egg. However, it has also been shown that exposure to adult hens greatly affected chick microbiota composition, implying vertical transmission through the egg is limited and direct contact with an adult is needed to facilitate transmission. Characterizing vertical transmission through the egg is important to develop methods that enhance the transmission of commensals while inhibiting pathogen transmission. Furthermore, identifying bacterial phylogenetic groups which do not transmit effectively through the egg is important, as this might affect the maturation of microbiota composition and function. Thus, we collected fertilized eggs from ten broiler breeders and grew their chicks for 14 days. We collected fecal samples from the hens during egg collection week and from the chicks at ages 2, 7, and 14 days. We then performed 16S rDNA sequencing and compared microbial communities. We found that out of 410 bacterial strains identified in hen feces only 25 were identified in the chicks by day 2 of life. Furthermore, out of the 410 strains, 250 did not appear at all in the chicks at least till day 14 of life and many of those who did appear were limited to just a few chicks. While we only measured colonization till day 14, this already represents about a third of the life span of broilers. Thus, most bacterial strains found in the hen do not efficiently transmit through the egg. Additionally, chick bacterial community analysis identified high levels of potential pathogens such as members of Proteobacteria. It is possible that the limited transmission of hen bacteria to the chicks, results in a non-mature microbiota unable to limit the growth of pathogens. Last, we identified a handful of bacterial strains common with the hens that did colonize the chicks efficiently. Interestingly, these were mainly Lactobacillus strains. This implies that perhaps Lactobacillus bacteria are not the optimal probiotic choice as at least some strains are naturally acquired efficiently. To conclude, further research is required to determine the impact of the lack of transmission of these gut commensals on commercial broilers, and if artificial exposure can positively impact broilers.

LACK OF CONSISTENT MICROBIAL TAXA ASSOCIATED WITH PRODUCTIVE PERFORMANCE IN COMMERCIAL BROILER FLOCKS

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The microbial communities of the gastrointestinal tract play an essential role in host health, nutrition, and physiology. However, there is a great variation in microbial taxa that have been associated with productive performance, which complicates the understanding of gastrointestinal microbial communities associated with performance outcomes in poultry [1]. Monitoring microbiota associated with production in non-invasive samples at a population level could reduce the noise of bird-to-bird microbiota variation [2]. Poultry dust has been used to investigate the microbiota of commercial chicken farms and offers promise as a tool for monitoring trends in the chicken microbiota at the flock level [3]. In this study, we further explore the stability of the microbial taxa in different chicken houses and in samples collected a year apart from the same farms. Poultry house dust was collected at days 14 and 35 of the production cycle from 38 commercial broiler flocks of three Australian integrator companies. The farms were ranked as low or high performers based on feed conversion ratio corrected for body weight by the integrator companies. Bacterial taxa of 248 dust samples were sequenced using 16S rRNA gene amplification and analyzed using DADA2/QIIME2 against the SILVA database. Permutational analysis of variance based on Bray-Curtis dissimilarities using abundance data for bacterial community structure results showed that company explained the most variation in the bacterial community structure (8%), followed by age (4%) and the least variation was explained by performance (1%), with significant interactions among these factors. No bacterial taxa in high or low-performing farms overlapped in all three companies or in successive flocks from the same company. Some taxa associated with high performance in a company were associated with low performance in another company (e.g., Jeotgalicoccus), corroborating previous studies highlighting the lack of universal microbial markers of productivity. In conclusion, there were no consistent microbial taxa across companies and flocks within a company. Research evaluating the directionality of change of major bacterial groups focusing on their function is warranted to further evaluate the usefulness of flock level microbiota testing.

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THE ASSOCIATION BETWEEN EARLY REARING ENVIRONMENT, GUT MICROBIOTA COMPOSITION, IMMUNE DEVELOPMENT AND WELFARE IN BROILER CHICKENS

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There is increasing evidence that gut microbiota, behaviour, and immune development are associated. Gut microbiota composition is affected by many factors and the environment is one of these. The aim of the present study was to determine the association between the early rearing environment, gut microbiota composition, immune development, behaviour and welfare in broilers. Broilers (Ross 308) were tested in a $2x^2$ factorial design (n=6 pens per treatment combination): they were either hatched in their home pen (OH) or at the hatchery (HH) and had a dark brooder until day (d) 14 as enrichment (EE) or no brooder (NE). Microbiota composition, histology and immune development were measured in the jejunum in two chickens/pen at d7, 14 and 35. In addition, microbiota composition was determined in pooled faecal samples of 6 chickens per pen at d14. Two chickens/pen were subjected to a novel environment test (NET) at d2 and d11 and behaviour was observed at d6, 13 and 33. At d35, composite asymmetry of metatarsus width and length and toe length was determined, and footpad dermatitis and hock burn was scored. NE had a higher microbiota diversity and different composition in faecal samples, and a higher diversity in jejunal samples compared to EE at d14 ($p \le 0.05$), and a tendency for a difference within OH was found in jejunal samples at d35 (p=0.068). At d7 and d35 the number of CD3+ cells, and at d35 KUL+ cells (indicator for macrophages) in jejunal villus mucosa were higher in NE than EE (p < 0.05). HH chickens showed more social reinstatement than OH chickens and EE chickens showed more social reinstatement than NE chickens in the NET (p < 0.05). In the home pen, NE chickens were sitting more than EE (p<0.001). At d6 NE showed more eating and litter pecking while sitting, but at d33 EE were eating more and litter pecking while sitting (age*enrichment: p < 0.05). Composite asymmetry scores were lower for EE than NE (p<0.05) and EE chickens also had less footpad dermatitis and hock burn than NE (p < 0.001), indicating better welfare for EE chickens. Our results suggest that especially the dark brooder affected the behaviour and had a positive effect on welfare, and that both microbiome composition and immune development were also affected by the brooder. It remains to be further studied whether the microbiome affects welfare and immune development, or that the early rearing environment affects microbiome, immune development and behaviour.

YEAST-BASED PREBIOTIC IMPROVES INTESTINAL MICROBIOTA AND SEROTONIN PRODUCTION OF BROILER CHICKENS EXPOSED TO EIMERIA CHALLENGE

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The correlation between welfare and serotonin production is recognized in the literature as 90% of serotonin is produced in the intestine, which might improve the intestinal guality of chickens, favoring the birds' well-being (Paz et al., 2019). The aim of this study was to determine the serotonin production and the intestinal quality of chickens supplemented with yeast-based prebiotics and exposed to Eimeria challenge. 1890 one-d-old male chicks were assigned to 6 treatments with 7 replicates (45 chicks/cage) for 41 days. Dietary treatments were: T1 - negative control, T2 - positive control (enramycin), T3 - yeast cell wall (Saccharomyces cerevisiae), T4 - yeast cell wall + free nucleotides, T5 - autolyzed yeast cell + postbiotic (250q/ton), T6 - autolyzed yeast cell + postbiotic (500q/ton). The control diet (antibiotic-free) was a corn-soybean diet formulated to meet the nutrient requirements of chickens according to the Brazilian tables. Wheat was used as a nutritional challenged to increase viscosity. Feed and water were available ad libitum and the production cycle was monitored. At d4, chicks from all treatments were orally inoculated with Bio-coccivet, (20x immunization dose, Biovet S/A). At d28, swab samples were collected (n=6/treatment) to understand the dynamic of gut microbiota. At d20 and d40, blood samples were collected (n=7/treatment) to a non-invasive evaluation of serotonin dosage. Serotonin data were analyzed by One-way ANOVA and Tukey test (p < 0.05). Microbiota was characterized by 16S sequencing on MiSeg Illumina® (Degnan and Ochman, 2012, and data were analyzed by the artificial intelligent platform EzBioCloud (https://help.ezbiocloud.net/ezbiocloud-16s-database/). Regarding the aut microbiota, a linear increase in relative taxonomic abundance was observed between treatments. The genus Turicibacter, a biomarker for host serotonin production, was identified only in T6 group (p-value 0.0271). Its abundance in T6 was 1.78% and the serotonin level observed T6 was 242.86 (at 20 days of age) and 302.71 (at 40 days of age) ng/mL of blood. In both periods, the chickens supplemented with yeast-based prebiotics showed the highest serum serotonin values, which is closely related to the welfare of the birds. All treatments differed statistically, in which T6 presented the best serotonin results, and the antibiotic (T2) the worst values for this parameter, possibly indicating more stressed chickens. These results are in accordance with the intestinal microbiota modulated by the additives and indicate better response to the challenge. Thus, the use of yeast-based additive is a promising alternative to replace antibiotics as feed growth promoters, inferring positively in chicken welfare and health.

Acknowlegment: FAPESP 2020/12641-4

keywords: Saccharomyces cerevisiae, Welfare, Microbiota, Chickens
26th World's Poultry Congress, abstracts selected in 2022

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META-ANALYSIS OF THE EFFECT OF A BACILLUS SUBTILIS STRAIN ON THE PERFORMANCE AND WELFARE OF BROILER CHICKENS

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Because of increasing pressure to ban or reduce the use of Antibiotic Growth Promoters (AGP) in poultry production, new alternatives such as probiotics increasingly come into consideration for animal feeding. Animal welfare is another important concern for the poultry industry as well as a major consumer demand that probiotics can respond too. The objective of the present study was to evaluate a probiotic solution Bacillus subtilis DSM 29784 (Bs29784) as an alternative to AGP. To that matter, a meta-analysis of experimental and field trials was performed to assess broilers performance and wlfare.

This meta-analysis involved three sets of trials performed from 2015 to 2020. The first is based on eight performance trials comparing the effect of different AGPs and Bs29784 on 6,896 broilers from hatch till slaughter. The second set is a compilation of four field trials in which performance on 1,557,280 broilers was measured following the dietary inclusion of Bs29784. Finally, the third trial set focused on welfare parameters (pododermatitis (FPD) and litter quality) scored on 347,360 broilers. After meeting a priori quality criteria, a factorial mixed model ANOVA was performed on the data using the procedure GLIMMIX in SAS.

Meta-analysis showed that Bs29784 improved FCR by 1.76% compared to nonsupplemented control (P<0.05) and was not significantly different from the AGP group. Bs29784 also increased the BWG by 1.60% (P<0.001) without affecting the FI (-0.16%; P>0.05). Regarding field trials, FCR was also enhanced by 3.17% with the supplementation of Bs29784. Finally, the probiotic supplementation significantly decreases the severity of FPD from 10 to 13 points, correlated with a better litter quality score.

Because the feed intake was not affected, we assumed that the systematic performances improvement with Bs29784 supplementation is due to an optimization of intestinal parameters. Indeed, Bs29784 has been shown to enhance the host response and to modulate the microbiota leading to a stronger intestinal barrier and a controlled inflammatory response as well. In conclusion, this meta-analytical study indicates that in experimental or field conditions, Bs29784 consistently improved the performance of the flocks associated with better animal welfare.

THE MICROBIOTA PROFILE AS A BIOMARKER IN DIFFERENTIATING THE "NO ANTIBIOTICS EVER" BROILER PRODUCTION

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Inappropriate use of antibiotics in poultry production has raised concerns in consumers worldwide. Broilers raised without antibiotics, "No Antibiotic Ever (NAE)", is an important claim, meaning no antibiotics used in broiler production at any stage from hatchery to abattoir. In our work DNA sequencing technology by 16S rRNA was applied to differentiate the microbiota fingerprint among the broiler flocks using antibiotics and those that never received antibiotic treatment. Six identical broiler farms were used, all part of the same integrator. Management practises were standardised across the farms, stocking densities were at 30 kg per m2. Moreover, all houses used the same chicks (Ross 308) and feeds from the same mill. During the examined period, the flocks of three houses (AntiNEG) did not receive any antibiotics, whereas the flocks of the other three houses (AntiPOS) received Doxycycline and Amoxicillin via water twice. From each house at day 42 of age, twelve chickens were randomly selected for Next Generation Sequencing (NGS) analysis. Digesta samples were collected from the jejunum and the caeca and their genomic DNA was extracted with commercial isolation kit (DNeasy PowerFood Microbial kit, Qiagen, Germany). The 16S rRNA primer pair was utilized to evaluate the microbial ecology of each sample on the MiSeq. Samples were sequenced utilizing the Illumina MiSeq chemistry following manufacturer's protocols. Statistical analysis was performed using XLstat, NCSS 2007 and NCSS 2010. Total observed phyla analysis in the jejunum showed that Bacteroidetes, Actinobacteria and Cyanobacteria percentages were severely limited in AntiPOS samples, while Dietzia, Salinococcus, Brevibacterium, Weisella and Anaerostipes were the bacterial genera markers detecting the AntiNEG status. In the caeca, the phyla differences between the groups were not so apparent, however Clostridia, Megamonas, Ornithinibacillus, Parabacteroides percentages were higher in the AntiNEG samples, whereas Akkermansia was only present in the AntiNEG samples. Bacterial taxonomy by NGS marks the antibiotic usage at farm level and detects NAE production.

Acknowledgments: This research has been co-financed by Greece and the European Union (European Regional Development Fund) in context of "Research – Create – Innovate" Operational Program "Competitiveness, Entrepreneurship and Innovation (EIIANEK)" of the NSRF 2014-2020. Project Code: T1E Δ K-03856 (MIS 5030740). Acronym: GREEN POULTRY MEAT ANTIFREE.

ESSENTIAL OILS AND TRIBUTYRIN EFFECTS ON INTESTINAL DIVERSITY DETERMINED BY AMPLICON SEQUENCING

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A feeding trial was performed to evaluate the dietary effect of essential oils and organic acids on broiler chickens' intestinal microbial populations by using next generation technology. 360 one-day-old male chicks were randomly allocated to 4 treatments (6 pens of 15 chicks per treatment). Control treatment (A) was fed standard diets. The diets of the other treatments were further supplemented with essential oils (B), tributyrin (C) or both (D). On day 38 intestinal samples from the jejunum and the caeca) were collected for analysis. A 16S rRNA primer pair was utilized to evaluate the microbial ecology of each sample on the MiniSeq, by singlestep 30 cycle PCR (HotStarTag Plus Master Mix Kit, Qiagen, Valencia, CA). All amplicon products were mixed, purified, and sequenced utilizing the Illumina MiniSeq according to manufacturer protocols. Statistical analysis was performed using XLstat, NCSS 2007, "R" and NCSS 2010. In the jejunum Firmicutes, Actinobacteria and Proteobacteria were the three phyla present. Treatment C had the lower percentage of Firmicutes (A 99.59%; B 98.07%; C 94.25%; D 97.01%) and the highest of Actinobacteria (A 0.19%; B 0.31%; C 5.60%; D 1.28%). Treatment A had the poorest microbial variability, since bacterial genera such as Veillonella, Lactococcus, Brevibacterium, Salinicoccus and Gallibacterium were not detected, whereas Staphylococcus, Streptococcus, Enterococcus were present in low numbers. Treatment group B had the higher biodiversity with 16 different genera of bacteria. In the caeca, 12 bacteria phyla were observed, while the relative abundance comparison showed that the treatment B had the lowest percentages of Actinobacteria, Cyanobacteria, Euryarchaeota, Synergistetes, Spirochaetes, Verrumicrobia, and Tenericutes. The taxonomic classification data indicated 35 predominant genera. Lactobacillus percentage was in higher percentages in treatments C (10.40%) and D (7.90%). Treatment B had the highest percentage of Bifidobacterium (1.85%) and Megamonas (12.51%). In conclusion, dietary essential oils and tributyrin can influence broiler chicken microbiota, towards a better profile in phylum, family and genera levels.

Acknowledgments: This research has been co-financed by Greece and the European Union (European Regional Development Fund), funded by the Operational Program "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020), Project code MIS 5029057. Acronym: FoodOmicsGR.

THE MODULATORY ROLE OF DIFFERENT SUPPLEMENTATION LEVELS OF ESSENTIAL OILS ON GUT MICROBIOTA DIVERSITY OF BROILER CHICKENS

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The aim of this trial was to investigate the effect of incremental amounts of dietary essential oils on broiler chicken gut microbiota diversity, by using amplicon sequencing - next generation technology. 288 one-day-old male chicks were randomly allocated to 4 treatments (6 pens of 12 chicks per treatment). Control treatment (TrA) was fed standard diets. Diets of other treatments were supplemented with an essential oils' mixture. On day 38 intestinal samples (jejunum and caeca) were collected for analysis. Appropriate 16S rRNA primer pair was utilized to evaluate the microbial ecology of each sample on the MiniSeq, by single-step 30 cycle PCR (HotStarTag Plus Master Mix Kit, Qiagen, Valencia, CA). All amplicon products were mixed, purified, and sequenced utilizing the Illumina MiniSeq according to manufacturer's protocols. Statistical analysis was performed using XLstat, NCSS 2007, "R" and NCSS 2010. In jejunum, identified phyla, were firmicutes, actinobacteria, proteobacteria and cyanobacteria. TrA had the lowest percentage of firmicutes (84.11%; 90.27%; 92.18%; 99.80%, respectively) and the highest percentage of actinobacteria (14.45%; 0.87%; 2.07%; 0.06%). TrD had the lowest percentages of proteobacteria (1.31%; 8.86%; 5.72%; 0.13%). On genus level, 16 bacteria were observed. TrD had the highest percentage of Lactobacillus (74.22%; 89.84%; 90.84%; 99.89%), the lowest percentages of potential pathogens such as Helicobacter, Escherichia, Corynebacterium and Staphylococcus, and absence of Shigella. In the caeca, 7 different phyla were observed with firmicutes having the highest percentage (50.48%; 72.61%; 82.18%; 74.18%). On genus level, 70 bacteria were identified, with Lactobacillus, Bacteriodes, Faecalibacterium, Megamonas and Clostridium being the most abundant. TrD had the highest percentage of Lactobacillus (8.00%; 19.38%; 11.42%; 22.27%). TrB had the highest percentage of Bifidobacterium (2.76%; 14.71%; 5.21%; 0.56%). Hierarchal clustering of predominant genera showed that control TrA results are distinct from the other three treatments.

Acknowledgments: This research has been co-financed by Greece and the European Union (European Regional Development Fund) in context of "Research – Create – Innovate" Operational Program "Competitiveness, Entrepreneurship and Innovation (EPAnEK)", NSRF 2014-2020. Project Code: T7ΔKI-00313 (MIS 5050735). Acronym: GreenPro.

EFFECT OF FEED EFFICIENCY ON CECAL MICROBIAL COMMUNITY COMPOSITION OF COMMERCIAL YELLOW BROILER CHICKENS

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INTRODUCTION: Feed efficiency, as an important indicator to measure growth traits, greatly affects the industrial benefits of broiler. In recent years, researchers have found that intestinal microorganisms play an important role in livestock and poultry digestion, nutrient metabolism and regulation of host intestinal immune response. The intestinal microbial concentration of mature chickens is the largest, which affects health and performance (Stanley et al., 2014).

MATERIALS AND METHODS: In this study, 270 yellow broilers males were selected and raised in the same environment. The feed conversion ratio (FCR) was calculated and ranked. A total of 60 sampled chickens were selected and divided into high feed efficiency (HFE) group and low feed efficiency (LFE) group. After euthanized on the day 64, cecum contents were collected and microbial genome DNA was extracted to construct the library which was sequenced on Illumina Hiseq2500 platform. Raw data was filtered with FLASH software. Using USEARCH software to clustered the similar tags to generate the operational taxonomic unit (OTU). Representative OUT sequences were compared to the Greengene V13_5 database by RDP classifier software. Taxonomic assignment of OTUs was categorized using Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations in PICRUSt.

RESULTS: Firmicutes, Bacteroidetes, and Actinobacteria were three main bacterial phyla in the cecal microbial community. There were differences of Ruminococcaceace, Rikenellaceae, Bacteroidaceae, and Bacteroides between HFE and LFE groups. Abundance of Bacteroides in HFE group was significantly higher than that in LFE group. The genus Bacteroides was negatively correlated (P < 0.05) with FCR. Predictive functional analysis showed that metabolic pathways such as "carbohydrate metabolism" were significantly enriched in HFE group.

CONCLUSION: We performed 16S rRNA gene sequencing and predicted the metagenome function. This study may increase the understanding of correlation between cecal microbiota and FE, and provide novel insight for improving growth performance of yellow broilers. In addition, Bacteroides may be used as a biomarker of to improve the performance of yellow broilers.

KEY WORDS: yellow broiler, feed conversion ratio, cecal microbiota, 16S rRNA

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MOLECULAR IDENTIFICATION OF ILEAL BACTERIA IN BROILER BIRDS ADMINISTERED ANTIBIOTIC USING BACTERIAL 16S DNA SEQUENCES

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The implication of antibiotic administration on broiler chicken ileal microbial ecology may be difficult to ascertain using culture – based approach. The several drawbacks of culture – based approach in studying poultry intestinal microbiome gives credence to culture – independent techniques. The anaerobic nature of most gut microbiota coupled with the fact that 50 – 90% of gut bacteria are yet to be isolated further necessitate the use of 16S – based investigations. This study was designed to identify ileal bacteria in broiler birds administered antibiotic using bacterial 16S DNA sequences amplified from ileal digesta. A total of two hundred and forty (240) day-old Broiler chicks (ANAK, 2000) were used for a growth experiment previously published (Ofongo – Abule et al. 2016). Digesta samples were collected from the ileum at six (6) weeks of age for DNA extraction, amplification and sequencing. Genomic DNA extraction, amplification and sequencing was carried out according to Otobo et al., 2018. One hundred sequences of DNA extracted from ileal digesta samples collected from broiler birds administered antibiotic (Dicoxin-plus) were interrogated using BLAST (Basic Local Alignment Search Tool) analysis on NCBI – National Centre for Biotechnology Information (Weisburg et al., 1991; Altschul et al., 1990). The BLAST analysis results produced a number of (57) uncultured bacteria; which indicated that a greater percentage of organisms tightly associated with the ileum have not been formally grown or have not been documented as cultured bacteria in a particular growth medium. Fifty-six (56) different strains of uncultured bacteria and one uncultured organism were identified. Thirty-six (36) different strains of Lactobacillus salivarius, one L. plantarum strain and five different strains of uncultured Lactobacillus were identified from the one hundred sequences interrogated. Eight of the L. salivarius strains identified, were complete genome: L. salivarius ZLS006; L. salivarius CICC 23174; L. salivarius JCM 1046; L. salivarius CECT 5713; L. salivarius UCC 118; L. salivarius; L. salivarius KLB39 and L. salivarius ATCC 11741. Conclusion: Antibiotics administration could be implicated in the prevalence of L. salivarius strains over other microbe ecology of the ileum. Acknowledgements: The authors gratefully acknowledge funding from TETFUND.

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PROBIOTIC CLOSTRIDIUM BUTYRICUM POSITIVELY IMPACTS MICROBIOTA, SCFA PROFILE AND TECHNICAL PERFORMANCE OF BROILER CHICKENS

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A specific probiotic strain of Clostridium butyricum (Miyairi 588) was evaluated for use in broilers, with the goal of improving technical performance and restricting potential caecal pathogens. Experimental set-up: Using the facilities of the University of Life Sciences (Prague, Czech Republic), 160 one-day old Ross 308 broiler chickens were divided at random in two groups (80 animals per group). Both groups were reared under the same commercial conditions, for a duration of 49 days. Treatments consisted of a control group, fed a basal diet, and a probiotic group, fed the same basal diet supplemented with 1 kg Miya-Gold®/mton of feed (5x1011 CFU C. butyricum Miyairi 588/mton of feed). Miya-Gold® is a single-strain probiotic formulation, commercialised by Huvepharma®. Average weights were recorded at day 1, 7, 10, 20, 35 and 49, with feed conversion ratio (FCR) calculated accordingly. Caecal microbiota was analysed in 5 individuals from each group at day 1, 10 and 42, with short-chain fatty acids (SCFA) measured in the caecum at day 42, via gas chromatography. All data was statistically evaluated with Statgraphics Centurion XV (version 15.2.05/2007, StatPoint Technologies, Inc., USA). After a Shapiro-Wilk test to check for normality, two-sample t-tests were used to compare both groups. Results: Final average weights were significantly higher in the Miya-Gold[®] group versus the control (3.23 kg vs. 2.78 kg, P <0.001), whilst FCR differed greatly as well (1.84 vs. 2.08). Additionally, significantly higher butyrate levels were present in the caeca of the C. butyricum group (132.12 mmol vs. 103.41 mmol, P < 0.05). This supports earlier work indicating that C. butyricum Miyairi 588 produces significant levels of butyrate in the distal stages of the gastrointestinal tract, where it is most useful. Finally, the microbiota was positively impacted in the probiotic group, with significantly lower E. coli levels from day 10 onwards (in log CFU/g caecal content: 8.47 vs. 7.29 on D10, 8.22 vs. 7.00 on D42) and significantly lower enterococci levels on day 42 (8.10 vs. 7.55 log CFU/g, P<0.05). Adding Miya-Gold® to the diet of broiler chickens positively impacted technical performance, whilst restricting potential pathogens in the caecum. As shown here, part of the probiotic's mode of action is due to its capability to produce butyrate in this location.

IN VITRO AND IN VIVO CHARACTERIZATION AND ANTI-SALMONELLA ACTIVITIES OF LACTIC ACID BACTERIA ISOLATED FROM CHICKEN FAECES

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Poultry is a natural host for Salmonella species. Lactic acid bacteria are among the beneficial organisms that play a pivotal role in maintaining gut health and have proven to be one of the most effective strategies for eradicating Salmonella infections on poultry farms. Effective isolates of lactic acid bacteria (LAB) from chickens faeces were screened for possible probiotic activities in vitro and in vivo experiments. The efficacy of isolated LAB on performance, lipids profile and Salmonella enterica ser. Enteritidis (SE) activity were detected in broiler chicks to confirm the probiotic potential of LAB isolates. A total of 31 of LAB strains were isolated and characterized for probiotic properties including microscopic, and the ability to produce H2O2, antagonism against SE, tolerance to acid and bile salts, survivability in simulated gastric juice, and antibiotic susceptibility test. All of the isolates were Gram positive, cocco-bacilli that produce H2O2. The results showed that one LAB isolate among 31 was chosen on the basis of its tolerance to pH 2.5 for 8 h, and bile salts at 0.3 % for 24 h. API 50 CHL and 16S rRNA sequencing indicated L. fermentum as the most promising LAB strain with excellent probiotic potential. It inhibited SE with zones of inhibition ranging from 10.5 ± 0.51 to 21 ± 0.1 mm, and it also exhibited a stronger antagonism against SE when coculture activity was examined. L. fermentum was found to be resistant to at least one antimicrobial agent in an antibiotic susceptibility test. The isolate was chosen as a promising probiotic for in vivo efficacy in broiler chicks based on in vitro criteria. In vivo study, 120 broiler chicks were orally inoculated with 250 μ l of 1 \times 106 cfu SE at 21 d of age. At 21 days following infection, the probiotic-treated group demonstrated a reduction in SE load in both the caeca and the carcass. In conclusion, the results of the in vitro and in vivo probiotic investigations strongly suggest that L. fermentum is potential candidate as probiotic for controlling Salmonella Enteritidis activity in chickens.

INFLUENCE OF LINCOMYCIN-SPECTINOMYCIN TREATMENT ON ENTEROCOCCUS CECORUM-INFECTIONS AND ON THE COMPOSITION OF CECAL MICROBIOTA IN BROILERS

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Enterococcus cecorum (EC) is one of the main causes for skeletal disease in meattype chickens. Infections lead to animal welfare problems and high economic losses in the broiler industry. Despite the global importance of the pathogen, data on the rapeutic options and possible intervention strategies is still limited. The aim of the present study was to investigate the effects of lincomycin-spectinomycin treatment in the first week of life on the enterococcal infection and on the composition of the cecal microbiota. A total of 383 one-day-old commercial broiler chicks was divided into four groups: non-infected and non-treated, non-infected and treated, EC-infected and non-treated, and EC-infected and treated. The ECinfected groups were inoculated orally with an EC suspension at the first (10^{4}) colony forming units (CFU)) and third day (10^8 CFU) of life. The two treatment groups were treated with lincomycin-spectinomycin via the drinking water (16.6 mg/kg lincomycin and 33.4 mg/kg spectinomycin) for six consecutive days, starting two hours after the first inoculation. Twenty birds per group were necropsied at study days 7, 14, 21, and 42, and samples were taken from the ceca and several extraintestinal tissues to detect EC via bacteriological cultivation and real-time PCR. In addition, cecal samples were taken for the analysis of the microbial composition. Typical symptoms of the EC-associated disease were observed, and the presence of EC was confirmed by bacteriological examination in approximately 25 % of the birds in the EC-infected, untreated group. However, birds in the EC-infected, treated group neither showed any clinical signs or gross lesions throughout the trial, nor was EC detected in extraintestinal tissues or in the ceca after treatment. The composition of the cecal microbiota was highly influenced by the antibiotic treatment, whereas oral EC-infection did not influence the development of cecal microbiota in the present study. Antibiotic treatment reduced the amount of potentially beneficial Lactobacillus spp. in the two treatment groups. In contrast, the relative abundance of the genus Escherichia/Shigella was enhanced after treatment. Overall, antibiotic treatment successfully prevented the onset of the EC-associated disease in this experiment. Nevertheless, it had a direct impact on the composition of the cecal microbiota. Further research on alternative pre- and intervention strategies is urgently needed to combat EC infections in broilers.

EFFECT OF MANNOSE-RICH FRACTION SUPPLEMENTATION ON THE INTESTINAL MICROBIOME OF COMMERCIAL BROILERS

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The broiler gastrointestinal microbiome has been described as a modulator of performance and reservoir for infectious agents to enter the food chain. The goal of this project was to characterise the effect of mannan rich fraction (MRF) on bacterial diversity and composition in the duodenum, jejunum, ileum and cecum of commercial broilers. Approximately 35,000 1-d old birds were placed into each of two sheds receiving a standard wheat-soya diet or a standard diet plus MRF (Alltech Biotechnology) at 1300:1000:600 g t-1 in starter, grower, and finisher rations respectively. At day 35 (post-hatch) intestinal content from the duodenum, jejunum, ileum, and cecum of 12 randomly caught birds per shed was collected. Following DNA extraction, sequencing libraries were prepared by amplification and barcoding of the 16S rRNA gene V3-V4 region. Resulting amplicons were sequenced on the Illumina MiSeq platform generating 10-50 k PE300 reads per sample and VSEARCH was used for anlaysis of 16S sequences. Results showed a divergent yet rich microbiome structure between anatomical sites and observed the explicit effect of MRF on community structure, diversity, and pathogen modulation. A Brunner-Munzel test was used to compare differences between datasets where $P \leq 0.005$ was considered statistically significant. MRF supplemented birds had significantly greater Chao1 richness in the cecum (P \leq 0.001, nFC = 0.1311) and significantly different bacterial community composition in each GI tract section was noted using PERMANOVA ($P \le 0.001$). Supplemented birds had lower levels of zoonotic pathogens Escherichia coli ($P \leq 0.001$) and Clostridioides difficile ($P \le 0.001$) in the duodenum, jejunum and ileum and Shigella dysenteriae ($P \le 0.001$) in the ileum and cecum highlighting the potential of MRF supplementation in food chain integrity. In this study, MRF addition was observed to lower the bioburden of pathogens in broilers and promote greater microbial diversity in the intestinal tract. Bacterial diversity is an important component of a healthy functioning microbiome with higher diversity correlated to a healthier state and lower diversity correlated with dysbiosis. By using a prebiotic such as MRF to enhance bacterial diversity and composition it may be possible to prevent dysbiosis and maintain a healthy balanced intestine. This study is the first, to our knowledge, to investigate the effect of MRF on the microbiome associated with different GI tract anatomical geographies.

EFFECT OF MANNOSE-RICH FRACTION SUPPLEMENTATION ON THE INTESTINAL MICROBIOME OF LAYERS DURING PEAK LAY

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Bacterial composition of the chicken intestinal tract has become a prominent research focus and has been extensively studied to understand its association with performance. Most previous studies of the intestinal microbiome of chickens have examined broilers, likely due to their economic importance and short growth cycles with few studies focusing on the layer microbiome. However, broilers and layers have different genotypes, different lifespans in commercial production, and different dietary requirements. Therefore, the composition of the gut microbiota in these two lines is likely very different. The gut microbiota composition can be enhanced and strengthened with the use of prebiotic supplements such as mannan rich fractions (MRF). This study focused on examining the impact of MRF on the cecal microbiome of layers during peak lay. A total of 344 Shaver female laying hens were randomly allocated to one of 2 experimental groups and fed either a control or MRF supplemented diet. The cecal content of 10 randomly selected birds per treatment per timepoint was collected into DNA/RNA shield. Subsequently, DNA was extracted sequencing was carried out using the Novoseg600 system using PE150 strategy. The resulting sequences were analysed using the phyloFlash software. Brunner-Munzel tests were used to compare datasets where $P \leq 0.005$ were considered statistically significant. Results showed MRF supplemented birds had higher Chao1 a-diversity in the cecum ($P \le 0.001$, $\eta FC = 0.07$). Bacterial β diversity at each time point was significantly different between groups (PERMANOVA, $P \leq 0.001$). Microbiome analysis showed that following 84 days supplementation during peak lay the pathogenic bacteria Listeria monocytogenes and Campylobacter jejuni were not detected in the cecum of MRF supplemented birds (P \leq 0.001). Enterococcus faecalis (P \leq 0.001, nFC = 1.945). and Clostridioides difficile were significantly lower in the MRF layer cecum ($P \le 0.001$, nFC = 0.399). Securing a balance between beneficial and pathogenic bacterial populations in the intestinal tract is critical for the health of the animal and consumer. In the present study, the role of MRF as a microbiome modulator has been characterized and shown to improve diversity, alter composition and reduce bacteria which are important from a food safety perspective. This provides an attractive tool for future potential approaches to improve layer intestinal health as producers are encouraged to reduce antibiotic use.

MODULATION OF GUT MICROBIOTA ASSOCIATED TO IMPROVED BROILERS PERFORMANCE WITH SACCHAROMYCES CEREVISIAE VAR. BOULARDII SUPPLEMENTATION

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Gut health is a major component of broilers zootechnical performance. Microorganisms that inhabit the gastrointestinal tract play a crucial role in the prevention of pathogen colonization, immune system modulation and gut health maintenance. Supplementation of diet with Saccharomyces cerevisiae var. boulardii CNCM I-1079 (LSB, Lallemand, 10⁹ cfu/kg feed) was previously shown to improve zootechnical performances of broilers raised in standard farm conditions (mortality, final weight, average daily gain, feed conversion ratio, homogeneity). LSB also increases the resistance to infectious bronchitis virus contamination (Barbé et al., 2018). The objective of this study was to assess whether beneficial effects of LSB on zootechnical performances were associated with gut microbiota modulation. 208 ROSS birds randomly allotted to control and LSB treatment groups were provided pelleted feed and water ad libitum. The feeding program was divided in starter (0-10d), grower (10-25d) and finisher (25-35d) phases. At 35 days, caecal and ileal contents were randomly sampled from 5 birds in each group. Microbial DNA was extracted from gut content samples with ZR-96 Soil Microbe DNA Kit™ (Zymo Research). High-throughput sequencing of the 16S rRNA gene V4-V5 regions was performed on Illumina MiSeg sequencer. Sequences were processed using FROGS pipeline. Beta-diversity was visualized using ordination with Bray-Curtis dissimilarity matrix and mixed linear model was applied to analyze the effect of diet and sampling location on microbiota alphadiversity and composition (R software). Richness and shannon indices were higher in birds fed with LSB diet (respectively 815 ± 77 vs 510 ± 124 and 4.5 ± 0.4 vs 3.3±0.7 in ileum, 614±2.1 vs 512±25 and 3.8±0.0.08 vs 3.5±0.09 in caecum, mean \pm SE, P<0.05). LSB tended to reduce the ileal microbiota heterogeneity between birds compared to the control group. The relative abundance of several genera was modulated by LSB supplementation: beneficial bacteria exhibited higher level in LSB group in ileum (P < 0.05) such as Megasphaera (0.6% vs 0.1%), Butyricicoccus (0.3% vs 0.06%), Oscillospiraceae (6.6% vs 3.0%). Enterococcus, which include potential pathogens, were lower compared to control group (1.0% vs 9.0% in ileum and 0.1% vs 0.9% in caecum, P<0.05). Beneficial effects of LSB on broilers performance may thus be partially mediated by beneficial gut microbiota modulation.

Barbé et al., Improvement of zootechnical performance in broilers raised in standard farm conditions and supplemented with Saccharomyces cerevisiae boulardii. Mediterranean Poultry Summit, 2018.

METAGENOMIC ANALYSIS OF GIT OF KADAKNATH CHICKEN USING 16S RRNA SEQUENCING

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Gut health and microbiota play a pivotal role in the health and production of bird. Microbiota of GIT is responsible for the competitive exclusion of pathogenic microbes and maintaining the integrity of mucosa. With the advent of NGS platforms, the study of whole GIT metagenome was made possible. Indigenous chicken is a rich repository of microbiota responsible for the resistance to harsh environments and pathogens. Metagenomic analysis of the whole gastrointestinal tract was done in the present study. Whole gut content from 6 birds selected randomly from a flock of 40 birds of 12 weeks old Kadaknath chicken was collected and DNA was isolated and outsourced for 16s rRNA sequencing of V1-V3, V3-V4, V4-V6 hypervariable regions. De novo whole genome metagenomics was performed by NovaSeq 6000 and paired end read of 150bp×2 was obtained. Taxonomic profiling of sequences was performed using NCBI data utilizing MEGAN and MG-RAST software. Functional annotation of contigs was done using MGA. Bacteria is the major domain in the microbiome of gut, archaea accounted for 0.4% of the total sequences. The major bacterial phyla in the gut microbiome was Bacteroidetes 45.69% followed by Firmicutes 35.66% and Proteobacteria 10.24%. 79 genera of bacteria showed >0.1 relative abundance constituting about 89.5 % of total bacteria present in the gut metagenome. 157 species recorded >0.1% of relative abundance constituting 81.18% of total bacteria. Lactobacillus genus accounted for 0.75% of total bacteria present in the gut metagenome. Among the Lactobacillus genus, 29 species were found in the whole gut metagenome of Kadaknath chicken. The major species include L. salivarius 18.78%, L. crispatus 9.63%. L.delbrueckii 7.52%, L. acidophilus 7.28%, L. johnsonii 6.69%, L. plantarum 6.60%, L. reuteri 6.57%, and L. fermentum 5.01 %. Functional annotation studies using bioinformatic tools KEGG and SEED analysis revealed that most of the functional pathways of the whole gut metagenome constituted for the synthesis of cofactors, vitamins, prosthetic groups, and pigments, followed by carbohydrate metabolism, whereas the reads accounting for the stress response and virulence were lowest among all other functional pathways. GIT metagenome constituted 28 phyla, 51 classes, 110 orders, 241 families, 595 genera, and 1483 species of bacteria. The pathogenic microbiota constituted the third major phyla however the majority of functional pathways constituted for metabolic pathways.

EFFECT OF A NOVEL PRECISION BIOTIC ON THE INTESTINAL MICROBIOME OF BROILER CHICKENS

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The objective of the studies was to evaluate the effect of a novel glycan-based precision biotic (SymphiomeTM, DSM Nutritional Products) on the growth performance and the metagenome of the cecal microbiome of broiler chickens. In study 1, day-old chicks were placed on a completely randomized block design with 2 treatments, 21 replicates, and 40 birds/replicate. The treatments consisted of a non-supplemented control or feed supplemented with 1.1 Kg/ton of feed of SymphiomeTM. Cecal content samples were collected at 24 and 42 d of age from 1 bird/pen, and frozen for microbiome analysis through whole genome sequencing (Illumina HiSeq 3000). The supplementation of SymphiomeTM improved body weight gain (BWG) and feed conversion ratio (FCR) of the birds (P < 0.05). Related to the microbiome analysis, SymphiomeTM increased (P < 0.05) the abundance of pathways related to energy production around the TCA cycle associated with a concomitant increase of acetyl-CoA, justified mainly by a significant acetylation of multiple amino acids and increased lipid biosynthesis. The increased carbon flow was also directed to the production of short chain fatty acids, mainly propionate by the acrylate pathway. About the nitrogen metabolism, ammonia was found to be largely detoxified through associated pathways to polyamines, i.e., through the uric acid cycle at day 24, and through the asparagine outlet at 42 days. In study 2, day-old chicks were placed on a completely randomized block design with 3 treatments, 10 replicates, and 25 birds/replicate. The treatments consisted of a non-supplemented and non-challenged control (CON), a challenged control (ChaCON), and a challenged group supplemented with 0.9 Kg/ton of feed of SymphiomeTM (ChaSYM). All the birds were vaccinated against coccidiosis on d 0 (Coccivac B52). The challenge consisted of Clostridium perfringens strain #6 given via drinking water to the challenged groups on d 15, 21, and 28. Growth performance parameters were evaluated on d 14, 28, and 45. On d 22 and 46, 1 bird/pen was randomly selected, and the cecal content was collected and frozen for microbiome analysis through whole genome sequencing (Illumina HiSeg 3000). The supplementation of SymphiomeTM recovered the FCR of the birds, similar to the CON birds (1.782a, 1.856b, 1.792a, for CON, ChaCON, ChaSYM, respectively, with P = 0.005), and reduced the NE associated mortality (P = 0.0002). Supplementation of SymphiomeTM appeared to restore several microbial pathways affected by the challenge including nitrogen, propionate, butyrate, fatty acid biosynthesis, and biosynthesis of cofactors. Some pathways suggest a link towards modulation of the immune response.

INOCULATION WITH A MICROBIAL COMMUNITY DERIVED FROM ADULT CHICKEN CECAL CONTENT ENABLES THE EARLY DEVELOPMENT OF A MATURE MICROBIOTA IN YOUNG CHICKEN.

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The gut microbiota plays a crucial role in maintaining gut health, modulating the immune system while preventing pathogen colonization. In nature, hatched birds in nest are raised in contact with their mother, whereas under standard farm conditions, eggs are hatched in clean environment, with no contact with adult hens. This greatly impacts gut microbiota establishment. Aviguard®(Lallemand) is a microbial community derived from healthy chicken, which efficiently prevents pathogen colonization, such as Salmonella, E. coli and C. perfringens, when administrated in hatched birds. The early colonization of a protective microbiota is a likely involved mechanism, however not fully understood. Our objective was to better describe the effect of Aviguard on the gut microbiota colonization in chicks. One day old birds were inoculated with Aviguard or water (control) and fed with sterilised feed and water for 7 days. Two boxes with 9 birds per boxes were used per treatment. At 7 days, caecal and ileal contents were randomly sampled from 5 birds per box. Microbial DNA was extracted from gut content samples with ZR-96 Soil Microbe DNA Kit[™] (Zymo Research). High-throughput sequencing of the 16S rRNA gene V4-V5 regions was performed on Illumina MiSeg sequencer. Sequences were processed using FROGS pipeline. Beta-diversity was visualized using ordination with Bray-Curtis dissimilarity matrix. Mixed linear model was applied to analyze the effect of treatment and sampling location on microbiota alpha-diversity and composition (R software). Aviguard had a major impact on microbial colonization in both caecum and ileum of young chicks (P<0.001, permanova). Shannon index was significantly lower in Aviguard treated birds $(2.15\pm0.15 \text{ vs } 3.10\pm0.06 \text{ in caecum and } 2.46\pm0.09 \text{ vs } 2.97\pm0.06 \text{ in ileum, mean}$ \pm SE, P<0.05). This suggests a rapid establishment of well-adapted bacteria, in comparison to the control birds' microbiota still in the process of selection. Aviguard treatment had a significant effect on the abundances of 41 genera (out of 58 tested), in caecum and/or ileum. Major genera increased with Aviguard includes Bacteroides (27% vs 3.4%), Megamonas (17% vs 0.02%), Olsenella (3.2% vs 1.6%) in both locations, Blautia (1.3% vs 0.3%) and Megasphaera (3.1% vs 0.8%) in ileum, while Lachnochlostridium was higher in control birds (13% vs 3.6%). Aviguard inoculation thus enables the early establishment of a mature gut microbiota, likely protective against pathogen colonization.

26th World's Poultry Congress, abstracts selected in 2022

WORKING GROUPS' sessions

26th World's Poultry Congress, abstracts selected in 2022

Construction of egg quality

Selected short communications

EFFECT OF CALCIUM PARTICLE SIZE ON PERFORMANCE, EGGSHELL QUALITY, BONE MINERALISATION, AND IN VITRO/IN VIVO SOLUBILITY IN LAYING HENS: A META-ANALYSIS APPROACH

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Several studies over the three last decades have studied the effect of coarse limestone on production performances, bone health, and calcium (Ca) digestibility in laying hens. However, there is no consensus on the advantages of coarse limestone and the underlying mechanisms of limestone particle size are still unclear. The objective of this study was to study the impact of limestone particle size on the digestive and metabolic fate of Ca to optimize calcium utilization by laying hen through meta-analysis approach. Fifty-eight publications published between 1971 and 2019 and including 192 experiments were included in the database. Four categories of dependent variables were identified: 1) Ca solubility (in vitro and in vivo Ca solubility and gizzard retention time), 2) production performance (laying rate, egg weight), 3) eggshell quality (eggshell specific gravity, eggshell thickness, eggshell breaking strength), and 4) bone mineralization (tibia breaking strength, tibia ash content). The independent variables tested were Ca particle size and age. Multiple regression models were fitted with the nlme package of R software with the random effect of the experiment. Results showed that the in vitro solubilisation of Ca linearly decreases with limestone particle size (P < 0.001; R2 = 0.95), while in vivo solubilisation linearly increases with limestone particle size (P < 0.001; R2 = 0.91). This result can be due to the longer retention of larger limestone particle in the gizzard (P <0.05; R2 = 0.65) that induced higher Ca solubilisation by gastric juices than fine limestone. Coarse limestone showed no effect on production performance while all eggshell quality parameters increased when hens received larger limestone particle (P < 0.001; R2 > 0.91), increasing eggshell specific gravity by 1%, eggshell thickness by 1%, and eggshell breaking strength by 3% when passing from 0.25 mm to 1.5 mm. The most relevant effect of large limestone particle was on tibia breaking strength (P < 0.05; R2 = 0.94) that is increased by about 4% when passing from 0.25 mm to 1.5 mm. This effect was however age-dependent (Age x Particle size, P < 0.05; R2 = 0.92) given that there was no significant effect of larger limestone particle on tibia breaking strength before about 60 weeks. The current study shows a priority for egg production and eggshell formation in older hens at the expense of bone mineralisation which can help reducing bone mineralisation problems occurring with ageing.

Key words: laying hen, coarse limestone, calcium solubilisation, eggshell quality, bone health.

THE RELATIONSHIP BETWEEN METABOLIC PROFILES, BONE STATUS AND EGGSHELL QUALITY IN AGEING LAYING HENS

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Eggshell production requires a daily mobilization of 30 to 40% of the calcium stored in the medullary part of long bones. Consequently, the high rate of egg production lead to skeleton changes in particular at the end of the laying cycle inducing bone fragility. Here in, to explore the interaction between bone and eggshell quality metabolic failures we have studied bone microarchitectural texture, eggshell parameters related to mechanical properties, blood and liver metabolic markers. A total of 67 laying hens with similar egg weight and laying rate were divergently selected on extreme egg strength and deformation among 450 animals at 84 weeks age and reared until 96 weeks. Laying hens were classified as low (33) and high (34) eggshell qualities. Egg quality parameters (egg weight, diameter, shape index, eggshell weight, strength, deformation, toughness and elasticity) were assessed during two weeks before sampling at 96 weeks of age. Birds were sacrificed and blood, liver and tibiae were collected and in utero egg removal occurred. Blood markers (1,25-OHD3, 25-OHD3, CTX, osteocalcin) were analyzed as well as liver markers of vitamin D metabolism, CYP27A1 and CYP3A4, tibia microarchitectural texture and eggshell ultrastructure and matrix proteins affecting shell texture. Hens age related changes were observed in eggshell physical parameters and in equipmental matrix proteins abundance associated to a decrease of eggshell quality. At 96 weeks of age, a vitamin D metabolism and bone resorption stimulation (higher level of blood 1,25-OH2-D3 and CTX as well as a lower ratio OC:CTX) were observed but only in hens with higher eggshell quality. Yet, liver CYP27A1 and CYP3A4 expressions remained unchanged suggesting the absence of regulation at this level. Additionally, a better eggshell quality was correlated with a higher overall bone density and a lower bone medullary density. The present integrative study provides a better description of the relationships between bone, eggshell quality and biological markers during laying hens' ageing. These findings have implications for understanding bone and eggshell quality degradation and suggest potential adapted nutrition or management practices. Further designed studies notably including longitudinal analysis of bone parameters, ultrastructure and eggshell composition are required.

EVALUATION OF ESSENTIAL OIL OF OREGANO (LIPPIA ORIGANOIDES) AS AN ALTERNATIVE TO THE USE OF ANTIBIOTIC GROWTH PROMOTERS IN ISA BROWN LAYING HENS

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The objective of this work was to evaluate the effect of the oregano essential oil (OEO) of the genus Lippia origanoides, on the productive parameters, egg quality and intestinal morphometry as an alternative to the use of growth promoting antibiotics (GPA) in Isa Brown laying hens. A total of 96 laying hens of the 70-week-old Isa Brown genetic line were used, in a completely randomized design with 4 treatments and 4 replicas with 6 hens for each replica. The diets were provided for 10 weeks and formulated according to the nutritional requirements of the genetic line and the production phase. These included a control diet: NC (without OEO and GPA), another including GPA (50 ppm Zinc Bacitracin) and 2 levels of OEO: 80 ppm and 150 ppm respectively. The body weight change (q) and the average eqg weight showed no differences between treatments (P > 0.05). The feed conversion was lower for 1500EO, 800EO and GPA compared to the control group (P <0.05). The mortality % of the birds was not significantly different between the treatments, however the 1500EO obtained a 0% mortality. The components of the egg (% yolk, albumin and skin) did not show differences between the treatments. The height of albumin and Haugh Units was significantly higher for 1500EO compared to the other treatments (P < 0.05). Likewise, the color of the yolk was higher for the groups 800EO and 1500EO (P < 0.05). The eggshell thickness was greater for the groups 800EO and 1500EO (P < 0.05), however the eggshell breaking strength did not present significant differences between the treatments (P > 0.05). In the coloration of the shell the value of L was lower in the treatments 800E0 and 1500E0 (P < 0.05) and the values of a * and b * were higher for 800EO and 1500EO (P < 0.05). The morphometric analyzes of the small intestine revealed significant variations in the height and width of the villi and depth of the crypt in Isa Brown hens. In the duodenum, significant differences in hairiness were observed in the 1500EO, 800EO and GPA treatments compared to the NC group (p < 0.05). Villus width and crypt height / depth ratio were higher for the 1500EO treatment compared to the GPA and NC treatments (p < 0.05). Likewise, the depth of the crypt was lower in the 1500EO group compared to the GPA and 800EO treatments (p < 0.05). In the jejunum, there were significant differences in the height and width of the villi in the 1500EO, 800EO and GPA treatments compared to the NC group (p <0.05). Crypt depth was lower in the 1500EO group compared with the GPA and 800EO treatments, but when comparing the height / depth ratio of the crypt, the 1500EO treatment was the one that presented a greater relationship compared to the other treatments (p < 0.05). In the ileum, height, villi width and crypt height / depth ratio showed significant differences in the 1500EO, 800EO and GPA treatments compared to the NC group (p < 0.05). No significant differences were observed in the depth of the ileum crypt (p > 0.05). The findings of our study indicate that 150 ppm OEO can be used as a substitute for GPA; it has a positive effect on the performance and quality of the egg, furthermore, parameters related to intestinal morphometry of laying hens improvements.

ESTIMATION OF GENETIC PARAMETERS OF EGG NUMBER AND EGG QUALITY TRAITS IN LONG PRODUCTION CYCLE

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The breeding goal, "producing 500 saleable eggs in 100 weeks", has drawn increasing attentions among layer breeders globally. So far, no genetic parameters of egg production and egg quality traits in 100 weeks have been reported. In this study, we estimated the genetic parameters of traits in 100 weeks in a pure line of Rhode Island Red (RIR). In total, 1256 RIR chicken was raised to 102 weeks and comprehensive traits were collected. In order to improve the accuracy of the measurement of egg quality traits, 3 consecutive eggs were collected for each individual during the 100th week and then subjected to egg quality test, and the average value was taken as the individual's trait phenotype value. Six traits, i.e. the cumulative number of eggs by the end of 100 week (EN100), egg weight (EW100), body weight (BW100), eggshell strength (ESS100), eggshell color (ESC) and Haugh Unit (HU100) were analyzed. The multi-trait repeatability animal model was applied to estimate the variance component of each trait and the covariance component between traits. Repeatability, heritability of each trait and genetic and phenotypic correlations between traits were calculated. Nearly half of the population (48%) produced more than 500 eggs. Genetic analysis showed that the repeatability of ESS100, EW100, ESC100, HU100 were 0.48, 0.83, 0.74, 0.56, respectively, indicating that single record for EW100 and ESC100 is quite representative, while multiple measurements for ESS100 and HU100 are necessary. The heritability for BW100, EN100, ESC100, ESS100, EW100 and HU100 was 0.347, 0.246, 0.370, 0.119, 0.282 and 0.106, respectively, and the standard error of the estimated heritability of each trait is between 0.06 and 0.08, suggesting that traits in the late production cycle were low to medium heritable. Medium genetic correlation between BW100, EW100 and HU100 were observed (0.41), while there exists a strong negative correlation between EW100 and HU100 (-0.63). Correlations among other traits were weak $(-0.17 \sim 0.25)$. The results from this study suggest that egg number and egg guality traits in late production cycle is partly under genetic control, and it's possible to further improve egg production and egg quality for a longer production cycle through proper genetic selection programs.

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Quality of egg products

Selected short communications

RELEVANCE OF NON-INVASIVE AND NON-DESTRUCTIVE IMAGING TOOLS TO ASSESS INTERNAL QUALITY OF TABLE EGGS

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Egg guality (shell strength, freshness and maintenance of physicochemical, technological and organoleptic properties, etc.) is essential to the economic viability of the table egg sector. Certain alterations, linked to external factors such as the hens age or the storage conditions for example, can in particular, have negative impacts from a health and economic point of view. Some internal egg quality indicators (characteristics of the yolk and white) are visible and are mostly measured after breaking the egg. Others, like the volume of the air chamber or the displacement of the yolk, are less easily measurable. The objective of this work is to evaluate the use of tomographic imaging tools (magnetic resonance imaging (MRI) for soft tissues, and computed tomography (CT)-scan for hard tissues) as non-invasive methods and non-destructive to characterize the quality of eggs. Consumption eggs from Lohmann Tradition hens were collected on the day of laying, and then stored for 7, 14 and 28 days at two temperatures (4 and 20°C). They were analyzed by MRI and CT-scan, and afterwards characterized with the standard measurement tools of egg quality after breaking. In total, 37 trays containing 10 to 15 eggs were imaged i) by CT-scan for assessment of the air chamber volumetry, and ii) by MRI for the characterization of the white and yellow volumes and for the mapping of T2 relaxation times. The images were processed with different software (ITK-snap, ImageJ, TableCurve2D, Matlab). Comparisons and correlations were made between the standard and the imaging methods. The volume measurements determined under the different storage conditions were thus studied using a comparison of groups, while the T2 relaxation times were studied i) by principal component analyzes (PCA) and ii) by correlations with certain standard parameters of the egg quality. The results obtained show that imagery can be used as a non-invasive tool for measuring internal egg quality while providing new possibilities for measuring criteria that are difficult to assess once the egg has been broken. The results of this study may ultimately allow the creation of a new egg quality referential.

OZONE TREATMENT ENHANCED PHYSICOCHEMICAL QUALITY OF EGGS

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The egg is a high-quality nutrient source in human diet; therefore, a rigorous control must be realized to ensure the microbiology and physicochemical quality to be preserved on the shelf life of eggs. The aim of this study was to evaluate the effect of ozone gas on egg shelf life for consumption. A total of 60 1-day-old fresh eggs from cage free hens were randomly allocated in two groups, five replicates per group: control - six eggs with similar weight placed in sterile recipient; ozone treatment (OT) - six eggs placed in sterile and sealed container and exposed to 57 mg/L using ozone generator (O&L1.5 RM, Ozone & Life[®]) during 7 days. Both groups were maintained at room temperature (24 °C) for all experimental period. Three eggs in each replicate per treatment were evaluated on 7d and 14d after the start of ozone treatment. Haugh unit (HU) and albumen pH were evaluated. HU was calculated by using the formula: HU=100×log (h -1.7W^{0.37}+7.6), where h is the height of the albumen (mm) and $\langle em \rangle W \langle em \rangle$ is the weight of the eqg (g). Albumen pH was done after separated from the egg and homogenized using a pH meter (Quimis[®], model Q400A). The experimental design was a factorial 2x2 (ozone and time). All data were analyzed using SAS 9.4 program, and means compared using LS means test, where differences were considered significant at P < 0.05. In the mains effects, no significant difference was observed between the treatments for the HU variable (P > 0.05), but pH was significantly lower in the ozone treated eggs (P < 0.05). There were significant interactions between ozone treatment and time on both HU and albumen pH parameters (P < 0.05). OT had eggs with lower HU at 7d compared to control, however, the HU was significantly higher in OT at 14d (P < 0.05). This finding indicated that ozone may decrease the effect of time in eqg protein degradation. Albumen pH, which tend to increase as eqg aged due the deterioration of albumen, was not significant different at 7d between control and OT, nevertheless it had significant difference at 14d, where OT group had a lower pH compared to the control group. In conclusion, this result may indicate that ozone may be beneficial to extend shelf life of eggs for human consumption.

THE STUDY OF THE PHYSICAL AND CHEMICAL PROPERTIES ON THE GLOSS OF CHICKEN EGGSHELLS

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Eggshell appearance generally does not determine egg's nutritional value. However, eggs with a shiny appearance and uniform color are more attractive to customers. Gloss is loosely defined as the specular component of light reflecting from an object. In the previous study, we found significant differences in the surface textures of the high- and low-gloss chicken eggshells. However, the mechanisms responsible for the gloss is unclear. In this study, 200 chicken eggs were collected from the 55-week-old Rhode Island Red hens. The eggshell gloss and cuticle coverage were measured with the reflectometer (Konica Minolta CM600). X-ray photoelectron spectroscopy (XPS)was used to examine the surface chemical composition (atom percentages, %) of the eggshell. Our results showed that the eggshell cuticle coverage was low ($\Delta E^*ab=21.86\pm5.09$), but positively correlated with the gloss (r=0.53, P<0.01). XPS analysis found that the inorganic composition of calcium and phosphate was negatively correlated with the eggshell gloss (r=-0.34, P<0.01) and cuticle coverage (r=-0.14, P<0.01), respectively. Calcium and phosphate attribute to the presence of hydroxyapatite, calcium phosphate and calcium carbonate accumulating on the outer surface at the final stage of eggshell mineralization. Moreover, two tailed analyses for the eggshell gloss and cuticle coverage also indicated that the composition of calcium and phosphate influenced the eggshell appearance. We speculated that the better cuticle coverage quality and less accumulation of calcium and phosphorus compounds on the eggshell surface would lead to higher eggshell gloss. These findings advance our understanding of the eggshell gloss and have the potential significance in breeding program for the improvement the eggshell quality.

Keywords: chicken, eggshell gloss, XPS, cuticle coverage, mineralization.

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Egg quality

Posters

EFFECTS OF LARD AND VEGETABLE OILS SUPPLEMENTATION QUALITY AND PROPORTION ON EGG QUALITY AND PRODUCTION PERFORMANCE OF HY-LINE BROWN

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In poultry breeding, oils as the energy feed, the type, concentration and guality of oils have a vital influence on the production performance and egg quality of laying hens. In the present study, we investigated the qualitative and amount effects of lard and vegetable (soybean) oils supplementation on production performance and egg quality of Hy-line Brown layers. A total of 720 283-days-old Hy-line brown layers were allocated into eight treatment groups following a $2 \times 2 \times 2$ factorial arrangement with two types (lard and soybean oils), two concentrations (0.5% and 1.5%) and two qualities (normal and oxidized). The normal treatment increased the average daily feed intake (ADFI), average egg weight (AEW), laying rate (LR), body weight gain (BWG) and decreased the feed conversion ratio (FCR), in which the BWG and LR increased significantly with the increasing concentration (P < 0.05). Moreover, the quality of supplementation also affected the parts of eqg quality to influence the freshness, nutrients and microstructure. Following the normal treatment, the albumen height (AH) and haugh unit (HU) of the high-dose soybean oil group was significantly lower than the high-dose oxidized soybean oil group (P < 0.05). And the oxidized treatment increased the HU and decreased the yolk color (YC) between low-dose normal and oxidized lard groups. Moreover, among the same quality groups, the high concentration treatment also affected the egg guality, with the decreasing AH and HU in the normal groups and decreasing eggshell thickness (EST) in the oxidized groups. However, among all the groups, the eggshell strength (ESS), eggshell index (ESI) and yolk sticky (YS) had no significant change (P > 0.05). In addition, the oxidized treatment significantly decreased the saturated fatty acid (SFA) and decreased the unsaturated fatty acid (UFA). Finally, the microstructure of cooked yolk of highdose oxidized lard group showed significant changes, the yolk spheres had been significant cross-linking.

In conclusion, the dietary supplementation of quality and proper proportions of lard and soybean oils could improve the egg quality and production performance of layers. We should avoid using oxidized oils, due to that could decrease the various production indicators and nutrient to influence the economic benefits

Key words: Egg quality, Lard, vegetable oils, Production performance, Oxidized, Hy-line brown

EFFECT OF HOUSING SYSTEM ENVIRONMENT ON BROWN EGG LAYER QUALITY (INTERNAL AND EXTERNAL).

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The housing system (HSy) is an external factor that influences both the performance of hens and the egg quality characteristics. Conventional cages have been banned in the EU since 2012, and the housing of laying hens is permitted only in enriched cages or in alternative systems, such as cage free housings, aviaries or free range, believed to improve the welfare of the hens. The objective was to evaluate the quality of eggs from brown laying hen strains based on HSy. Analysis were made of eggs from major brown commercial strain in the US and EU market, housed in five systems: Cage Free (CF), Enrichable Cage (EC), Enriched Cage (ErC), Conventional (CvC), and Free Range (FR). Eggs were evaluated in three Period, 3 (25-28 wks.), 5 (33-36 wks.), and 9 (49-52 wks.), in each period 744 eggs, randomly chosen from each HSy, were evaluated in the proportion of the house size (CF 120, EC 192, ErC 192, CvC 192, and FR 48 eggs resp.). In each period all eggs were evaluated for a set of physical characteristics: shell color, egg weigh, albumen high, Haugh unit, yolk color (TSS, York, England), shell strength and elasticity, and vitellin membrane strength and elasticity (Texture Analyzer, Surry, England). Statistical analysis was done using JMP 14 and significant means differences between period and HSy were determined using Tukey's HSD. The differences in the three periods were highly significant (P<.0001) in all characteristic evaluated, as are expected as hens age. Shell color was darker brown in EC, than the others HSy (P<.0001), and in the FR system eggs were heavier (P<.0001) than other HSy, Haugh unit was low in CF, (P<.0001), and better yolk color was showed in FR HSy (P<.0001). Correlation of the shell quality, showed that eggs from HSy CF and FR, have greater shell breaking strength (P<.0001), requiring more force to break, followed by EC, ErC, and CvC showed a very similar shell quality, respectively. Shell elasticity was significantly (P<.0001) better in eggs from hens housed at CF and FR system than ones in the EC, ErC, CvC. The vitellin membrane strength and elasticity were not affected by the HSy, with P values of 0.1182, and 0.3264, respectively. In conclusion, the housing systems of CF and FR, can improve the external and internal eqg quality, which may be due to the better interaction of the layer hens with some beneficial variables from the environment, expressing a normal behavior, like walking, nesting, coprophagy, and access of pastures.

CHANGES OF THE DUCK EGGS QUALITY DEPENDING ON THE TIME AND TEMPERATURE OF STORAGE AND COVERING THE EGGSHELLS WITH CHITOSAN OR SILICONE LAYER

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Egg washing is the recommended method of sanitizing duck eggs, but removing the mucin layer may cause a decrease in egg guality during its storing. The aim of the study was to examine changes of physical and chemical indicators of duck egg quality depending in length (LS) and temperature (TS) taking into account using silicon or chitosan preparation that recreates the surface eggshelllaver Hatching eggs from 27. and 60. weeks old parental flocks of Cherry Valley Pekin ducks (Gardawice farm, Poland) were delivered to Lab weekly in batches of 450 eggs by 4 weeks. The eggs were randomly divided into 3 groups and weighed, and then washed in 1% aqueous solutions (T 37-39°C): silicone (Dergall) or chitosan (Chitozan D) preparation (ICB Pharma, Poland). The compounds were characterized by the cleaning and disinfecting properties and simultaneously creating a protective layer on the eggshell surface. The control group was not washed but only disinfected by ozonation. Next, the eggs were placed in four climatic chambers (Iglotech Kraków, Poland) and stored at 8, 12, 18, or 22° C. Analyze of physicochemical characteristics such as eqg weight; shell strength; shell deformation; egg white height; Haugh Unit; strength of vitelline membrane; yolk colour on the Rochea scale; yolk height; yolk width; yolk index; egg protein pH; air chamber diameter was taken with a texture-meter (TekstureAnalyser, TA.XT Express) and ph-meter (Mettler-Toledo, Poland) at 0, 7, 14, 21 and 28 storage days. The results were statistically analyzed using three-way ANOVA and Tukey post hoc test. There was found that chitosan increased shell strength compared to the control and silicone group (P < 0.001). Moreover, the egg white quality and strength of the vitelline membrane decreased significantly with increasing TS (P <0.05), LS (P <0.001), and the age of the flock (P <0.001). It was observed that the egg white quality of ozonated eggs was higher than those covered with silicone or chitosan (P = 0.025). In conclusion, the quality of duck eggs was significantly reduced when stored for more than 14 days at 18 and 22°C, regardless of the use of covering eggshell preparations. The storage of eggs at the temperature of 8 or 12° C allowed to maintain the right quality for up to 28 days. Covering the eggs with a silicone or chitosan layer doesn't improve the physicochemical properties of the egg during storage.

The study was financed by European Funds for the project no POIR.01.01-00-1010/17 by the National Centre for Research and Development, Republic of Poland.

EFFECT OF SODIUM BUTYRATE PROTECTED WITH SODIUM SALT OF MEDIUM-CHAIN FATTY ACIDS ON EGG QUALITY IN AGED HENS

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Laying hens face more challenges as they age (Foley et al. 2021), poor nutrient utilization and health status result in a decreased egg quality. However, many nutrient strategies are currently being studied to counter the negative effect of the late laying period hens. Among them, butyric acid and medium-chain fatty acids (MCFA) have been described as candidates for alternative non-antibiotic feed additives that can promote gut health. Besides that, the use of salt forms and their combination may contribute to protect their immediate absorption on the intestinal tract, promoting beneficial effects along the gut. In this sense, the aim of the present study was to evaluate the effect of protected sodium butyrate by sodium salt of MCFA from coconut distillates (DIC) on egg quality in aged hens. A total of 720 ISA Brown laying hens aged 52 weeks were randomly assigned to three experimental treatments (8 replicates/treatment) for 11 weeks: a basal diet (CTR), the last diet supplemented by 0.5 kg/T (0.5DIC), and 1 kg/T (1DIC). Diets, in mash form, and water were provided ad libitum. At the end of week 62 of age, seven eqqs from each replicate were sampled to measure egg quality parameters. Data was analysed using a linear model. Tukey's test was performed to determine differences between treatments. There were no differences in egg, yolk and albumen weight, neither in Haugh Units. However, the results revealed that the 1DIC group had higher total eggshell weight (P = 0.025) and thicker eggshell (P = 0.059) than the CTR group. These findings agree with different authors who demonstrate that butyric acid and MCFA participate in calcium metabolism and could increase its absorption improving egg quality (Sobczak and Kozlowski, 2016; Liu et al., 2020). In addition, changes in yolk colour (P = 0.027) and luminosity (P = 0.039) were described in 1DIC in comparison with the CTR group. It may be attributed to the better gut health status enabled by the DIC supplementation. In this sense, butyric acid and MCFA improve the absorption of nutrients as minerals and lipid-soluble carotenoid pigments (Kaczmarek et al., 2016; Zhao et al., 2019). Therefore, the present study demonstrated that the sodium butyrate protected with MCFA sodium salts improved egg guality in aged laying hens.

EFFECTS OF LENGTHENING THE LAYING PERIOD OF LAYING HENS ON EGG QUALITY

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Egg quality is influenced by various factors, such as the hen age and the egg storage conditions, which may have significant sanitary and economic consequences. Controlling egg quality is therefore a major challenge for the table egg industry. Progress in genetic selection for egg guality and production has made possible to lengthen the laying cycle of hens, which is also in line with the sustainability of laying farms. For example, the egg production period, which used to last about one year has been extended by six months (+ 150 eggs/hen). However, there is relatively little information available on the consequences of such an extension on the quality of table eggs and its evolution during storage. Results of our study on Lohmann Tradition hens reared to 101 weeks of age showed a decrease in laying rate (-30% between 45 and 101 weeks of age) and an increase in average egg weight (+7%) during the laying cycle. This increase in weight mainly results from the increase in yolk weight (+13%) and albumen weight (+7%), while the shell weight decreased slightly over the studied period (-6%). Several other quality parameters decreased significantly with hen age, notably shell strength (-24%), yolk index (-5%) and Haugh units (-7%). These livestock monitoring data are fully consistent with the expected results. Eggs from hens of 30-32, 70-74 and 94-100 weeks of age were stored from 0 to 28 days and then characterized. As expected, the storage time influences the internal egg quality, but the degradation kinetics of the internal egg quality do not seem to differ when comparing 94-100-week old hens to younger hens (30-32 and 70-74 weeks). Additional analyses are in progress (antimicrobial assays, proteomics, etc.). The results of these analyses will be integrated in order to have an in-depth view of the effects of the lengthening of the laying cycle on the overall health of hens, and on physicochemical and molecular egg characteristics.

SELENIUM AND PHYSICAL-CHEMICAL INDICATORS OF HEN'S EGG QUALITY

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Egg is a foodstuff that is nutritionally balanced, affordable and used daily in the household. The egg can be enriched with various nutricines by designing mixtures for laying hens. Therefore, the aim of this study was to produce eggs enriched in selenium at two levels, lower and higher, and to examine its effect on egg quality and freshness indicators during storage in a refrigerator at 4 °C. Physicochemical properties of eggs and TBARS values, as indicators of oxidative processes, were investigated. 120 laying hens of Tetra SL hybrids were used in the research. The experiment lasted 5 weeks. At the beginning of the experiment, the laying hens were 30 weeks old. Two groups of hens, E1 and E2 were formed, which were fed with two mixtures. Each group was housed in 6 cages with 10 laying hens. The feed mixtures fed to the laying hens differed in the content of organic selenium (Sel-Plex). Feed for laying hens from E1 group contained lower levels of selenium (0.348 mg/kg), and laying hens from E2 group consumed feed with 0.447 mg Se/kg of the mixture. At the end of the experimental period, a total of 120 eggs were collected (E1 60 eggs and E2 60 eggs). Eggs belonged to class L (egg weight of 63-73 g). The analysis of the results did not reveal significant differences between the examined groups in the eggshell strength and thickness, weight of eggs and share of basic parts in the egg (P>0.05). Selenium levels in feed and time of analysis influenced the increase in pH in egg yolk and albumen (P<0.001). The time of analysis significantly influenced the reduction of albumen height (mm) and HU (P<0.001) while the color intensity was influenced by the time of analysis and interaction (P<0.05). Lipid oxidation in egg yolks of the examined groups was uniform, no statistically significant differences in the obtained values were found (P>0.05). The level of selenium in feed mixtures for laying hens significantly affected its content in egg albumens and yolks. In albumen, the selenium content increased from 107.86 to 162.48 μ g/kg (E1 compared to E2; P<0.001) and in yolk from 644.11 to 748.05 μ g/kg (E1 compared to E2; P=0.032). The research established the possibility of increasing the selenium content in eggs by using the organic form of selenium in the feed mixture for laying hens. It was also observed that higher levels of selenium in feed have a positive effect on indicators of egg quality and freshness.

Keywords: selenium, eggs, physical properties, lipid oxidation

ALTERNATIVE SOURCES OF PIGMENTS AND FATTY ACIDS IN LAYING HENS' DIET

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Eggs are consumed all over the world as extremely nutritious products of animal origin. The aim of this study was to examine the possibility of replacing synthetic pigments, which are nowadays used in conventional egg production, with natural sources of pigments and their influence on yolk colour, as well as the possibility of changing the egg nutritive profile by adding co-extrudates rich in omega-3 fatty acids in laying hens' nutrition with the aim of functional eggs production.

The experiment was conducted on 180 Lohmann Brown laying hens for one month. Hens were divided into six treatments (2 control (C1 and C2) and 4 experimental (E1-E4)) with a total of thirty hens (six replicates with five birds in cages). The laying hens were fed with corn-soybean meal-based diet (C1 and C2). Flax-corn meal co-extrudate was added in the amount of 13.5% (E1) and 22.5% (E2), while camelina-corn meal co-extrudate was added in the amount of 16.6% (E3) and 27.6% (E4) in basal diet. C1 contained up to 3% fat without added pigments, while C2 contained up to 5% fat and synthetic pigments. The same amount of natural pigments (1% carrot and 0.5% paprika) was added in all experimental treatments. Content of fat in E1 and E3 was 3%, while in E2 and E4 was 5%.

It was found that the content of PUFAs, especially ω -3 fatty acids, was significantly higher (p<0.001) in eggs from all experimental treatments than those in eggs originated from hens fed with control diets. The ω -6/ ω -3 ratios were significantly lower (p<0.001) in treatments E1, E2, E3 and E4 (1.43, 1.01, 1.74 and 1.73, respectively) than in treatments C1 and C2 (9.40 and 8.88, respectively). The addition of co-extruded flaxseed in treatments E1 and E2 had a negative effect (p<0.05) on the sensory quality of the eggs (taste of eggs) while the addition of co-extruded camelina seed did not influence taste or smell of eggs (p>0.05). No significant changes (p>0.05) on the internal egg quality characteristics were observed for eggs from the experimental treatments when compared with eggs from control treatments. All experimental treatments achieved desirable color demanded by consumers, from 12.67 to 13.28 RYCF value (Roche Yolk Color Fan).

Hence, the obtained results showed the possibility of producing functional egg with desirable colour and fatty acid composition by adding selected co-extrudates and natural pigments in hen's diet.

Keywords: co-extrudates, camelina seed, flaxseed, natural pigment, functional eggs

EFFECTS OF CASSAVA STARCH COATING ENRICHED WITH ESSENTIAL OILS ON INTERNAL QUALITY OF TABLE EGG

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Coatings are materials that can be used to maintain microbial safety and food quality longer. Therefore, the aim of this study was to evaluate the effect of cassava starch coating enriched with different essential oils on the eggshell microbiota and maintenance of internal egg characteristics measured by the Haugh index. A total of 285 brown eggs were obtained from Embrapa 051-line laying hens (same age) and distributed in the following treatments: uncoated eggs, coated with cassava starch + Ginger essential oil, coated with cassava starch + Lemongrass essential oil and coated with cassava starch + Tahiti lemon essential oil. The cassava starch coating enriched with essential oils was sprayed onto egg surface using a hand sprayer. All eggs were stored for 35 days at 20°C. To obtain Haugh unit (HU), the logarithmic relationship between albumen height (mm) and egg weight (g) was taken into account, applying the formula $HU = 100\log (H + 1)$ 7.57-1.7W0.37). Eggs were classified as AA, excellent quality (\geq 72); A, high quality (71–60); B, average quality (59–31); and C, low quality (\leq 30). A completely randomized design was used. Statistical procedures were performed with SAS Studio University Edition (Inst. Inc., Cary, NC, USA). The data were analyzed by analysis of variance (PROC GLM), and means were compared using Tukey's test. Statistical significance was considered at p < 0.05. The total aerobic mesophilic bacteria count was significantly lower (p < 0.05) for coated eggs (mean $0.70 \pm 0.37 \log 10$ CFU/mL) than for uncoated eggs ($2.21 \pm 0.17 \log 10$ CFU/mL). Eggs on day zero had HU of 87.08 ± 2.97 (classified as AA, excellent guality). HU differed significantly (p < 0.05) between treatments from day 21 of storage. At 35 days, treatments with coated eggs had similar and higher means (mean 70.61 \pm 5.35) and were classified as A (high quality), while uncoated eggs had lower means (51.60 ± 4.28) and classified as B (average guality). Cassava starch-based coating enriched with essential oils is recommended to ensure better internal quality of table eggs during their shelf life.

PRODUCTIVE PERFORMANCE, EGG QUALITY, CHOLESTEROL CONTENT AND YOLK FATTY ACID PROFILE OF LAYING HENS FED VARIOUS DIETARY INCLUSION LEVELS OF FREE FATTY ACID

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Certain types of fat by-products, such as soybean acid oil (AO), are an example of an unconventional feed resources that present a growing interest due to market availability, competitive price, and environmental sustainability potential. AOs are fat by-products from the chemical refining processes of edible oils and are characterized by having high proportions of free fatty acids (FFA). The objective of the study was to assess the effect of the dietary FFA content on hen production performance, egg quality traits, cholesterol content and fatty acid (FA) composition of the yolk with the aim of support the nutritional value of these unconventional fat sources. The experimental diets resulted from replacing crude soybean oil with increasing amounts of soybean AO. A total of 72 Lohmann Brown-Classic hens (19 weeks of age) were randomly assigned to four treatments with 10%, 20%, 30% and 45% FFA, respectively. Each treatment had six replicates with three hens each. Throughout the 15-week experimental period, productive performance was recorded weekly. At the end of the trial, eggs were subjected to quality assessment. For statistical analysis, one-way ANOVA using the GLM procedure has been performed. Orthogonal polynomial contrasts were used to determine the linear effect of increasing inclusion levels of FFA. Increasing inclusion levels of FFA resulted in lower egg production but with a notable increase of egg weight with higher albumen, and lower yolk and shell proportions (linear, P < 0.01). No significant differences were found on average daily feed intake, egg mass and feed conversion ratio (P > 0.05). Increasing the dietary FFA% reduced the Haugh unit values linearly (P < 0.01). However, yolk colour, shell thickness, shell breaking strength, egg chemical composition (dry matter, ash, crude protein, and ether extract) and yolk cholesterol content did not differ among treatments (P > 0.05). Regarding lipid composition, increasing the dietary FFA content did not have a relevant impact on the yolk FA profile. Only a reduction in the n-3 PUFA% content was observed with the inclusion of soybean AO (linear, P < 0.001), but this was related to a lower linolenic acid content in the soybean AO added to the feed. Despite having found some differences, eggs of all treatments presented suitable quality for their commercialization. Thus, the present results demonstrate that AOs may have high potential for to be supplied as an alternative fat source for laying hens.
FATTY ACIDS COMPOSITION AND ANTIOXIDANT POTENTIAL IN EGGS OF HENS FED DIETS CONTAINING RAW AND FERMENTED RAPESEED CAKE.

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The rapeseed cake residual oil may improve the profile of fatty acids in eggs due to high concentrations of polyunsaturated fatty acids (PUFAs), unfortunately the use of rapeseed products in poultry nutrition is limited due to the presence of antinutritional factors. Fermentation can reduce the content of undesirable compounds in rapeseed feed, therefore the aim of this study was to determine the effect of raw and fermented rapeseed cake on hens4 performance, fatty acids composition and antioxidant status of eqgs. A total of 216 Hy-Line Brown layers at 36 weeks of age, kept in battery cages, were divided into 3 dietary treatment groups (36 replicates of 2 birds each). For 12 weeks, the birds were fed isonitrogenous and isocaloric diets. In the control group (C), soybean meal was the main source of dietary protein, the experimental groups were fed diets containing 20% of raw rapeseed cake (RRC) or fermented rapeseed cake (FRC). Performance was measured during the experiment. On the last day of the experiment, 10 eggs were collected from each group to determine the fatty acid profile and selected antioxidant status indicators. The differences between group means were determined by ANOVA and Tukey's test with the use of Statistica 13.1. In comparison with hens fed with RRC, birds from group C and FRC achieved significantly higher laying performance (P=0.001). Regardless of the form, inclusion of rapeseed cake in the diet for laying hens, contributed to a significant decrease in the levels of saturated fatty acids (SFA) and an increase the content of C18:2; (n-6), C18:3 (n-3) and C22:6 (n-3) in eqg yolks. Eqg yolks from RRC and FRC groups were also characterized by a significantly higher proportion of n-3 and n-6 PUFAs in total FA pool (P=0.001) and more desirable n6/n3 PUFA ratio (P=0.001). Both RRC and FRC significantly reduced SOD activity in egg yolks (P=0.001) and CAT activity was higher in group FRC than in group C and RRC (P=0.002) The content of lipid oxidation products (LOOH) increased significantly in the volks of equs laid by hens fed RRC (P=0.001). Irrespective of its form, RC had no effect on the concentrations of MDA and GSH/GSSG in eqg yolks. It could be concluded that fermented rapeseed cake, compared with raw cake, contributes to more desirable performance parameters in laying hens. The inclusion of raw and fermented rapeseed cake in diet of laying hens has a beneficial influence on the fatty acid profile and antioxidant potential of egg yolks.

Project financially supported by Minister of Education and Science in the range of the program entitled "Regional Initiative of Excellence" for the years 2019-2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN.

EVALUATION OF PRODUCTIVITY AND EGG QUALITY TRAITS IN TWO ITALIAN CHICKEN BREEDS AND THEIR CROSSBREEDS KEPT IN ENRICHED CAGE AND FREE-RANGE REARING SYSTEMS

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The diffusion of local chicken breeds is threatened by the widespread use of highlyselected commercial hybrids, which can lead to a progressive reduction of biodiversity due to the loss of genetic variability. A valuable strategy to improve the utilization of local breeds could be the crossbreeding with more productive genotypes, allowing the preservation of genetic variability while achieving acceptable productivity. The aim of this study was to evaluate productivity and eqg quality traits of two Italian chicken breeds (Robusta Maculata and Bionda Piemontese) and their crossbreeds, obtained by crossing local breed cocks with hens from a selected hybrid strain (Sasso), in respect to a commercial laying hen hybrid (Lohmann brown) raised in both enriched cage and free-range rearing systems. A total of 300 pullets (3 replicates of 10 birds/genotype/farming system) were housed at 16 wks of age both in outdoor pens and enriched cages and fed the same commercial feed. Productive performance was evaluated on a fortnight basis during the deposition cycle. At peak production, 100 eggs/experimental aroup were collected for the determination of the main quality traits, including eggshell breaking strength (EBS), Haugh index (HI) and yolk-albumen ratio. As expected, some important productive traits such as egg deposition rate and egg weight resulted higher in the commercial hybrid, which exhibited better feed conversion ratio compared to the alternative genotypes. In addition, egg deposition rate was greater in crossbreeds than in local breeds as well as in hens housed in enriched cages rather than in free-range system. Eggs from local breeds had lower egg weight as well as albumen and eggshell weight compared to crossbreeds and commercial hybrid. Local breeds and crossbreeds laid eggs with higher yolk/egg ratio and lower EBS than commercial hybrid. In addition, Lohmann brown's eggs showed the highest HI value. Other ongoing analyses will provide a more detailed overview concerning the quality traits of eggs from these local breeds and their crossbreeds. In conclusion, crossbreeding local breeds with a more productive genotype determined higher productive efficiency. Robusta Maculata and Bionda Piemontese as well as their crossbreeds produced eggs with some valuable quality traits which could be useful to valorize and conserve these Italian breeds for food production.

INFLUENCE OF DIRECT-FED MICROBIALS ON MECHANISMS OF PHOSPHORUS ABSORPTION AND METABOLISM IN CHICKENS

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About 80% of phosphorus is excreted in manure into the environment. Phosphorus excreted into the environment causes environmental pollution while adding inorganic phosphorus into poultry diets is an additional production cost. Some microbes such as Aspergillus ficus have been used to increase phosphorus absorption in poultry with a beneficial effect on production efficiency. Three probiotic bacteria, bifidobacterial, enterococcus and lactobacillus were cultured, multiplied, encapsulated, and re- introduced to the diet of 400-Day old Ross bird through the feed to enhance phosphorus absorption and utilization in poultry. 20 % of the birds were slaughtered for analysis of carcass characteristics.

The metagenomics assay of the GI tract of chicken yielded a total of 8.082 sequences, with 61% representing 5.030 species of different organisms. Massila 48% was the dominant microorganism followed by Bacteroides 9%, Streptomyces 6%, bacillus 6%, and 18 different microorganisms each having percentage lower than 5%. Probiotic bacteria did not have any significant effects (P < 0.05) on feed consumption, body weight gain, and feed conversion ratio and carcass characteristics of the broiler chicken. Also, there was no significant difference (P<0.05) between phosphorus in feed and feces. Probiotic bacteria lactobacillus and Enterococcus at the rate of 100mg/kg feed significantly (P<0.05) reduced phosphorus in the manure.

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Construction of meat quality

Selected short communications

EFFECT OF VITAMIN D STATUS ON CHICKEN GROWTH AND BREAST MEAT QUALITY

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Vitamin D status, which depends in part on the form distributed (vitamin D3 or 25-OH-D3), may influence muscle development and chicken meat quality. Our objective was to evaluate the impact of maternal and dietary supplementation of vitamin D3 or 25-OH-D3 on vitamin status, growth and chicken meat quality. Two groups of female breeders were supplemented with vitamin D3 (3,200 IU) or with 69-µg 25-OH-D3 + 440 IU vitamin D3 for 2 months. The chicks from these two groups received either 5,000 IU of vitamin D3 or 69-µg 25-OH-D3 + 2,240 IU of vitamin D3 at hatching. Four experimental groups were thus raised from hatching to 42 days. The concentrations of the two hormonal forms, [D3] and [25-OH-D3], were measured in egg yolk and plasma at different stages. [25-OH-D3] doubled in the yolk of eggs from 25-OH-D3 breeders, while [D3] was more than 3 times higher in those from D3 breeders. At hatching, chicks from 25-OH-D3 breeders had three times lower plasma [25-OH-D3] than those from D3 breeders. However, they responded better to 25-OH-D3 feed supplementation with a higher plasma [25-OH-D3] at D6. During postnatal growth, plasma [25-OH-D3] was two to three times higher in 25-OH-D3 supplemented animals than in those receiving vitamin D3. Maternal 25-OH-D3 supplementation improved the growth of animals up to 21 days. The amount of 3 proteins (FN1, NCAM, MYH15) associated with lesions observed in White Striping and Wooden Breast defects (necrosis, fibrosis, regeneration) was reduced, with a trend toward a reduction in the percentage of severe form of White Striping at D42 (from 9 to 3%, P = 0.09), in animals given 25-OH-D3 after hatching. Interestingly, the fillets of the animals that received 25-OH-D3 through maternal nutrition and post-hatch diet had a 2-point higher technological performance than the other three groups. This study indicates that the vitamin D status of animals is influenced by maternal (via egg yolk) and postnatal nutrition and may have consequences for chicken growth and meat quality.

EFFECT OF FEEDING AN OLIVE POMACE EXTRACT FROM OLEA EUROPAEA TO BROILERS ON GROWTH PERFORMANCE AND CARCASS QUALITY UNDER CHALLENGING CONDITIONS

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Selection for accelerated growth rate and high breast yield in broiler chickens has been associated with carcass quality problems. By-products from olive oil mill processing are rich in bioactive substances, including polyphenols and triterpenes, with anti-inflammatory and antioxidant properties. The present study aims to evaluate the effects of a standardized olive pomace extract (Luctalife OE poultry, OE, Lucta S.A., Spain) on performance, carcass and meat quality in broiler chickens. A total of 840 1 d-old male Ross 308 chicks were housed in floor pens (60 birds/pen) until 41 d of age. Birds were randomly distributed in 2 treatments (6-8 pens / treatment): control (CON, without OE, n = 6) and OE (1250 ppm) during the first 5 wks, n = 8). During the trial, animals were submitted to various stress factors (heat stress, high human interventions and less digestible diets). Body weight (BW), average daily gain (ADG), feed intake and feed conversion ratio (FCR) were recorded on weekly basis. At the end of the trial animals were slaughtered and 12 carcasses per treatment were used to determine carcass quality, wooden breast and white striping myopathies, evolution of lipid oxidation (TBARS) throughout display in film and fatty acids (FA) profile in the breast. Performance data were analyzed using a mixed-effects model (SAS software). Carcass and meat quality traits were analyzed using a General Lineal Model (SPSS 26.0), myopathies were assessed with Mann-Whitney tests. OE group presented a higher (P<0.05) accumulated ADG until 35 d of age compared to CON, resulting in higher BW at d28 (1735 vs 1609 g, OE and CON, respectively; P < 0.05). Accumulated FCR was lower (P<0.01) in OE compared to CON during the first 21d of age. No differences were found in carcass weight, breast yield or myopathies, but OE showed lower lipid oxidation after 8 d of display (P<0.05) even with a higher polyunsaturated fatty acids (PUFA) composition (23.4% vs 25.7%, CON and OE, respectively; P<0.01), for both n-6 and n-3 PUFA. Under the current challenging trial conditions, the use of OE showed positive effects on performance and in final carcass quality traits.

DIETARY SUPPLEMENTATION OF A VASODILATOR OR AMINO ACID/ANTIOXIDANT BLEND IMPROVE BROILER FEED EFFICIENCY AND REDUCE WOODY BREAST SEVERITY AT HARVEST

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A recent myopathy of the pectoralis major known as woody breast has become a key economic and welfare concern to the poultry industry. Woody breast causes varying degrees of rigid, pale breast fillets characterized by replacement of lean muscle protein with abnormal connective tissue growth in the live bird. This phenotype is characterized by hypoxia and oxidative stress in a muscle unsupported by adequate circulatory supply. Multiple factors may be involved in woody breast development, and interventions to prevent or reduce this myopathy are of great interest. Hence, the current work objectives were to supplement broiler diets with ingredients aimed to improve circulation and oxidative status. 1344 male Ross 708 broilers were randomly assigned to one of four diets for 49d: a basal control diet (CON), or the basal diet plus a blend of supplemental amino acids and an antioxidant (BLEND), 0.1% vasodilator ingredient (VD), or 0.002% Astaxanthin (AsX). At d14, d28, d42, and d49, performance outcomes were collected on all birds and serum from 16 broilers/treatment (n=64) was analyzed for creatine kinase and myoglobin. Once weekly beginning on d28, a subset of 192 broilers were measured for breast width, and on d42 and 49, breast fillets from 16 broilers/treatment (n=64) were palpated for woody breast severity (0-2 score), weighed, and analyzed for compression force (1d post-mortem) and water-holding capacity (2d post-mortem). Data were analyzed using Proc Mixed (SAS Version 9.4) with the fixed effect of dietary treatment. Feed conversion ratio (FCR) was higher in birds fed the AsX diet compared to CON d0-14 but was improved in both the BLEND and VD-fed birds d42-49, each by over 2 FCR points (P<0.05). Breast width was increased in the CON on d42 compared to the VD and AsX-fed broilers (P<0.05). Serum creatine kinase and myoglobin, indicators of muscle damage, were not affected by dietary treatment. Weight of breast fillets at d42 and d49 were unaffected by diet, nor were raw fillet water-holding capacity and compression force. There was a greater distribution of fillets scored 0 (normal, unaffected by woody breast) in birds fed the BLEND diet at d42 (12.5% increase), and a greater percentage of normal scores in birds fed the VD at d49 (12.9% increase) compared to the CON. These results indicate that the BLEND and VD diets simultaneously improve feed efficiency in birds approaching market weight while reducing woody breast prevalence.

REGENERATION PROCESSES IN THE PECTORALIS MAJOR MUSCLES OF BROILER CHICKENS SELECTED FOR DIFFERENT GROWTH-RATES: THE IMPORTANCE OF VIMENTIN AND DESMIN

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Intermediate filament proteins exert a relevant role in maintaining musclecytoarchitecture. Among them, desmin (DES) and vimentin (VIM) have been deeply studied in myopathic and dystrophic muscles and are considered reliable marker of regeneration. In this context, in light of the similarities existing between the growth-related abnormalities affecting broilers Pectoralis major muscles (PMM) (i.e., white striping – WS and wooden breast – WB) and human myopathic disorders, the present study aimed at quantifying VIM and DES proteins in PMM of broiler chickens belonging to a fast- (FG) and a medium-growing (MG) genotype, with the first exhibiting features ascribable to WS and WB whereas the second considered as a control group. For this purpose, a total of 30 PMM were selected along different developmental ages of the birds (i.e., at 28, 35 and 42 d) (5 PMM/genotype/sampling age) and VIM and DES guantified through western blot analysis. At each sampling, differences in VIM and DES content between the genotypes were assessed by using the non-parametric Mann-Whitney U test. Immunoblots revealed the presence of two bands: one ascribable to the native proteins (either VIM or DES), whereas the second associated to the development of an omo- or hetero-dimeric form at the Z-disk. At 28 d, a significantly higher (p<.01) VIM content was found in FG if compared to MG with the first exhibiting a 2- and a 3-fold increase in the content of the native and of the dimeric form, respectively. This finding corroborates the hypothesis of intense regenerative processes taking place within the PMM of FG as well as of mild regeneration ascribable to a physiological turnover in MG. On the other hand, no differences were observed in DES. Intriguingly, at 35 d, a remarkably lower (p<.01) VIM content was found in FG, either in the native (-63%) or in its the dimeric form (-65%). This was mirrored by a 2.5- and 5-fold increase (p<.01) in the content of the native and dimeric DES. These findings may be attributed to a progression of the regenerative processes that, at a later stage, require the synthesis of DES, replacing the pre-existing VIM-based network to support the sarcomere architecture. At last, no significant differences in VIM and DES between the genotypes were observed at 42 d. Overall, VIM and DES may be considered reliable markers for monitoring the occurrence and assessing the stage of the regenerative processes taking place in broilers PMM.

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Meat quality – slaughter and processing

Selected short communications

ELECTRICAL STUNNING OF POULTRY: INFLUENCE OF ANIMAL SEX AND WEIGHT

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Stunning in slaughterhouse is an essential stage before bleeding and allowing to maintain animal unconscious until it dies. This practice is under the regulation N 1099/2009. In France, electrical water bath stunning is the most widespread method to stun broiler chickens, for which the regulation imposes a minimum current that must be distributed to every chicken, according to the applied frequency. According to the literature, the unconsciousness of animals is better guaranteed for applied frequencies lower than 200 Hz and an intensity applied by animal above 120 mA, while products quality is better for frequencies above 1000 Hz and an intensity lower than 100 mA. However, other parameters than the electric parameters may influence stunning efficiency and the carcasses quality such as the sex of animals and their weight. In our study, the impact of the sex of the animals and their weight was evaluated in an experimental station on Ross 308 broilers stunned at electrical parameters respecting the regulations (150mA/400Hz) or used in ritual slaughter (90mA/400Hz and 90mA/600Hz).To assess the impact of sex, 216 male and 216 female chickens were slaughtered individually by electronarcosis with water bath, at 33 and 36 days of age respectively and at an average live weight of 1.8 kg. To assess the impact of animal weight, 144 male chickens with an average live weight of 1.3 kg, 144 male chickens with an average live weight of 1.9 kg and 144 male chickens with an average live weight of 2.5 kg were slaughtered individually by waterbath electronarcosis. The sex of the animals did not have a significant effect on the efficiency of stunning for any of the parameters tested. However, for the 90mA/400Hz parameter, females had more red heads than males (10/48 vs 1/48 respectively). Animal weight influenced stunning efficiency and carcass quality. Regardless of the electrical parameters applied, the heavy chickens (2.5 kg) had fewer severe class red wing tips than those in other weight categories (3/48 vs. 15/48 for the medium 1.9 kg and light 1.3 kg). In addition, with the 90mA/ 400Hz treatment, the heavy chickens after bleeding had more head (21/48, 13/48 and 4/48 for heavy, medium and light respectively) and wing (22/48, 17/48 and 7/48 for heavy, medium and light respectively) movement. With the 150mA/400Hz treatment, the chickens were all sufficiently stunned without effect of sex and weight. To conclude, with the below regulation parameters, the carcass guality of the females appeared to be more degraded than that of the males. At these parameters, the heavier the chickens were, the more signs of waking up were present and the less carcass quality was degraded.

SUSCEPTIBILITY OF POULTRY PROCESSING SALMONELLA ISOLATES TO LOW AND HIGH PH

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In various parts of the world chemical processing aids are used to reduce the levels of pathogens on raw poultry products. Among these chemical processing aids, peroxyacetic acid (PAA) is the most common and is used at different concentrations at multiple points during poultry processing. Additives are added with PAA to adjust the pH of the treatment to either low pH (≥ 2) or high pH (≤ 12). It has been suggested that continued use of high or low pH adjusted PAA has led to pH resistance. This study aimed to determine the ability of poultry processing Salmonella isolates to grow at a pH range from 2 to 12. Eleven Salmonella isolates, 5 from poultry processing wastewater, 4 from carcass rinses, and 2 laboratory research strain were used for pH susceptibility evaluation. To determine pH susceptibility, pH of Mueller Hinton Broth (MHB) was adjusted to pH 2, 4, 6, 8, 10, and 12 using HCl or NaOH. In three replicate wells of a 96-well plate, 990 µL of the series of MHB adjusted to different pH levels were added (18 wells). Then 10 µL of a 105 CFU/mL Salmonella inoculum was added to each well. The same design was used for each of the 11 Salmonella isolates. Following 24 h of static incubation at 37°C, optical density was recorded. Optical density (OD) values were analyzed using PROC ANOVA of SAS using Tukey HSD for mean separation. All Salmonella isolates were susceptible to pH 2 and OD did not differ from the negative control. At pH 4, 9 of 11 Salmonella isolates grew as well as the positive control, and 2 were more susceptible ($P \le 0.0174$). The 2 susceptible isolates at pH 4 also had a lower OD at pH 6 than the positive control ($P \le 0.0224$). At pH 8, 8 of 11 isolates were more susceptible than the positive control ($P \le 0.0486$). Increasing pH from 8 to 10 reduced OD for all 11 isolates ($P \le 0.0094$). The further reduction in OD for all isolates ($P \le 0.0067$) was observed when pH increased from 10 to 12, but not to the level of the negative control ($P \le 0.0008$). These findings indicate that Salmonella isolates collected from poultry processing wastewater, carcass rinses, and research strains are susceptible to a pH of 2 but can grow well at pH 4, and high pH (>8) significantly reduces Salmonella growth. Processing aids at low pH should be well controlled to ensure a pH of less than 2 to maximize antimicrobial effects. High pH processing aid efficacy may increase with increasing pH levels.

EFFECT OF BAKING AND GELATIN ADDITION ON 3D PRINTED FUNCTIONAL CHICKEN MEAT-BASED PRODUCT

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Three-dimensional (3D) food printing is an emerging computer-aided additive manufacturing approach used to design new food products with special characteristics not attainable by conventional processing technologies. The aim of this study was to produce a functional chicken meat-based product with a given form, flavor, shape and size using 3D food printer and to investigate the effects of 1.79% gelatin addition and baking on some product characteristics. Product formulation consisted of cooked boneless chicken thigh, chicken broth, celery root, carrot, onion, turmeric powder, garlic powder, salt, olive oil, carrot fiber and kappa-carrageenan. Gelatin was added at 1.79% (w: v) level determined in our previous study to obtain the best printability characteristics. A control group was also formulated without gelatin addition. Dynamic rheological properties (G': storage modulus, G": loss modulus) were determined before printing. The two groups of printed products were baked at 170°C for 1 h. Moisture, protein, fat and ash contents (% in dry matter), pH value, water activity (aw), instrumental color (CIE L*, a*, b* values) before and after baking, and texture profile and sensory evaluation were performed in baked products. The use of 1.79% gelatin increased G' and G'' values (p < 0.05) indicating that this group with enhanced structure was suitable for 3D printing. Baking resulted in increased protein content, and decreased pH, aw, fat and moisture contents (p<0.05). In gelatin added baked product, lower a* value, and higher b* value, protein content, hardness, gumminess and chewiness values were obtained in comparison to the control (p<0.05). The group with 1.79% gelatin possessed 30.94% moisture, and 45.61% lipid, 26.05% protein and 3.62% ash contents in dry matter. In sensory evaluation, 3D printed 1.79% gelatin containing group received higher scores than the control for all the attributes evaluated (p < 0.05). This study indicates that 3D printing enables to develop an innovative chicken meat-based product with enhanced nutritional value and acceptable sensory characteristics. In global food market, 3D food printing offers a perfect alternative to process chicken products that could drive the poultry meat industry to become more competitive. This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK-Project# 2180017).

PRELIMINARY CHARACTERIZATION AND PATHOGENESIS OF DORSAL RECUMBENCY SYNDROME IN BROILER FLOCKS FROM ONTARIO, CANADA

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A dorsal recumbency syndrome has been recently described in broiler chickens. Affected birds are flipped onto their backs and unable to right themselves, suggesting the descriptive name "turtle chicken". Previous reports suggested that breast myopathies may be associated with this syndrome, as a cause of impaired wing movement (necessary for the upright position). Breast myopathies are characterized by myodegeneration, inflammation, and scarring of the pectoral muscles. In this study, we aimed to characterize the clinical, pathological and serum chemistry changes of affected broilers. Between March and October 2020, 18 recumbent and 18 unaffected broilers (Ross 708) were sampled at approximately 45 days of age from a commercial farm. Clinical signs were recorded, serum was collected, and birds were euthanized for postmortem analysis and histopathology. Detailed sampling from the Pectoralis major of 24 birds (13 affected, 11 unaffected) were scored histologically to determine the presence and severity of myopathies. Serum from 4 affected and 4 non-affected chickens was submitted for a full chemistry profile, while the remaining 32 samples were used to detect the levels of aspartate aminotransferase (AST) and creatine kinase (CK). Student's t test and Wilcoxon rank-sum test were used to compare the amounts of serum analytes and histological scores between affected and unaffected birds (p < 0.05). Sampled flocks were mixed-sex, and the live weight at slaughter was approximately 2.8 kg. All recumbent birds were bright and alert, could move the legs but had difficulties moving their wings. Hydropericardium was observed in 38.4% and 18.2% of affected and unaffected birds; right ventricular hypertrophy was observed in 23.1% and 9.1% of affected and unaffected birds. Compared to unaffected birds, recumbent birds displayed a higher severity (p < 0.05) of myodegeneration, inflammation, and accumulation of fibro-fatty tissue in the P. major muscle. The level of AST and CK in recumbent chickens was higher (p < 10.05) compared to unaffected birds, suggesting muscular damage. In conclusion, serum chemistry and histopathological changes in the P. major suggest an association between breast myopathies and dorsal recumbency syndrome. Recumbent birds had a higher frequency of hydropericardium and right ventricular hypertrophy, suggesting these lesions may lead to death of recumbent birds. Keywords: biochemistry; breast myopathy; broiler; cardiomyopathy; histopathology

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Meat quality

Posters

UNDER INDIAN SCENARIO, STUDYING IMPACT OF TRANSPORT DURATION ON BROILER BIRD'S MEAT QUALITY

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Many studies find that the quality of meat will be completely deteriorated due to stress faced by the birds during transport or due to feeding withdrawal before slaughter. Especially in developing countries like India, where a tropical environment, as well as poor transport facilities, makes the condition worse. This tends to hamper sensory qualities such as color, appearance, flavor, texture, juiciness, & overall acceptability while physical meat quality attributes such as Shear Force Value (SFV), pH value of meat and drip loss. To find the effect of increasing transportation time and its impact on the meat quality of broilers the experiment was executed with 480 marketable commercial broiler chickens which were transported for 0, 2, 4 and 8h. A control group was kept without transport in crates. For the parameters study, a total of ten breast meat samples were taken from birds from each treatment group after slaughter. We found that different transit duration had no significant (P>0.05) impact on the SFV ie. Tenderness and drip loss. Variation in transport time had a high significant (P < 0.001) effect on the pH of the muscles. The pH was higher (6.22) in 8h transport group (T4) which will lead to poor keeping qualities. All the meat sensory qualities were significantly (P<0.05) affected except flavor, by transport time of the broiler birds. Birds that are transported for a longer duration exhibit poor sensory meat quality parameters, which possibly affect the consumer preference (Scoring was 5.4, 5.2, 5.4 and 5.6 for appearance & color, texture, juiciness, and overall acceptability respectively). Transport duration of 8h had a significant (P<0.05) impact on body weight loss. Finally, it can be concluded that a transport time of more than 4h is not recommended under Indian conditions as it directly affects the meat quality parameters.

EFFECT OF FEEDING AN OLIVE POMACE EXTRACT FROM OLEA EUROPAEA TO BROILER CHICKENS ON GROWTH PERFORMANCE AND CARCASS QUALITY

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Selection for accelerated growth rate and high breast yield in broiler chickens has been associated with carcass quality problems. By-products from olive oil mill processing are rich in bioactive substances, including polyphenols and triterpenes, with anti-inflammatory and antioxidant properties. Previous studies using a standardized olive pomace extract in broiler diets reported improvements in performance, gut integrity and carcass guality when animals were challenged. The present study aims to evaluate the effects of a standardized olive pomace extract (Luctalife OE poultry, OE, Lucta S.A., Spain) on performance and carcass quality in broiler chickens without any controlled challenge. A total of 992 1 d-old male Ross 308 chicks were housed in floor pens (62 birds/pen) until 41 d of age. Birds were randomly distributed in 2 treatments (8 pens / treatment): control (CON, withoutOE) and with OE (500 ppm, during all experimental period). In this study animals were not challenged. Body weight, average daily gain, feed intake and feed conversion ratio were recorded on weekly basis. At the abattoir 100 carcasses per treatment were evaluated for incidence and severity of myopathies (white stripping, WS; wooden breast, WB; spaghetti meat, SM), and other 16 carcasses per treatment randomly selected were used to determine general carcass quality traits and lipid oxidation evolution under high oxygen modified atmosphere during display for 12 days (TBARS). Performance data were analyzed using a mixedeffects model with repeated measures and incidence of myopathies analyzed using a non-parametric Wilcoxon test (SAS software, v.9.4). Carcass guality traits were analyzed with a General Lineal Model (SPSS 26.0). No differences in performance were observed between treatments. At slaughterhouse, a decrease in the incidence of total myopathies was observed in OE compared with CON (63% and 77%, respectively; P=0.03), and also OE tended to have a lower total incidence of WS than CON (55% and 68%, respectively; P=0.06). A significant treatment per display interaction was observed in lipid oxidation (P<0.01), where OE presented lower TBARS compared to CON after 4 days of display (0.049 vs 0.058 mg malondialdehyde/kg muscle, P<0.01). Under the current trial conditions, the use of OE improved carcass quality, reducing the incidence of myopathies and slowing down meat lipid oxidation at short display times.

THE EFFECT OF LIGNOCELLULOSE SUPPLEMENTATION IN DIETS ON MEAT QUALITY AND ANTIOXIDATIVE STATUS UNDER HIGH STOCKING DENSITY CONDITION OF BROILER CHICKENS

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Objective

Fast growing broilers in commercial farms are stressed throughout their short live time, e.g. by sup-optimum housing conditions, an unphysiological rapid growth or high stocking densities. These internal and external stressors negatively affect the growth performance and meat quality and are thus of economic relevance. This study tests the hypothesis that a supplementation by lignocellulose (LC) an improved stress resilience. Material contributes to and methods A total of 294 one-day-old male Ross308 broiler were kept under controlled conditions either under normal or high stocking density (13 or 18 birds/m²) for a 35-day trial duration. Birds were fed a basal diet based on corn and soybean meal. The birds were allocated randomly to 3 treatments \acute{a} 6 replications: NSD: standard diet; normal stocking density; HSD: standard diet; high stocking density; LIG: standard diet supplemented with 8 g LC/kg feed on top (OptiCell®; agromed Austria GmbH); high stocking density. As expected, a high stocking density caused a reduction in growth performance: Final body weight of 2.53 kg observed for HSD birds was significantly lower than in NSD group (2.69 kg; p=0.027). A supplementation of LC tended to increase the final body weight (2.66 kg; p=0.075). No differences in FCR were observed in between the treatments, however meat quality was strongly affected by both stocking density and LC supplementation: water holding capacity of breast meat expressed in the parameters of drip loss, cooking loss and thawing loss was significantly impaired in HSD compared to NSD (p < 0.05). LC supplementation compensated the negative effect of high stocking density, as all mentioned parameters were significantly improved in LIG when directly compared to HSD (p<0.05). Moreover, shear force values as measurement of meat's tenderness were significantly lower in NSD and LIG (8.56 N and 8.54 N, respectively) compared to 11.02 N of HSD (p<0.01 each). These results are in concordance with a reduced Thiobarbituric Acid Reactive Substances (TBARS) concentration of 0.19 and 0.18 mg/kg in breast meat for NSD and LIG, respectively, compared to 0.38 mg/kg in HSD. Statistical analysis was performed using SAS University Edition (SAS Institute Inc., 2021). Data of this study suggest a potential of the used LC to improve meat quality and counteract negative effects of stress exposition in broilers. A reduction of TBARS concentration give proof of the anti-oxidative mode of action.

INVESTIGATING DIFFERENCES OF PECTORALIS MAJOR MUSCLE VIMENTIN AND DESMIN GENE EXPRESSION BETWEEN BROILERS SELECTED FOR DIFFERENT GROWTH-RATES

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Vimentin (VIM) and Desmin (DES) genes encode for homonymous proteins with pivotal roles in maintaining cytoskeletal stability of muscle fibers, both in humans and chickens. Recent studies have evidenced that VIM and DES proteins can be considered reliable markers of the regenerative processes taking place within the Pectoralis major muscle (PMM) of modern broilers affected by the growth-related myopathies, known as White Striping (WS) and Wooden Breast (WB), but also in those having macroscopically normal appearance. In this context, the present study aims at investigating the VIM and DES gene expression looking at multiple steps of PMM development in broilers belonging to both fast- (FG) and medium-(MG) genotype, considering the latter as a control group in light of its lower growth rate. In particular, this work was designed to evaluate differences in VIM and DES normalized transcript levels between FG and MG at different stages of breast muscle growth. For this purpose, chicken PMM samples (5/each genotype) were collected at 28, 35, and 42 days of age (d). gRT-PCR of VIM and DES mRNA was performed using RPL4 and GAPDH as normalizing genes. At each sampling time, differences between genotypes in VIM and DES mRNA guantification were assessed by using the non-parametric Mann-Whitney U test. The VIM gene showed an expression level higher in FG than in MG broilers at 35 d (P<.05), and the difference was close to being significant at 28 and 42 d (P=.09). DES mRNA level was significantly higher expressed in FG than in MG broilers at 28 d (P<.05) and 42 d (P<.05). At 35 d, no significant differences in DES transcription level were observed. The present results agree with our previous findings by evidencing an increased expression of these genes in FG vs MG. The outcomes confirm the role of VIM and DES as markers of muscle regenerative processes, in the light of the higher amount of VIM and DES mRNA in FG compared with MG. Taking into account the progression of muscle development at different stages, these findings highlight that muscle regeneration takes place in FG broilers already at least from 28 d. It is widely known that differences in the expression level of DES and VIM proteins and coding genes can be ascribed to a progression of the regenerative process, since DES filaments replace the pre-existing VIM network during muscle development. Accordingly, a progression of the muscle regeneration process from 28 to 42 d in FG might be hypothesized.

DEVELOPMENT OF A QUANTITATIVE ELISA FOR A CHICKEN SPECIFIC TROPONIN-T PEPTIDE IN SKELETAL MUSCLE TCA EXTRACTS WITH POTENTIAL USE IN FOOD INDUSTRY

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Proteolytic degradation of muscle during postmortem aging, results in the production of protein fragments that may affect meat qualitative specifications. Troponin T (TnT) is an important regulatory and structural component of skeletal muscle thin filaments and particular TnT fragments represent a widely reported marker of meat aging in different animal products intended for commercial distribution in the food industry. Based on our previously reported competitive enzyme-linked immunosorbent assay (ELISA) for the quantification of TnT (16-31) fragment identified in trichloroacetic acid (TCA) soluble beef skeletal muscle extracts, we developed herein the respective ELISA for chicken specific TnT peptide. We used a synthetic 21aa TnT chicken-specific peptide (TnT-21), previously identified by Wei Z., et al. and related to meat quality characteristics. High affinity polyclonal anti-TnT-21 antibodies were generated in rabbits and isolated from total IgG on a TnT-21 immunoadsorbent. Affinity-purified anti-TnT-21 antibodies were used for coupling with HRP, using glutaraldehyde. A competitive ELISA was developed based on inhibition of binding of the labeled antibodies to immobilized TnT-21 by soluble TnT-21 peptide at serial dilutions (standard curve). Twenty post mortem skeletal muscle extracts (10 Ross 508 genotype chickens and 10 Sasso genotype chickens) were produced according to our previous reported TCA extract protocol and used to evaluate the specificity and sensitivity of our method. We developed a sensitive and specific ELISA for the detection of TnT peptide fragment in a useful range 20pmol to 3nmol TnT-21/ml of aqueous solution. Our preliminary data indicate the presence of TnT-21 peptide in all TCA extracts, ranging from 300pmol to 2nmol/ml depending on the extract origin. The peptide concentration was significantly higher in extracts derived from Ross 508 genotype chickens although further analysis with larger sample size and an aging standard protocol are needed. Conclusions: Our guantitative competitive ELISA for specific TnT-21 fragment in range poultry will be further used for the correlation analysis between TnT-21 concentration and gualitative characteristics of the meat during postmortem aging. This guantitative ELISA may prove advantageous for future use at both research and industrial level.

CAN THE DIFFERENT COOKING METHODS AFFECT THE MEAT PROXIMATE COMPOSITION IN BROILER CHICKENS FED LIVE INSECT LARVAE AS ENVIRONMENTAL ENRICHMENT?

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Introduction. Cooking methods have been reported to influence nutrient composition of meat and fish [1], but no information is available in poultry. Since insect-based products may affect meat quality in broilers [2], this study investigated the effects of Tenebrio molitor (TM) and Hermetia illucens (HI) live larvae as environmental enrichment on proximate composition of cooked meat in broiler chickens. Material and methods. A total of 180 4-day-old male broiler chickens were randomly allotted to 3 groups (6 pens/treatment, 10 birds/pen): a) control group (C, commercial feed), b) HI group (commercial feed + HI live larvae) and c) TM group (commercial feed + TM live larvae). At 38 days of age, 2 birds/pen were slaughtered, and right and left breasts were collected. Breasts were divided in three portions by performing two cuts: i) cranial, ii) intermediate, and iii) caudal. Cranial and caudal portions were submitted to 4 cooking methods (oven [O], bainmarie [BM], plate [P], grill [G]) following a randomization model. Proximate composition (dry matter [DM], crude protein [CP], ether extract [EE], ash) of raw (intermediate portion) and cooked meat was evaluated. Data were analyzed by GLM (SPSS software, P<0.05). Results. The P and G cooking overall led to higher meat DM, CP and ash contents than the other methods (P<0.001), thus reflecting the increase in nutrient concentration due to the highest moisture losses [3]. Live larvae administration did not influence the proximate composition of raw meat (P>0.05), while, independently of the cooking method, the breast of the larvaefed broilers showed decreased ash content (P<0.001). The breast of the larvaefed birds also displayed lower DM, CP and ash contents than the C group when submitted to O and P cooking, with BM decreasing ash content as well (P < 0.05). These outcomes were reasonably related to the different nutritional profile and predominant water content of the insect larvae. Differently, no significant differences related to diet, cooking method and their interaction were underlined for EE content (P>0.05). Conclusion. Cooking methods may influence meat proximate composition in broilers as a consequence of the differences in moisture losses, also exacerbating the impact of the different nutritional profile of the insect larvae.

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QUALITY TRAITS OF CHICKEN BREAST MEAT OBTAINED BY ITALIAN LOCAL BREEDS AND THEIR CROSSBREEDS

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The production of poultry meat and eggs is currently almost solely based on high performance strains reared in intensive farming systems with increasing concerns for biodiversity, animal welfare and product quality. Thus, lately, there has been a renewed interest towards local breeds (LB), whose utilization can preserve genetic variability while fulfilling consumers' expectations in terms of animal welfare perception and product quality. Due to their slow growth rate, LB usually exhibit poor productive performances, facet that may be improved through crossbreeding. Within this context, this study aimed at evaluating the main quality traits and technological properties of male chickens' breast meat of two Italian LB (Bionda Piemontese, BP and Robusta Maculata, RM) and their crossbreeds obtained by crossing LB cocks with hens from a selected hybrid (Sassò, S), in respect to a commercial broiler hybrid (Ross 308, R308). The obtained crossbreeds (BP×S and RM×S) and their relative LB were reared in an indoor farming system and slaughtered at 86 d of age, while R308 chickens were sacrificed at commercial age (42 d). For each experimental group, six breasts (P. major muscles) were selected and used to assess proximate composition, technological properties (pH, color, and water holding and binding capacity) as well as oxidative profile (TBARs and carbonyls). Data were analyzed through One-Way ANOVA, considering the genotype (R308, BP, RM, BP×S and RM×S) as the main effect. Overall, if compared to their respective LB, BP×S and RM×S did not show any remarkable difference neither in terms of technological properties nor of chemical composition. However, when compared to R308, the LB and their crossbreeds exhibited improved water holding and binding capacities as proven by the significantly lower (P<0.001) cooking losses and higher (P<0.001) marinade uptake. As concerns proximate composition, with respect to R308, alternative genotypes showed lower moisture and higher collagen content (P<0.05), the latter likely due to their higher slaughter age. Muscles from LB and their crossbreeds also showed significantly lower TBARs concentration (P<0.05) if compared to R308. In conclusion, despite of their slow growth rate, male chickens from selected LB and their crossbreeds showed guite favorable meat quality traits, and their possible use for meat production could represent an important step towards the conservation and utilization of indigenous breeds.

COMPARATIVE MEAT QUALITY ASSESSMENT FROM FAST-GROWING AND SLOW-GROWING CHICKEN.

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During the last few years, alternative slow-growing chickens' production has increased in response to consumer's pressure about fast-growing conventional broilers. Nevertheless, the meat characteristics from these new chickens has been less studied than that from broilers. The aim of this work was to compare meat quality parameters from a commercial fast-growing broiler and a slow-growing genetic. A total of 696 one-day-old chicks from each of the following treatments were housed in 36 boxes (58 birds/pen): Ross 308 males, Ross 308 females, and mixed Ranger Classic (RC) males and females. Two ages of slaughter were defined in the three groups (44 and 57 days). Animals that were slaughtered at 44 days were fed with a fast-growing conventional diet versus the slow-growing diet that 57 days animals received. RC carcasses were sexed at the processing plant after slaughtering. Twelve randomly carcasses were sampled from each genetic and sex at each slaughter age 24 hours later (making a total of 8 treatments) and the dissected. Colorimetric parameters with 600d breast was а Minolta spectrocolorimeter were evaluated in fresh meat, and proximate composition and texture with a TA-XT2i texturometer were assessed after freezing. Effects of genetic, sex and their interactions were analyzed with a General Lineal Model (SPSS 26.0). Ross 308 meat had less dry matter with less protein percentage than RC meat (20.99 vs 22.86%; $P \le 0.001$). However, the intramuscular fat percentage was higher in Ross 308 animals (2.37 vs 1.53%; $P \le 0.001$), showing male Ross 308 the highest intramuscular fat of all treatments at an older slaughter age $(3.16\%; P \le 0.05)$. RC breast was darker (L* 53.2 vs 55.7; P \le 0.001), with lower redness (a*-0.90 vs -0.15; P \leq 0.001) and yellowness (b* 5.80 vs 8.38; P \leq 0.001), with less saturation (5.9 vs 8.4; $P \le 0.001$) and more Hueo (99.2 vs 91.5; $P \le 0.001$) than Ross 308. The R630-R580 index as a good indicator about less myopathy's prevalence was lower in RC, females and younger animals. However, RC meat was more tender (8.32 vs 11.46 N; P \leq 0.001), gummier (3.54 vs 4.45 N/cm2; P \leq 0.05) and more cohesive (0.416 vs 0.380; $P \le 0.001$) and elastic (0.621 vs 0.569 cm; $P \le 0.001$) than Ross 308 meat. Younger animals and females showed less adhesive and more elastic meat too ($P \le 0.001$). It is important to continue with a line of research that allows improving animal welfare with new genetics but also ensuring the improvement of final product quality.

ORGAN AND REGIONAL COMPOSITION, PH AND WATER HOLDING CAPACITY OF FAST-GROWING AND SLOW-GROWING CHICKENS

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Interest in alternative chicken's production has increased in recent years as an answer to consumer's demand for better animal welfare conditions. It is necessary to know the final product characteristics obtained with these alternative systems in comparison with current fast-growing systems. In this study, we have assessed organ composition, carcass and meat quality from commercial fast-growing broilers and a slow-growing genetic. A total of 696 one-day-old chicks from each of the following treatments were housed in 36 boxes (58 birds/pen): Ross 308 males, Ross 308 females, and mixed Ranger Classic (RC) sexes. Two ages of slaughter were defined (44 and 57 days). Animals that were slaughtered at 44 days were fed with a fast-growing conventional diet versus the slow-growing diet that 57 days animals received. RC carcasses were sexed at the processing plant after slaughtering. Twelve randomly carcasses were sampled from each genetic and sex at each slaughter age before eviscerating (making a total of 8 treatments). Evisceration was carried out manually with the hot carcass and organs were weighed. Regional composition was assessed in cold carcasses 24h later. In other 12 clean carcasses per treatment, the breast pH was measured, and drip loss was assessed after 48h. Genetic, sex and their interactions were analyzed with a General Lineal Model (SPSS 26.0). RC carcasses were 1kg lighter but with higher abdominal fat, gizzard, spleen, neck, wings, and skin percentages than Ross $(P \le 0.001)$. Abdominal fat percentage increased with age in RC but did not in Ross $(2.29 \text{ vs } 2.64\% \text{ in RC}; 1.90 \text{ vs } 1.72\% \text{ in Ross}; P \le 0.05)$. Gizzard percentage decreased with age, at a higher rate in RC (1.00 vs 0.74% in RC; 0.70 vs 0.61% in Ross; $P \le 0.001$). The breast percentage of the whole carcass was 4 points lower in RC than in Ross (24.90 vs 28.97%; P≤0.001), without significant differences between both sexes in RC. Nevertheless, thigh percentage was lower in females of both genetics. Ross showed higher pH (5.92 vs 5.82; $P \le 0.001$) and drip loss (2.01) vs 1.72%; P \leq 0.05) than RC. In addition, RC showed a lower incidence of low pH meat (2.1 vs 20.8 %). Slow-growing genetics produces less meat at the same slaughter age than fast-growing animals, with a lower breast development, higher fatness and better meat quality indicators. Therefore, it is important to continue with a genetic selection that allows improving quality parameters with these new genetics without penalizing productive data.

CANNABIS-DERIVED CANNABIDIOL IMPROVES BREAST MEAT QUALITY IN CHICKENS SUBJECTED TO DIFFERENT CHALLENGE MODELS

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Based on the previous research results, it may be assumed that cannabidiol (CBD) may have a beneficial influence on the mechanisms regulating the immune system of birds, and therefore may reduce the harmful effect of stress factors on the physicochemical properties of chickens' meat. The experiment was conducted on 204 Ross 308 broilers divided into 6 treatment groups, 34 birds each, according to the scheme:C (control group);C+CBD (cannabidiol added to feed, 30g/kg feed);C+LPS (birds infected with E.coli lipopolysaccharide administered per os at a dose of 250µg/k body weight);C+C.perfringens (birds infected with C.perfringens bacteria dose exceeding108CFU); at а not C+CBD+LPS;C+CBD+C.perfringens(respectively: infection model as above+CBD dietary supplementation). At 42 days of life, 10 birds were selected from each group and dissected to obtain breast muscle samples for the analysis. The basic chemical composition of the breast muscle was determined with standard methods. Twoway multivariate analysis of variance (MANOVA) was applied to determine the effect of CBD dietary supplementation on dressing percentage and broiler meat quality. There was noeffect(P>0.05) of CBD in the diet of chickens on the slaughter value of birds, which ranged from 69.73 to 71.22 %. A significant correlation was found in the chemical composition of the breast muscles.CBD supplementation significantly (P < 0.01) decreased content of collagen in the C. perfringes subgroup, reduced water, and increased fat in the analysed breast muscles in Csubgroup. Our findings confirm that since a mild infection with eitherC.perfringensor LPSendo toxin may occur without causing visible abnormalities in the muscle tissue, such meat could stand as a potential carrier of pathogens to the food chain. The addition of CBD in the diet of chickens alleviated the consequences of adeteriorated health status of birds, increasing the collagen contentin the breast muscle tissue,

with no adverse effect on selected slaughter values, yet beneficial impact on individual parameters of the chemical composition of the breast muscles.

Grant No.2018/29/B/NZ9/0135

26th World's Poultry Congress, abstracts selected in 2022

Education

Selected short communications

VICKY THE VIRTUAL CHICKEN

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As part of Poultry Hub Australia's training initiative, we have developed a virtual chicken experience. 'Vicky' the virtual chicken allows users to experience all parts of the chicken from inside out. This project aims to provide not only an interactive learning tool but also an engagement tool that will facilitate a greater understanding and interest in the Poultry Industry.

The experience is run through virtual headsets and responds directly to the user ensuring each experience is unique. Both meat and egg chickens can be explored separately allowing the user to experience what each bird looks like inside. It is possible to explore different layers of the chicken such as organs, skeletal, muscle, separately just as you would if doing a live dissection. The meat chicken focuses on the different cuts of meat and the laying hen highlights the reproductive system allowing users to explore where an egg is made. There is also a testing function that asks the user to select a specified part of the chicken with tweezers and place on the tray, providing direct feedback to the user on a screen that indicates if they selected the correct part. The testing function can be played in a challenge mode providing an output score and printable certificate. The testing function collects metrics that may be used by an assessor to evaluate a user's competency.

This interactive tool has applications across all levels of education. We have already received fantastic feedback from users within Australia and we look forward to bringing this resource to the international poultry industry and public.

THE VALUE OF VIRTUAL EDUCATION IN INFLUENCING PRACTICE CHANGE

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Effective extension programs use different approaches depending on the stage of adoption (awareness, interest, evaluation, trial, adaptation, adoption) for new technologies. Mass events are effective to raise awareness of new information. In 2020/21 in-person extension events have been limited, virtual events became the norm. Maintaining an effective extension program that produces the desired results has been challenging. A free monthly 1h webinar series was created in February 2021 by the Poultry Innovation Partnership (PIP) to raise awareness of cutting-edge poultry research and influence practice change amongst producers. Several methods were used to facilitate engagement and learning including greeting guests, providing Q&A sessions and providing downloadable presentation copies so participants can follow along. Webinar series effectiveness was evaluated on attendance metrics (registration and attendance, number of questions asked and time in the webinar) and post-event surveys. Attendees are automatically directed to the survey when they leave the webinar. To date, 11 webinars have had 431 total attendees; attendees were present for 79.6% of total webinar time. 100% of attendees would attend another PIP webinar. Average registration was 64.9/event with 39.2 attending (59.2%) with 8.8 questions/event. 159 attendees have completed the post event survey; 32.8% are producers with an additional mixture of industry, government and academia. Producer respondents were asked how well they understood the topic before and after the webinars; 31.3% were not at all familiar, 56.3% were somewhat familiar and 12.4% were very familiar. Postevent 51.7% were somewhat familiar and 48.3% were very familiar, indicating that the goal of increasing awareness was met. 74.1% of producers indicated that they intended to or would consider making changes on their farm due to the webinar, indicating that practice change is possible using webinars. Interestingly, producers were asked to rate the value of the webinars using a sliding scale between 0-\$100. The average value to producers was \$56. A subsequent question asked if attendees would pay to attend similar webinars (\$0, 15, 25, and 35). 60.7% of producers would not pay, 28.6% would pay \$15, 10.7% would pay \$25 and 10.7% would pay \$35. As funding for extension services diminishes, bridging this gap between perceived value and willingness to pay is a key requirement for extension professionals.

A NEW GLOBAL POSTGRADUATE COURSE IN POULTRY HEALTH SCIENCES: E-LEARNING SUPPORTED WITH ONSITE PRACTICAL TRAINING

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Advanced training courses and workshops enable employees of veterinary practices, pharmaceutical companies, animal feed and animal feed additive companies, research institutes and universities to further develop their expertise in poultry health sciences and subsequently improve their career prospects in the poultry sector. However, most short technical trainings are limited in time and focus only on the newest trends in poultry health issues. Therefore, this postgraduate course was developed by the chair of Poultry Health Sciences to offer an in-depth educational program to educate fundamental and applied knowledge, to bridge the gap between research and field expertise. This new postgraduate in Poultry Health Sciences of Ghent University is a three years program offered as an online course supported with in total 5 weeks of onsite practical training and 2 weeks of externship. By combining this online course with short periods of onsite practical trainings, students will be able to acquire both in-depth theoretical and practical knowledge. In the first year (10 ECTS credits), students will learn basic avian sciences: embryology, anatomy, physiology, immunology, breeding & genetics; poultry production, management, husbandry and biosecurity, and gut health & nutrition. In the second year (10 ECTS credits), students will acquire knowledge and skills in the field of diseases of poultry, treatment and prevention, and scientific methods & reasoning. In the last year (10 ECTS credits), students will gain important knowledge and skills in the field of poultry behaviour & welfare, and food safety. Finally, during an externship the student will be able to apply his/her acquired knowledge in practice. This postgraduate course is designed to suit those in continuing employment or with other commitments. Courses are organized as instructor-led and facilitated e-learning in the period October – April. Students will be able to study and review course materials during their own time. Practical exercises are offered at a set time in enterprises in situ though, within periods of one week once or twice per academic year (AY). The programme in Europe and Asia started in AY 2021-2022. In the AY 2022-2023, the programme will start in Latin America, with onsite practical exercises in Brazil. Finally, in the AY 2023-2023 the programme will also start in Middle-East / North Africa.

QUALIFICATION AND CONTINUING EDUCATION FOR PEOPLE WORKING IN THE POULTRY SECTOR

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The framework of the poultry production is constantly changing. New production systems and techniques, an increasing importance of animal welfare as well as management tools and the current level of scientific knowledge are challenging the competences of people who work with poultry at different stages of production. Furthermore, qualification and continuing education in order to approve, expand or renew knowledge becomes more and more important in quality assurance schemes (e.g. supplier contracts) and is even required e.g. in the German Animal Welfare Act. Therefore, on the one hand people are 'required' to participate in further training and on the other hand people participate voluntarily in order to improve their competences. Thus, continuing education – and the certificates to validate that it has happened - is therefore mandatory for those involved in the poultry industry. This emerging demand opens the discussion for a closer integration of vocational training, continuing education and advanced further education. Presently, the "in" catchwords are "Dual Course Studies" and "Open Universities". Within a project, funded by the German Federal Ministry for Education and Research (BMBF), the University of Applied Sciences Osnabrück founded the "Osnabrücker Poultry Academy" (OPA) which worked out a concept that may meet all demands and requirements and may be best explained using the example of the certificate course "Poultry Professional" (PP). The PP is an onthe-job certificate course targeting people working in the poultry sector who want or need to approve, renew or expand their knowledge and skills. The course, which comprises 300 hours workload and covers level 5-6 of European Qualification Framework (EQF), is designed as a blended learning education program combining online digital media with traditional classroom methods. The gained certification "Poultry Professional" and acquired knowledge must be maintained by collecting 20 continuing education credits (CEPS) within 2 years. Therefore the "Poultry Professionals" have state-of-the-art knowledge. However, the same program can also be completed on EQF level 6-7 which includes further examinations and workload (600 hours). Continuing education offers quality assurance in the whole production chain and addresses inter alia the sensitive issues of food safety and animal welfare and thus, may promote the public image of the poultry industry.

NATURAL PREVENTION AND TREATMENT FOR NECROTIC ENTERITIS IN BROILERS WITH CIMENOL RING

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Necrotic enteritis (NE) is one of the major diseases that affect poultry farms globally and is caused by Clostridium perfringens (CP). Cimenol ring (CR) is an active molecule from plant extracts with a strong antimicrobial effect against CP. A trial was conducted to evaluate whether CR is effective as a prevention and as a treatment for NE in experimentally challenged broilers. 480 one-day-old broilers were raised for 40 days and distributed into 4 groups with 6 replicates each. Treatments were an uninfected healthy control (NC); an infected control (PC); an infected group with CR as a treatment in the drinking water at 1 ml/l for 7 days post-infection (CR1); and an infected group with CR as a prevention in the feed at 0.5 kg/t continuously during all the trial (CR2). NC and PC did not receive any antimicrobial. CP was administered to all birds in the infected groups on days 14, 15 and 16 of age. Results were statistically analyzed, and P values below 0.5 were considered statistically significant. On day 21, PC and CR1 obtained significantly lower weights than NC and CR2 due to the infection. The growth rate in CR2 was like that of healthy birds (NC). At the end of the trial, CR1 and CR2 obtained significantly higher weights than NC and PC, since CR promotes the balance of the gut flora and prevents CP overgrowth. Feed conversion rate was also better in the groups with CR at the end of the trial, compared with NC and PC. Significant differences were observed between CR1 and the control groups NC and PC. In week 3, mortality increased in PC and CR1 (P<0.05) but remained low in CR2. In week 5, mortality was similar (P>0.05) in CR1 and CR2 compared to that of healthy birds in NC, which indicates that CR succeeded in controlling the infection, and significant differences were observed between NC and PC only, the latter with the highest mortality. Log10 CP counts per gram of caecal content on day 17 were 0.73, 4.85 and 1.93 in NC, PC and CR2 (prevention), respectively, with significant differences between all of them. In terms of number of CFU/q of caecal content, there was a 61% reduction of CP counts in CR2, compared to CR1. In summary, CR works as a prevention and as a treatment for NE as it prevents the negative consequences on performance results. Besides, CR obtains good results in challenged birds, like those of healthy birds, thanks to promoting the balance of the gut flora. Besides, CR is natural, does not create resistances nor needs withdrawal period.

26th World's Poultry Congress, abstracts selected in 2022

Feedstuffs

Selected short communications

MACRO ALGAE ULVA RIGIDA AND SOLIERIA CHORDALIS PRODUCTS IN BROILER DIETS: IN VITRO AND IN VIVO DIGESTIBILITY AND HEALTH RELATED EFFECTS

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Novel feed ingredients for the production of meat, milk and eggs are needed, which do not compete for currently used resources. Seaweed could be such a novel feed ingredient. This study aimed to determine nutrient digestibility and health-related effects of desalted seaweed co-products, and evaluate the effects of enzymatic hydrolysis (proteases Alcalase and Neutrase) on nutrient digestibility. A 2 \times 2 experimental design (seaweed speciesxenzymatic hydrolysis) plus added control was conducted. In total, 360 14-day old Ross308 male broilers were housed in 30 floor pens (0.96m2) with 12 birds per pen and 6 replicate pens per treatment. The basal diet (control) was diluted with 100 g/kg of one out of four seaweed products. Feed and water were available ad libitum and intake and bird weight were measured weekly. Faeces was collected qualitatively at pen level at d 26-27-28. At d 28, birds were euthanized and ileal digesta was collected. The villus height: crypt depth ratio (VL:CD) was analysed in duodenal tissue, IL13 and haptoglobin levels were analysed in blood plasma and jejunal content pH and gizzard weight were measured. Data were analysed using ANOVA with pen as experimental unit and significance stated at P=0.05. Body weight was not affected, but the feed conversion ratio (d14-21) was lower in birds fed untreated U. laetevirens (1.77) vs all other diets (1.84-1.94, P = < 0.001). For all nutrients, the apparent pre-caecal digestibility of the basal diet vs seaweed containing diets was higher (P<0.001), with a higher digestibility for U. laetevirens vs S. chordalis products. The enzymatic hydrolysis decreased digestibility of ash, nitrogen and multiple amino acids (P<0.05). Gizzard weight, jejunal pH and cytokine levels were not significantly affected by inclusion of seaweed products. Numerically, a 59% lower IL-13 level was observed in birds fed the untreated U. laetevirens diet vs the basal diet. Birds fed U. laetevirens vs S. chordalis diets had 11% shorter villi (P<0.001) and 10% lower VL:CD (P=0.006). Birds fed the treated vs untreated U. laetevirens diet had 8% deeper crypts, whereas the opposite was observed for birds fed S. chordalis diets (-4%; Seaweed×Enzyme effect, P=0.035).

The seaweed digestibility coefficients indicate that these products interacted with the basal diet. Based on this experiment, U. laetevirens is more suitable for inclusion in broiler diets, and the enzymatic treatment was not suitable for increasing digestibility.

DIETARY REPLACEMENT OF SOYBEAN WITH MICROALGAE MEAL DURING GROWER AND FINISHER PHASES: EFFECTS ON GROWTH PERFORMANCE, OCCURRENCE OF FOOT PAD DERMATITIS, BREAST MEAT QUALITY TRAITS, AND PLASMA AND CECUM METABOLOME OF BROILER CHICKENS.

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Optimizing the dietary concentration of novel protein sources is crucial for a sustainable use of these feedstuffs. This study was carried out to evaluate the effects of the dietary replacement of soybean with microalgae meal during grower and finisher phases on productive performance, occurrence of foot pad dermatitis (FPD), breast meat quality traits, and plasma and cecum metabolomics profile of broiler chickens. A total of 1,000 one-d-old Ross 308 male chicks were divided into 5 experimental groups (8 replicates of 25 birds/each): CON, fed a commercial soybean-based diet throughout the trial (0-41 d); F3 and F6, fed CON diet up to 28 d of age and then a finisher diet (29-41 d) with 3 and 6% of microalgae meal, respectively; and GF3 and GF6, receiving CON diet until 14 d and then diets with respectively 3 and 6% of microalgae meal in grower (14-28 d) and finisher (29-41 d) phase. All diets were isoenergetic and isonitrogenous. Productive performances were recorded on a pen basis at the end of each feeding phase (13, 28 and 41 d). Blood and ceca content were collected at 21 and 41 d for NMR metabolomic analysis (2 birds/replicate). At slaughtering (41 d), the occurrence of FPD was assessed on all processed birds while 15 breasts/group were collected for the evaluation of the main technological properties of meat. At 41 d, CON showed higher body weight than F6 and GF6, with F3 and GF3 presenting intermediate values (2.54 vs. 2.45 vs. 2.41 vs. 2.45 vs. 2.38 kg, respectively for CON, F3, F6, GF3 and GF6; P<0.05). GF6 exhibited the highest feed conversion ratio, while F3 did not present significant differences in respect to CON (1.785 vs. 1.810 vs. 1.834 vs. 1.886 vs. 1.934, respectively for CON, F3, F6, GF3 and GF6; P<0.01). The occurrence of FPD was similar among groups. As for breast meat color, yellowness (b*) was higher in groups receiving the microalgae meal, either considering raw or cooked meat (P < 0.001). Other meat quality traits were only slightly affected by the dietary treatments, whereas metabolomic analyses are still in progress. In conclusion, the administration of 6% microalgae meal during grower or finisher phase impaired the growth performance of broiler chickens, whereas a 3% inclusion level was partially tolerated. Regardless of the dosage, microalgae meal showed excellent meat pigmentation capacity. Ongoing analyses will provide further insights on the metabolic aspects of broiler chickens fed on diets containing microalgae meal.

BIOACCESSIBILITY OF MAIZE CAROTENOIDS AS A PREREQUISITE FOR THEIR UTILIZATION IN EGG YOLK PIGMENTATION

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Yellow maize is recognized worldwide as a source of carotenoids, fat-soluble pigments that contribute to the pigmentation of poultry products and improve their health. At high levels in hen diet, maize is a natural alternative to synthetic pigments in egg yolk pigmentation. Besides carotenoid profile, maize pigmentation potential is determined by the release of carotenoids from the grain matrix and incorporation into micelles during digestion. The aim of the study was to investigate the carotenoid profile and their digestibility and bioaccessibility in commercial maize hybrids. Representative samples of 105 maize hybrids were collected from nine seed companies in the same county in Central Croatia during 2019 season. Carotenoid profile was determined by reversed-phase HPLC method, which was also used to determine the profile of digested and micellar carotenoids in a standardized INFOGEST in vitro procedure mimicking digestion in the stomach and small intestine. Possible relationships between the levels in grain and digested/micellar carotenoids were tested using the SAS statistical package. The tested hybrids showed the following ranges (µg/g DM): 3.47-13.04 for lutein, 4.72-23.87 for zeaxanthin, 0.30-3.34 for a-cryptoxanthin, 4.47-4.62 for β cryptoxanthin and 0.21-2.09 for β -carotene. The proportions of both digested and micellar carotenoids decreased in the order: $|utein>zeaxanthin>\beta$ -carotene> β cryptoxanthin>a-cryptoxanthin (on average 62.2, 50.4, 42.5, 32.0 and 30.5% for digested and 52.7, 42.8, 33.6, 26.9 and 25.6% of grain content for micellar, respectively). The amount of both digested and micellar carotenoids increased with increasing content in the grain (r>0.60 for all carotenoids, P<0.0001), indicating that hybrids with higher carotenoid content will have their higher content for absorption and deposition in the yolk. However, the slopes of a linear regression between grain and digested/micellar carotenoids are below 0.5, indicating that the efficiency of digestion and micellarization decreases with increasing content in grain. In conclusion, the variability in the carotenoid profile of the tested hybrids shows a high potential for yolk pigmentation. Lutein and zeaxanthin have high digestion and micellarization efficiency, suggesting that high amounts of these carotenoids could be deposited in the yolk. Less polar a- and β -cryptoxanthin and β -carotene are less bioaccessible but could still contribute to yolk pigmentation. This work has been fully supported by Croatian Science Foundation under the project ColourMaize (IP-2019-04-9063). The work of doctoral student Dora Zurak has been fully supported by the "Young researchers' career development project - training of doctoral students" of the Croatian Science Foundation.

EVALUATION OF BLACK SOLDIER FLY LARVAE OIL DIGESTIBILITY IN ADULT COCKERELS

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Insect farming is considered a sustainable alternative to conventional feed ingredient production, as it requires little land and low water supply, while producing less greenhouse gas emissions. Among other insect sources, Black Soldier Fly larvae (BSFL) oil was identified as a promising alternative to plant-based fat sources such as soybean oil, especially in poultry diets applications. BSFL oil is rich in medium-chain triglycerides such as lauric acid (~42%), known for its favorable impact on animal health. The objective of this study was to evaluate the digestibility, as well as the apparent metabolizable energy of this oil in adult cockerels.

This study was carried out following the European reference (Bourdillon et al. 1990). Cockerels were fed either with a control (CTRL) diet (97% corn; 3% feed supplements) or the same diet where 10% of corn were replaced with 10% BSFL oil (TEST diet). For 72h, cockerels were fed ad libitum with TEST and CTRL diets respectively, and total feed intake was then measured. After 24h fasting, all feces were collected, dried, weighted and grinded. Gross energy of TEST and CTRL diets were measured using an adiabatic calorimeter, allowing the BSFL oil ingredient digestibility calculation. For each individual, apparent metabolizable energy (AME) is calculated as the gross energy of the feed consumed minus the gross energy contained in the excreta. Digestibility coefficients of energy and lipids were also calculated.

The average value of BSFL oil apparent metabolizable energy was found to be 9043 ± 123 kcal/kg DM. Digestibility coefficient of energy was calculated and an excellent value of $98.9 \pm 1.3\%$ was obtained. Digestibility coefficient of the oil ingredient itself was particularly high as well, as shown by the average value of $106.3 \pm 0.6\%$. This value was above 100%, which could be explained by the uncertainty brought by the oil incorporation rate of only 10% in the diet, and the different analytical methods used (measurement of fat content through hydrolysis for oil vs. direct measurement for corn and feces). However, this study showed an complete digestion of BSFL oil in adult cockerels. Ingredients' digestibility can impact FCR, and other fatty acids absorption such as Omega-3. These energy and digestibility results are therefore particularly encouraging to consider the inclusion of BSFL oil in poultry diets as a performant and sustainable alternative to current fat sources.
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Feedstuffs and feed technology

Selected short communications

ALTERNATIVES TO SOYBEAN MEAL IN STANDARD BROILER DIET

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Reducing soybean meal (SBM) incorporation in animal feed has become one of the main current challenges in order to reduce soya dependence and to meet consumers' expectations on more locally grown feedstuffs. Indeed, the importation of genetically modified soya from deforested areas is not socially acceptable anymore, but only a few efficient alternatives are available on the market today. Replacement of SBM in broiler diets is costly and impairs zootechnical performance. The objective of this trial was to study the effect on broiler performance of the inclusion of different alternative raw materials currently used in feed mills, with a partial or full replacement of SBM. 1120 Ross 308 broilers from 1 to 35 days old were fed with a 3-phase program: starter (S: 1-10d), grower (G: 10-21d) and finisher (F: 21-35d). They received 8 dietary treatments: PC with standard SBM; low (LSF: 5%, 10%, 15% for S, G and F, respectively) and high (HSF: 10%, 18%, 25%) inclusion of sunflower Hipro, similar inclusions for low (LRS) and high (HRS) rapeseed meal, DDGS (5%, 10%, 15%). C1 and C2 are 2 different protein concentrates of vegetable source providing essential amino acids. Growth performance and feed efficiency were studied in each phase. At d35, PC birds weighed 2551±83g (BW) with a feed conversion ratio (FCR) of 1.54±0.03. LSF and HSF did not present any statistical differences compared to PC at d35 $(BW: LSF = 2541\pm133g; HSF = 2565g\pm84g; FCR: LSF = 1.54\pm0.04; HSF =$ 1.54 ± 0.03). At d35, performance of LRS was statistically similar compared to PC, whereas it was significantly depreciated for HRS (P<0.01) (BW: LRS = 2444 ± 130 g; HRS = 2341 ± 91 g; FCR: LRS = 1.54 ± 0.02 ; HRS = 1.59 ± 0.04). DDGS had a slightly lower performance than PC at d35 (BW= 2507±84q; FCR = 1.56 \pm 0.03; P = NS). Finally, BW and FCR obtained with C1 or C2 were not statistically different to PC at d35 (BW C1 = $2575\pm123q$; C2 = $2535\pm55q$; FCR C1 $= 1.53 \pm 0.06$; C2 $= 1.55 \pm 0.04$). This study confirms the possibility of a partial or full replacement of standard SBM, even in the starter phase, without impairing growth performance and feed efficiency of broilers. Inclusion levels of different tested feedstuffs in this study allow a higher maximum inclusion and give options to limit feed cost. However, the full replacement of SBM in broiler feed remains costly, application is possible only if the consumers are willing to pay the additional price.

VARIATION OF AMINO ACID DIGESTIBILITY AND METABOLISABLE ENERGY OF SOYBEAN MEAL IN CAECECTOMISED LAYING HENS

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Variation of amino acid (AA) digestibility and nitrogen-corrected metabolisable energy (MEN) of solvent-extracted soybean meal (SBM) in laying hens is unknown although SBM is a standard ingredient of poultry diets worldwide. This study examined the AA digestibility and MEN in 18 SBM samples (6 European, 7 Brazilian, 2 Argentinian, 2 North American, 1 Indian). Maize/SBM-based experimental diets consisted of 300 g/kg of either one SBM sample or maize starch. All diets met or exceeded the nutrient supply recommendations. Pelleted diets were fed to 10 caecectomised LSL-classic laying hens in two 5×10 row-column designs so that 5 replicates were obtained from each diet in 5 periods. The daily feed allowance was 115 g while the hens were housed individually in metabolism units for 8 days. Total excreta were collected during the last 4 days of each period. The AA digestibility of the soybean samples was determined using a regression approach. The average digestibility of first-limiting AA in SBM was 91% for Met, 77% for Cys, 89% for Lys, and 84% for Thr. The AA with the highest digestibility (94%) and the lowest range in digestibility (4 %-units) was Arg while the AA with the lowest digestibility and the highest range in digestibility (23 %-units) was Cys. The range in digestibility of the other AA was 6–12 %-units. MEN of the SBM samples ranged 7.5–10.5 MJ/kg dry matter with an average value of 9.4 MJ/kg dry matter. Differences in AA digestibility and MEN between countries of origins did not exist except for low AA digestibility of the 2 Argentinian SBM. Indicators of SBM guality such as trypsin inhibitor activity (range 0.8-4.5 g/kg dry matter), protein dispersibility index (range 6.1-20.3%), KOH solubility (range 36-71%), and urease activity (<0.05 mg nitrogen/g/min in all samples) correlated significantly with AA digestibility (P<0.05) only in few cases. Variation of AA digestibility is relevant to the industry and more work is necessary to allow for rapid prediction of AA digestibility of SBM. Effects other than country of origin, trypsin inhibitor activity, protein dispersibility index, KOH solubility, and urease activity must have caused the variation of AA digestibility.

EFFECT OF THE SATURATION DEGREE AND THE ACIDITY LEVEL OF ADDED FAT IN BROILER CHICKEN DIETS ON PERFORMANCE PARAMETERS, ABDOMINAL FAT DEPOSITION AND FATTY ACID PROFILE OF LEG MEAT

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The present study evaluated the effect of the saturation degree and acidity level of supplemented fat in broiler diets on performance parameters, fat deposition and fatty acid (FA) profile of leg meat. A total of 528 1-d-old female broiler chickens (Ross 308) were randomly allocated into 48 cages (11 chickens/cage) and 8 treatments (n = 6 cages/treatment). Eight experimental diets resulted from replacing soybean oil with soybean acid oil, or palm oil with palm fatty acid distillate in different proportions. Thus, there were 4 soybean (S) and 4 palm (PO) diets with 6% of added fat varying in their free FA (FFA) composition (5%, 15%, 35% and 50%). Performance parameters, i.e., BW, ADG, ADFI and FCR, were measured. Animals were slaughtered at 37 d, and from 3 animals per cage (18 animals/treatment) both carcass weight (CW) and % of abdominal fat depot (AFD) were calculated. Additionally, samples of deboned leg meat with skin were collected and homogenized for the FA profile analysis. Data was analyzed by a two-way ANOVA using the statistical software R. Regarding the saturation degree of added fat, chickens fed S showed higher BW and lesser AFD (P < 0.001) than those fed PO, independently of the percentage of FFA. Concerning acidity, animals fed with moderate acidity levels (15% of FFA) had the highest BW, ADG and CW (P < 0.01). Moreover, as acidity increased, AFD decreased (P = 0.047). Deboned leg meat with skin reflected different FA profile according to the dietary fat source added: S showed a lower content of SFA and monounsaturated FAs (MUFA) and a higher content of polyunsaturated FAs (PUFA) than PO (P < 0.001). Animals fed with S, at high levels of acidity (50% of FFA), showed an increase (P = 0.005) on SFA and a decrease (P < 0.001) on MUFA and PUFA. This effect did not occur on animals fed with PO, in which no differences were observed between the different levels of acidity. These results suggest that palm oil and soybean oil included at 6% with moderate acidity levels (15% of FFA) could be suitable for poultry, since they showed the highest growth rates. Moreover, high levels of acidity (50% of FFA) in PO diets did not change the FA profile of deboned leg meat with skin, but these levels in S diets led to a decrease in PUFA and an increase in SFA and MUFA.

Keywords: free fatty acid, leg meat, fatty acid profile, abdominal fat deposition, broiler.

EVALUATION OF PARTICLE SIZE, FEED FORM AND PELLET DIAMETER ON PERFORMANCE AND NUTRIENT DIGESTIBILITY OF BROILERS AT 39 D OF AGE

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The objective of this study was to evaluate the effect of corn particle size and feed form on broiler performance and nutrient digestibility from 1 to 39 d of age. A total of 1800 d old male Cobb 500 broilers were randomly distributed among 9 dietary treatments with 8 replicates per treatment and 25 birds/pen. The experiment consisted of a 3×3 factorial arrangement of 3 corn particle sizes (750, 1150 and 1550 µm) and 3 feed forms (mash, 3- and 4-mm pellets) provided from 1 to 39 d. Pelleted treatments were offered as crumbles from 1 to 17 d. Titanium dioxide (TiO2) was added as an indigestible marker (0.5%) from 27 to 39 d of age to determine nutrient digestibility. Feed intake and BW were determined at 17, 27 and 39 d of age and FCR corrected for mortality. Data were statistically evaluated using the GLM procedure of JMP and means were separated by Tukey's HSD with statistical significance considered at $P \leq 0.05$. Broilers fed 3- and 4-mm pellets had higher feed intake, BW and lower FCR (P<0.05) than broilers fed mash diets at 39 d of age. Broilers fed diets with 750 μ m corn particle size had higher (P<0.05) BW than broilers fed diets with 1550 µm, but similar to broiler fed diets with 1150 µm. Corn particle size did not influence FCR at 39 d of age (P>0.05). Broilers fed 3- and 4-mm pellets had higher (P < 0.05) apparent ileal digestibility (AID) of protein, energy and fat than broilers feed mash diets. Significant interactions were observed between feed form \times corn particle size on AID of protein, energy, and fat. Corn particle size didn't influence AID of protein and energy when diets were fed as mash or 4 mm pellets; however, when broilers were fed 3 mm pellets, AID of protein and energy increased (P<0.05) as corn particle size increased from 750 to 1550 µm. Furthermore, AID of fat increased (P<0.05) in mash diets when corn particle size was reduced from 1550 and 1150 to 750 μ m; however, in broilers fed pelleted diets AID of fat was unaffected by corn particle size. The results of this study demonstrated that pelleting improves broiler performance and nutrient digestibility and the optimum corn particle size is influenced by feed form. Key words: Pellet diameter, Cobb, nutrient digestibility

EFFECTS OF DRYING TEMPERATURE AND STORAGE ON NON-STARCH POLYSACCHARIDE CONTENT OF TWO VARIETIES OF CORN DIFFERING IN KERNEL HARDNESS

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Temperatures applied to dry corn and storage time have been reported to affect starch forms, nutrient digestibility, and energy values. However, little is known about the impact of these factors on non-starch polysaccharides (NSP). Understanding these effects can be useful to improve nutrient utilization and gut health of poultry and livestock. This study evaluated the effects of kernel hardness, drying temperature, and storage time (2 and 29 wk post-drying) on total and insoluble NSP, arabinoxylan, arabinose:xylose ratio (A:X), arabinose, xylose, mannose, galactose, and glucose. Six treatments per storage period resulted in a factorial arrangement of two kernel hardness (average and hard) and three drying temperatures (35, 80, and 120 oC). Five samples per treatment were ground and scanned in a FOSS NIRS DS2500. The non-standardized spectra of each replicate were submitted to the AB Vista calibration model to predict NSP profiles. Data were analyzed in a completely randomized design using ANOVA that contained kernel hardness, drying temperature, and storage, with all possible interactions. Postharvest and before drying, the variety with average kernel hardness had more (P<0.01) insoluble and total arabinose, mannose, A:X, and less (P<0.01) NSP, xylose, arabinoxylan, and galactose than the hard kernel variety. Corn storage increased (P<0.01) the compounds detected as NSP, but the A:X ratios were similar. In corn stored for two wk, interactions were observed (P<0.01) for insoluble NSP, A:X, xylose, mannose, and galactose fractions, plus for total A:X, mannose, and galactose. After 29 wk of storage, significant interactions (P<0.001) were detected in all parameters evaluated. Drying corn with average hardness at 120oC decreased the insoluble NSP, xylose, and galactose, and total arabinoxylan, xylose, and galactose increasing the A:X ratios, while the hard kernel remained unchanged. After 29 wk of storage, the lowest values for all NSP fractions in the average hardness corn were observed when kernels were dried at 120oC. In corn with hard endosperm, drying at 80oC resulted in lower values for total arabinoxylans, total and insoluble glucose, and xylose, and drying at 120oC had lower galactose content. In conclusion, these corn varieties differed in NSP content, storage time increased NSP content regardless of kernel hardness and using elevated drying temperatures decreased NSP of average hardness corn, but this effect was different in hard endosperm corn.

EFFECTS OF MIX TIME ON COEFFICIENT OF VARIATION (MIX UNIFORMITY) AND BROILER PERFORMANCE DURING THE STARTER, GROWER, AND FINISHER PERIODS

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Previous research has reported that mixing uniformity is inversely proportional to mixing time. The majority of feed manufacturers use the same mixing time throughout the growing period regardless of age and feeding phase. However, throughout this period, birds may require a more or less uniform mix depending on consumption patterns. The objective of this study was to evaluate the effects of mix uniformity on broiler growth performance. A total of 640 male Ross × Ross 308 broiler chicks were randomly distributed in 40 floor pens with 10 replicate pens/treatment and 16 birds/pen. Feed was manufactured at the North Carolina State University Feed Mill Education Unit utilizing a 2 Ton counterpoise ribbon mixer. Two batches of feed were mixed for 4.5 min (3 min dry mix and 90 s of wet mix) and 30 s (0 s dry mix and 30 s wet mix) to obtain a Uniform (UM) and a Nonuniform (NUM) mix, respectively. Each pen was randomly assigned to 1 of 4 dietary treatments: 1) UM from 1-42 d, 2) UM from 1-28 d and NUM from 28-42 d, 3) UM from 1-14 d and NUM from 14-42 d, and 4) NUM from 1-42 d. Experimental diets were provided in mash form to prevent further mixing of the feed. Ten samples were collected at equally spaced time intervals from the mixer discharge conveyor after each mixing period and analyzed for coefficient of variation (CV) using multiple tracers (chloride ion concentration using Quantab Test Strips, L-Lysine-HCl, Quantum phytase, D-L Methionine, Microtracer F-Red#40(count), and Microtracer F-Blue#40(count)). Data were statistically evaluated using ANOVA and means were separated by Tukey's honestly significant difference. The experimental design consisted of a 1-way treatment structure using a randomized complete block design with pen location being the blocking factor. There were no statistical differences between the treatments on BW, FI, and FCR during the starter, grower, and finisher periods (P>0.05). In addition, mix uniformity did not influence individual bird BW CV from 1 to 42 d of age (P>0.05). Broilers were able to consume diets with a shorter mix time during the grower and finisher periods without any adverse effects on growth performance, which may save a significant amount of mix time in high-feed manufacturing facilities hence improving feed throughput and labor hours.

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Feedstuffs, Feed Technology, Feeding in alternative production systems

Posters

USE OF MEDIUM CHAIN FATTY ACIDS AS REPLACEMENT TO ANTIBIOTICS IN BROILERS DIET

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This study examined the influence of dietary supplementation of medium chain fatty acids (MCFA) on growth performance, gut health and economics in broilers. The day-old chicks were equally distributed into five dietary treatment groups viz. control group (T0), basal diet with antibiotic of BMD group (T1), basal diet with 0.25% lauric acid (T2), basal diet with 0.25% capric acid (T3) and basal diet with 0.25% lauric and 0.25% capric acids (T4). The birds fed diet containing lauric acid, capric acid or their combination recorded significantly higher (P<0.01) body weight and gain in weight compared to control. However, feed intake was comparable in all treatment groups though numerically increased feed consumption due to lauric and capric acid supplementation was evident. FCR was improved in broilers fed with MCFAs either singly or in dietary combination. The reduction in mortality was recorded in groups fed combination of MCFAs. The coliform count was numerically reduced, whereas, the total viable count in group fed MCFAs and their combination were substantially reduced. The HI titers did not show significant differences between treatments. The dietary supplementation of MCFAs was found to improve profit margin in broiler production. In conclusion, the results indicated MCFAs singly or in combination at 0.25 % level in diets improved growth performance, gut health and found to be economically beneficial.

EFFECT OF THE DIETARY ADMINISTRATION OF PREMIUM YEAST FRACTIONS ON THE PRODUCTIVE PERFORMANCE OF BIRDS VACCINATED AGAINST COCCIDIOSIS

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When coccidiosis control strategy is switched from coccidiostat supplementation to vaccination, it is generally observed birds face a disruption in performance, which is affected by disturbances such as bacterial enteritis. Yeast fractions (YF), thanks to support to gut integrity, reduction of pathogen pressure and effect on microbiota, can be good candidates to prevent a decrease in performance on vaccinated birds. The aim of the study was to investigate how YF could help maintain similar performance to coccidiostats on vaccinated birds. A total of 1,600 one-day-old male broilers (ROSS 308) were divided in 4 experimental groups (10 replicates of 40 birds/each): NC (negative control), fed a commercial basal diet free of coccidiostats, PC (positive control) receiving the NC diet supplemented with narasin and nicarbazin, VAC (vaccine) receiving the NC diet and vaccinated against coccidiosis, VACYF (vaccine + YF) receiving the NC diet supplemented with a premium YF (1-14d 250g/t, 14-25d 500 g/ton, 25-35d 250g/t) and vaccinated against coccidiosis. Treatments were organized in randomized block design and analyzed by ANOVA. Productive traits were determined on a pen basis at 4 different ages (14, 24, 28 and 35d). The random effect of the pen was included in the statistical analysis for D35 individual BW. At any age, there is a significant effect on collective body weight, groups PC and VACYF being significantly higher (P<0,001) at 24 and 28 days. Final individual body weights showed a significantly higher value for groups PC (2327g) and VACYF (2306g) compared to NC (2238g) and VAC (2238g), (P=0,006). Body-weight-adjusted FCR was significantly lower (P<0,001) for PC group (1.50) compared to VACYF (1.53), which was numerically lower than VAC (1.54) and NC (1.55). Surprisingly, mortality rates were significantly (P=0,025) lower for VAC group (1,3%) compared to all other groups (NC 4,0%, PC 4,8%, VACYF 4,8%), without any explanation having been identified. Consequently, administration of premium YF to birds vaccinated against coccidiosis leads to an enhancement of birds performance, reaching levels close to what can be observed on birds supplemented with coccidiostats.

SPONTANEOUS AND ENZYMATIC FERMENTATION OF RAPESEED CAKE FOR BROILER NUTRITION

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This study evaluated the effect of partial substitution of soybean meal (SBM) in broiler diets with (bio)technologically processed rapeseed cake on growth performance and nutrients digestibility. Rapeseed cake (RC) was treated with fermentation without (WR) or with enzymes (ER, phytase, pectinase and βglucanase). The experiment consisted of 5 experimental diets, 8 pens per diet and 10 birds per pen. A standard corn-SBM diet was produced as control group (CON). Three other experimental diets were produced using different RC products as substitutes for SBM at 15% inclusion level. Fifth diet (IFE) was produced using 15% untreated RC and in-feed inclusion of phytase, pectinase and β -glucanase (RONOZYME HiPhos and VP, DSM). Diets were isonitrogenous and isocaloric. Performance variables were recorded during starter (d 1-21) and grower (d 22-35) periods as well as at the end of the experiment (d 35). Apparent ileal digestibility (AID) of nutrients was determined at d 35. Data were subjected to ANOVA using the GLM procedure. Body weight gain and feed intake of all the experimental groups were similar during different experimental periods (P > 0.05). There were no differences among feed conversion ratio (FCR) of the experimental groups during the growing period (P>0.05). At the end of the starter period and whole experiment, FCR of broilers fed IFE diet was better compared with those received CON or untreated RC diets (P<0.05). At the end of the starter period, birds in ER group displayed better FCR than those in the CON group, while at the end of the experiment, birds fed ER diet showed better FCR compared with those received untreated RC diet (P<0.05). AID of crude protein and fat was identical for all the experimental groups (P>0.05). ER and IFE diets had higher AID of P compared with WR, CON and untreated RC diets (P<0.05). IFE diet showed higher AID of Ca compared with WR and untreated RC diets, while untreated RC diet had lowest AID of Ca among the experimental diets (P<0.05). In conclusion, partial replacement of SBM with enzymatically fermented RC or adding enzymes to diets containing 15% untreated RC can improve AID of Ca and P as well as feed efficiency in broiler nutrition.

MICROENCAPSULATED FORMULA OF ESSENTIAL OILS IMPROVED GROWTH PERFORMANCE OF BROILER CHICKENS UNDER EIMERIA SPP. INFECTION

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The objective of this study was to evaluate the effectiveness of a protected formula of essential oils (P(EO)) containing thymol, eugenol, and vanillin on the growth performance and intestinal health of broilers under Eimeria spp. challenge. Oneday-old male Ross 308 broiler chicks (720) were randomly assigned to 3 dietary treatments (8 pens/treatment; 30 birds/pen) from 1 to 35 d. The birds were vaccinated against coccidiosis at the hatchery (Coccivac-B52). The treatments were: non-supplemented and non-challenged control; non-supplemented and challenged control; and challenged supplemented with 600 mg/kg of P(EO). The basal diet contained corn and soybean meal, and the P(EO) was supplemented from 1-35 d. On d 1, the chicks in the challenged groups received 22,000 sporulated oocysts of E. acervulina, E. maxima and E. tenella via feed. Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), and the European Efficiency Production Factor (EEPF) were recorded and calculated. On d 15 and 29, one bird/pen was necropsied to evaluate the degree of lesions throughout the gastrointestinal tract by using the I See Inside methodology. Excreta samples were collected on d 10, 17, 24 and 31 for the determination of oocysts per gram (OPG) of excreta. Growth performance was analyzed by One-way ANOVA and the means separated by Tukey's test (P < 0.05). The lesion scores and the OPG data were analyzed by Kruskal-Wallis' test (P < 0.05). The challenge negatively affected BWG, FI, and FCR (P < 0.05) throughout the life of the birds. However, from 1 to 35 d, the inclusion of P(EO) improved the FI of birds by 3.4% (P = 0.02), the BWG by 4% (P = 0.01), and the FCR by 2.2% (P = 0.03) when compared to nonsupplemented and challenged control birds. Additionally, the EEPF was improved by 7.8% in challenged supplemented vs. challenged and non-supplemented birds and was similar to the non-challenged group. On d 29, the dietary supplementation of P(EO) tended to reduce the gizzard erosion scores (P = 0.07), and the inflammation of the ileal serosa (P = 0.08) when compared to the nonsupplemented group. Although not different between groups, the peak of oocyst shedding was observed on d 17 whereas the supplementation with P(EO) reduced the OPG by 59%, and by 67% on d 31. In conclusion, the dietary supplementation of P(EO) attenuated the negative effects of coccidiosis on the growth performance and reduced the OPG shedding in vaccinated and challenged broiler chickens.

EFFECT OF MANIHOT ESCULENTA LEAF MEAL INCLUSION IN DIETS OF BROILER CHICKEN ON GROWTH PERFORMANCE, HEMATOLOGICAL AND SERUM BIOCHEMICAL VALUES

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The increase in cost of feeding in broiler industry have given rise to continuous demand for alternative sources for poultry feed incorporation ingredients, this has resulted in search for feed additives from plants such as Manihot esculenta leaf meal. This experiment was conducted to investigate the effect of Manihot esculenta leaf meal (MELM) inclusion in diets on blood parameters and growth performance of Cobb 500 broilers. A total of 300 day-old chicks were allotted to three dietary treatment groups: 0% (ME0), 2,5% (ME2,5) and 5% (ME5) addition of MELM, with 5 replicates of 20 birds each. During the experimentation, feed intake (FI), live weight, and feed conversion ratio (FCR) were recorded weekly. At 6 wk of age, blood samples were collected from 30 birds per treatment for haematological and biochimical analysis. Results showed that feed intake (FI) was higher (p<0.05) in ME5 group compared to other treatments. Birds in the ME5 group showed highest feed conversion ratio value (p<0.05). Average live weight and weight gain of birds in the MEO and ME2.5 groups were similar and statistically higher (P<0.05). Carcass weight of group ME2.5 were higher those in groups ME0 and ME5 (p<0.05). The liver, heart and intestine weight showed not significant (p<0.05)differences among treatments. Except abdominal fat in the ME2.5 and ME5 group which were lower compared control group. Total Cholestérol, Triglycéride and Uric acids level decreased significantly (p < 0.05) in birds fed 5% of MELM. Red blood cells, white blood cells (WBC), packed cell volume and lymphocytes, hematocrit, heamoglobin increased with the increase in the Manihot esculenta inclusion levels (p < 0.05). It was concluded that supplementation with Manihot esculenta leaf meal in diet of broiler chicken enhanced the growth performance of birds without any deleterious effect on health status of the birds.

Keywords: Manihot esculenta, boilers chickens, growth performances, haematology.

EFFECTS OF FEEDING OATS AND BARLEY AT DIFFERENT INCLUSION RATES ON THE PRODUCTION TRAITS OF BROILER CHICKENS

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Oats and barley grains with hulls are fibrous feedstuffs that are not used at high inclusion rates in poultry diets. However, the recent findings on the importance of structural fibers and coarse particles on gizzard function mean, that feeding these cereals at higher inclusion rates could be beneficial. The present study was aimed to find out the effects of feeding relative high amounts of oats and barley with glucanase (Econase GT) supplementation, on the performance of broiler chicks. A total of 600 one day old male Ross 308 broiler chickens were divided randomly into 25 floor pens with 24 birds each. Beside a corn, wheat and soybean meal based control diet (C) two oats and two barley containing diets were fed in 5 replicate pens. The starter, grower and finisher diets were fed between 1-10, 11-24 and 25-39 days respectively. The inclusion rates of oats were 10 (010) and 20% (O20), those of barley 20 B20) and 40% (B40) in each feeding phase. The diets were isocaloric and isonitrogenous, their nutrient content were adjusted to the requirements of the Ross 308 broilers. The only difference in the nutrient contents was the higher crude fiber and beta glucan contents of the oats and barley containing diets. The results showed that treatments had no effects on the feed intake of birds in the starter and grower phase. In the finisher phase chicks from the B40 diet eat significantly less, compared with the other treatments. No significant effect in weight gain during the starter phase was found. In the grower phase O20 and B40 treatments resulted significantly less gain. On the other hand, in the finisher phase the two oats containing diets resulted significantly higher live weight gains. No significant differences in the feed conversion were found in the starter and grower phases. In the finisher phase FCR of birds fed the oat and barley diets were better than that of the control group. Significant differences were found between treatments O10, O20, B40 and the FCR of the control group. From the results it can be concluded, that broiler chick can tolerate relative high ratio of oats and barley even in the starter phase. Oat containing diets could improve the production traits of chickens mostly in the finisher phase. The reason for this can be partly the more intensive gizzard function and partly the prebiotic effects of oats beta glucan in this age interval. The research was supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project, which is co-financed by the European Union and the European Social Fund

ENDOGENOUS ILEAL AMINO ACID LOSSES RESPONSE TO NITROGEN-FREE DIETS WITH DIFFERENT RATIOS OF DEXTROSE TO CORN STARCH IN BROILER CHICKENS

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This experiment was conducted to determine the response of the AA composition of ileal endogenous amino acids (IEAAs) of broilers to nitrogen-free diets (NFD) formulated with different ratios of dextrose to corn starch. A total of two hundred and ten 28-day-old broiler chickens with similar weight were allocated to 5 groups in a randomized complete block design for a 3-day trial to estimate IEAAs. The ratios of dextrose to corn starch investigated here were 1, 0.6, 0.33, and 0.14 in groups A, B, C, and D, respectively. Birds fed basal diet was regarded as the control group (CT). All diets contained 0.5% titanium dioxide as an indigestible marker. On day 31, birds were euthanatized with pentobarbital sodium, the digesta, mucosa, and tissue of ileum were collected. The digestibility of dry matter (DM), the content of IEAAs, activity of digestive enzymes, the mRNA expression of Mucin2, intestinal morphology, and the goblet cells were evaluated. Data were analyzed by one-way ANOVA performed with SPSS 17.0 software. The results showed that the content of Asp, Thr, Ser, Glu, Gly, Ala, Val, Ile, Leu, Tyr, Arg and Pro composition of IEAAs in groups A and B were significantly higher than that of groups C and D (all P<0.05). The highest level of the ileal endogenous output for Phe was found in group B (P < 0.05), and Lys was highest in group A (P < 0.05). Moreover, group A had the highest DM digestibility which was 1.35 times higher than that of group D (P<0.001). For the activity of digestive enzyme, group A had the highest activity of a-amylase, lipase, sucrose, and maltase (P<0.05), the activity of chymotrypsin in group B and D were higher than that of group A and CT (P<0.05). There was no significant difference in digestive enzymes activity in group C when compared with group CT. Besides, the number of ileal goblet cells and the gene expression of Mucin2 were higher in group A than groups C and CT (P<0.05). In conclusion, our results indicate that a high level of dextrose in NFD increased the IEAAs flow by increasing the secretion of mucus and digestive enzymes. Given that the observed data of group C were similar to that of group CT, we suggest the ratio of dextrose to corn starch in NFD at 0.33 might mimic the IEAAs flow of birds fed a basal diet.

Keywords: Broiler; Ileal endogenous amino acids; Nitrogen-free diets

SCREENING OF SPECIFIC XYLAN-DEGRADING ENZYMES BASED ON CORN AND RELATED BYPRODUCTS IN AN IN VITRO DIGESTION MODEL OF THE BROILER CHICKENS GASTROINTESTINAL TRACT

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An in vitro experiment was conducted in two parts to assess hydrolysis effects of xylanase on different feedstuff types and screen specific xylan-degrading enzymes complex based on corn and related byproducts. Reducing sugars (RS) and dry matter digestibility (IVDMD) were measured. Part ONE, corn-soybean meal-DDGS, corn hull, and wheat bran substrates were hydrolyzed in the presence or not of two types of microbially derived xylanases (EX1/EX2). Results showed that RS acting on each of the above substrate types reflected a significant guadratic curve change (P<0.05) accompanied by suitable levels of Aspergillus niger-derived EX1 54 U/g, 54 U/g, 15 U/g, respectively, and 80 U/g, 40 U/g, 40 U/g for the appropriate addition dosages of Trichoderma-derived EX2. However, changes in degradation degree of the xylanases acting on different raw materials were not the same using IVDMD as a response indicator. Shifts were also different for diverse feed substrates, showing that net increase of RS or IVDMD distinctly elevated when enzymatically degrading wheat bran regardless of xylanase sources (P<0.05). Part TWO, corn-soybean meal-DDGS were tested by various levels of xylanase, arabinofuranosidase (EA), and ferulic acid esterase (EF). Then the quadratic regression rotation orthogonal combination design was applied to establish regression relationships between RS or IVDMD and three exogenous enzymes. We detected the xylooligosaccharides composition, concentrations of total starch, and alucose in the digestion incubated with enzyme complex. There was a significant quadratic relationship between RS or IVDMD and enzyme addition levels (P<0.05). The determined suitable doses were 54 U/g EX, 5.0 U/g EA, and 0.4 U/g of EF, respectively. The optimal enzyme combination profile was EX 69 U/q, EA 6.7 U/q, and EF 0.57 U/g based on IVDMD screening (R2=0.9089, P<0.001). Digestive substrates were composed of mere parts of xylose, and the total starch content markedly reduced as well as glucose content increased (P<0.001). Conclusively, the efficiency of xylanase supplements may vary, depending on the structure and composition of arabinoxylan in multiple cereal products and cultivars. In vitro assay is a convenient and rapid method of screening for xylan-degrading enzymes complex, yet corn and its related byproducts are poorly affected by xylanase-based degradation enzymes.

Keywords: In vitro digestion; Corn; Xylan-degrading enzymes; Screening

EFFECTS OF WHOLE PADDY RICE MIXING RATIO ON PRODUCTIVE PERFORMANCE IN NATIVE CHICKENS

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We investigated the effects of whole paddy rice mixing ratio on productive performance in native chickens (Hyogo-Ajidori: obtained from a 3-way cross between a White Plymouth Rock and a Hyogo, itself a 2-way cross between a Satsumadori male and a Nagoya female). One hundred and ten birds were divided into four experimental groups and a control group. Twenty-five, fifty, seventy-five and a hundred percent of the corn in the formula diet for the experimental groups was replaced by whole paddy rice and fed during the later term of fattening. The formula diet for the control group contained 65% of corn with no rice. Statistical analysis was performed using SAS GLM procedure. The following results were obtained. All rice mixing ratio diets were completely consumed, and the health condition of birds was good. The average body weight was significantly lower in the 100% group than in the other groups (p < 0.05). The feed conversion rate and production score were slightly higher in the 50% group and the control group than in the other groups. The meat yield was significantly lower in the 75% group than in the 25% group, 50% group and the control group (p < 0.05). The ratio of gizzard weight to live body weight was significantly higher in all of the experimental groups than in the control group (p < 0.05). The ratio of abdominal fat weight to live body weight in the 75% group was significantly higher than that in the 25% group and the control group (p < 0.05). The meat color became lighter with increasing rice mixing ratio. The percentages of oleic acid and monounsaturated fatty acid of fat in the thigh meat were significantly higher in the 100% group than in the 25%group, 50% group and the control group (p < 0.05). The concentrations of glutamic acid and total amino acid in the thigh meat was not different among the five aroups. The cost of feed per bird was lowest in the 100% group, and the cost of feed per 100g meat was lowest in the 50% group. These results suggest that the suitable whole paddd rice mixing ratio for feed efficiency in Hyogo-Ajidori chickens is 50%, and that for meat quality is 100%.

DIETARY SUPPLEMENTATION WITH A STANDARDISED GRAPE EXTRACT IN BROILERS: POTENTIATION OF THE ENDOGENOUS ANTIOXIDANT DEFENCES WITH AN EFFICIENT DIVERSIFICATION

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Whether they are fat-soluble (vitamin E), water soluble trace-elements (selenium) or secondary metabolites from plants (flavonoids), dietary antioxidants are crucial to provide protection against free radicals in broilers. Whilst recent research has shown that grape polyphenols act in synergy with vitamins E and C and that linear increase in vitamin E has limited effect over performances, the current study aimed at comparing the effect of a high level of dietary vitamin E in grower and finisher diets with a low dose of standardised grape extract (SGE) in finisher diet only on performances and antioxidant parameters in broilers. 480 day-old all-male Ross 308 chicks were randomly divided into 3 groups of 8 replicate of 20 birds each. All groups received the same starter diet (0-10D, 15IU vitamin E). Vitamin E level in CTL and NG group were 15 IU in grower (11-20D) and 10IU in finisher (21-42D) diets. NG group had additional 30ppm of SGE (Nor-Grape®, Nor-Feed, France) in finisher phase only. VE group received 100IU vitamin E from grower to finisher periods. Growth and FCR were monitored per period. Blood samples were drawn at D20 and D42 in all groups to measure total glutathione (GSH), glutathione peroxidase (GPx), oxidised lipids (TBARS) and DNA (8-OHdG). The VE diet had no impact on the studied parameters (growth or antioxidant defences) compared to the CTL group. The improvement of body weight and FCR with the use of SGE in the diet did not show to be a statistically significant vs. CTL or VE. Moreover, no differences were observed between groups and periods of blood sampling in terms of oxidative damages, showing low oxidative pressure. However, results showed that the VE diet had no effect on the studied endogenous antioxidant defences, whilst only the addition of SGE to the CTL diet resulted in a significant increase in GSH-PX (+23.3%; P<0.05) and GSH (+49.8%; P<0.05) at D42. These results confirm that may be successful to use different dietary antioxidants to produce a synergistic effect due to the occurrence of a simultaneous antioxidant mechanism. Using this strategy can be possible to use low dose of the single antioxidant with a potentiation of the antioxidant system.

Keywords: broiler, antioxidant defence, vitamin E, grape extract, antioxidant synergy

EVALUATION OF TWO COATING DENSITIES ON CALCIUM BUTYRATE EFFECTS IN EXPANDED BROILER FEEDS

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Butyrate supplementation has been recognized for its widespread positive effects on animal performance, gut morphology and function, and coating in a fat matrix greatly enhances its efficiency1. Moreover, expansion of broiler feeds is becoming more common practice in Belgium. This research aims to evaluate the influence of two coating densities on calcium butyrate (CB) effectiveness in expanded broiler feeds, hypothesising that a larger coating provides better protection and results in better performance. Therefore, a trial was carried out with 240 day-old, Ross 308 broiler chickens (10 pens/treatment; 8 birds/pen) at Poznan University of Life Sciences. Experimental diets were provided during starter (S; d1-10) and grower phase (G; d10-d28) and consisted of a basal diet (control group (CON)), basal diet supplemented with CB coated with 14% fat (CB14; S: 350mg/kg, G: 250 mg/kg), or CB with a thicker fat coating of 45% (CB45; S: 560 mg/kg, G: 400 mg/kg). Treatment diets were iso-butyric. All feeds were expanded with mean expander temperatures of 95.5°C and 97.5°C for S and G feeds, respectively. Growth and feed intake were evaluated for each phase. Additionally, on d10 and d28, ten birds per treatment were sampled for histo-morphological measurement on ileal sections, and for the determination of % Firmicutes by Real Time-PCR (d28 only) in ileal and caecal contents. Data were analysed using the GLM procedure in R with the "Agricolae" package on the 5% significance level followed by Tukey's post-hoc tests. During the S, no significant differences were observed between treatments. In the G, coated CB significantly increased average daily gain (CB groups: 66 vs CON: 64 g), decreased daily feed intake (89 vs 95 g) and as a result, improved feed conversion (FCR; 1.33 vs 1.48). The better FCR was also observed over the whole trial period and there was a tendency (p = 0.055) towards higher weight gains in treatment groups. No performance differences were observed between coating types. Numerical improvements were observed in the CB groups with regard to ileal histomorphology and % Firmicutes. In conclusion, the two coated CB forms almost equally improved the performance of broilers in expanded feed as compared to a control diet, indicating that a thicker coating does not necessarily imply better results.

1 Guilloteau et al. (2010) Nutrition research reviews, 23(2), 366-384.

EFFECTS OF DIETARY 25-HYDROXY VITAMIN D3 (25-OH-D3) ON BROILERS PERFORMANCE, MEAT COLOR AND BONE STRENGTH

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Due to genetic selection, modern broilers have the capacity to grow faster and develop more muscle than in the past. This situation has caused an imbalance between growth, meat yield, and bone development resulting in a lower bone density, increased locomotion problems, higher incidence of lameness and carcass condemnations that reduce overall performance and economical margins. Additionally, during the first weeks, vitamin D3 absorption and the low hepatic enzyme production in birds worsens this scenario. The objective of this study was to evaluate the effect of the dietary inclusion of 25-OH-D3 on broiler performance, meat guality and yield, and bone strength. A total of 1,200-day-old Ross 308 broiler males, were distributed across 3 treatments, 8 replicates of 50 birds each (10 birds/m2) in a completely randomized design. Broilers were fed with a basal diet based on corn-soybean meal formulated to meet breed nutritional recommendations. Treatments were: (T1): Basal diet + 4,000 IU/Kg of Vitamin D3 until processing (49 d); (T2): as T1 + 69 μ g/Kg 25-OH-D3 until day 21, and then as T1; and (T3): as T1 + 69 μ g/Kg of 25-OH-D3 during the whole experimental period. Feed intake, body weight gain, feed conversion ratio, bone breaking strength, and meat color and yield were assessed weekly and at processing. Data were analyzed using one-way ANOVA and means were compared by the Tukey test; effects were considered significant at $P \le 0.05$. At processing age, T3 birds were significantly heavier than T2 (P<0.05), and breast yield showed a trend (P<0.10) in favor of T3. At 35 and 42 d tibia strength was greater in T3 (P=0.004; P=0.022, respectively). At 35 and 49 d luminosity (L*) of leg meat (frozen and cooked) was significantly higher in T3 (P < 0.05). At same ages, T2 and T3 had the lowest redness (a^*) in leg meat and femur and tibia bones (P<0.05). It was concluded that dietary 25-OH-D3 during the whole production period positively influenced growth and meat yield. Moreover, the greater bone strength, higher meat luminosity and lower meat redness is likely related to the inclusion of 25-OH-D3, that influences bone density and skeletal integrity reducing bone marrow pigment migration to leg meat, which has a positive effect on consumer acceptance of broiler meat.

EFFECT OF OLIVE PASTE FLOUR ON A-TOCOPHEROL AND PERFORMANCE IN BROILERS OF A POULTRY FARM UNIT

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Olive as well as several olive byproducts have been used to supplement animal feed due to their antioxidant content.1 However, the impact of such byproducts on broiler antioxidant status and performance are differentiated depending on the composition, the process, and the storage of the byproduct, 2 as well as the experimental conditions.3 In this study we recorded the effect of an olive-paste based product on plasma and muscle tissue total antioxidant capacity (TAC), plasma a-tocopherol, organoleptic characteristics, and growth performance in chickens fed with 5% olive paste flour (OPF) on top of the commercial diet in largescale production on a poultry farm unit. Two different groups of chickens were raised under the same conditions in an agricultural poultry unit, in large-scale production, until the slaughter for the proceedings of the experiments. Group A fed with conventional food of the poultry farm, while group B fed with 5% OPF on top of the commercial feed. TAC was assessed in plasma and muscle tissue following the DPPH method. Plasma a-tocopherol was determined according to fluorescence measurements. Body weight (BW) and liveability were recorded daily, and feed conversion ratio (FCR) and European production efficiency factor (EPEF) were calculated at the end of the experiment. Organoleptic characteristics were evaluated in roasted chicken breasts by many tasters. Statistical analysis followed the independent t-test. a-tocopherol was significantly higher for the group B than for the group A (p<0.05), while TAC was not significantly affected (p>0.05). FCR was significantly higher but EPEF lower for the group B compared to the group A (p<0.05). Group B smelled more intensely and were more flavour than the aroup A, however other organoleptic characteristics did not differ. The present study shows that the supplementation of 5% OPF in chicken diet increases plasma atocopherol levels, implying that it may be transferred from olive into chicken plasma through diet. These data could be exploited in large-scale poultry production.

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GROWTH PERFORMANCE AND BLOOD BIOCHEMICAL PARAMETERS OF BROILERS FED WITH OR WITHOUT PLANT-BASED ISOQUINOLINE ALKALOIDS

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Plant-derived isoquinoline alkaloids have shown improvements in growth performance, gut integrity (Kikusato et al., 2020) and intestinal parameters (Xue et al., 2017; Khadem et al., 2014). The objective of the present experiment was to determine the effects of a feed additive containing plant isoquinoline alkaloids on performance and blood biochemistry of broilers up to 42 days of age, 2,688day-old male Ross 308 broilers were randomly assigned to 7 treatments with 16 pens/treatment and 24 birds/pen. Floor pens were bedded with deep litter of clean wood shavings. The basal diet was supplemented with a standardized preparation of plant-derived isoquinoline alkaloids (Sangrovit® Extra, Phytobiotics GmbH, Eltville, Germany) at 0 (T1), 45 (T2), 60 (T3), 90 (T4), 120 (T5), 150 (T6) and 3000 mg/kg (T7). T7 was considered an overdosage. Birds were fed a mash starter (0-21 days) and grower diet (22-42 days) based on corn, wheat and soybean meal. Birds had ad libitum access to mash feed and drinking water. Performance parameters were determined at 21 and 42 days and haematological and blood biochemical parameters were determined using 2 birds/pen at 42 days. Data were analysed by one-way ANOVA using the General Linear Model function in SPSS. Pen was the experimental unit. Multiple comparisons between treatments were executed using the Tukey test. Significant differences were declared at $p \le 0.05$. In comparison with the control (T1), mortality-corrected FCR was improved (p < p0.0001) by supplementation with the plant-based additive in all dosages by 5-7%. Mortality-corrected ADG was increased by 5% in T2 and T4 (p < 0.005). The European Production Efficiency Factor (EPEF) was improved by 14, 12 and 10% in T2, T4 and T5, respectively (p = 0.0006). Overall mortality did not differ between treatments (p = 0.898) and ranged between 1.0 and 2.1%. There were no major effects on blood biochemical parameters. All haematology and clinical blood chemistry values were within reference ranges for healthy broiler chicks. In conclusion supplementation with the plant-based isoquinoline alkaloids improves growth performance and particularly feed efficiency in broilers, thus representing a dietary strategy to improve productivity in commercial poultry meat production. Furthermore, all zootechnical and blood data support that dietary supplementation with the additive is well tolerated by broilers up to 3,000 g/t.

A TRIPLE STRAIN PROBIOTIC DEMONSTRATES HIGH SIDEROPHORE ACTIVITY: A NEW STEP TOWARDS THE UNDERSTANDING OF PATHOGEN INHIBITION IN POULTRY

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Iron is a vital nutrient for virtually all forms of life. The requirement for iron is based on its role in cellular processes ranging from energy generation and DNA replication to oxygen transport and protection against oxidative stress. Bacterial pathogens are not exempt from this iron requirement. However, these organisms must acquire iron from within their animal hosts in order to replicate and cause disease. Chr. Hansen probiotic strains produce siderophores and are screened for this feature. One known siderophore in Bacillus subtilis is called bacillibactin. Research was conducted to evaluate siderophore production in a new 3-strain Bacillus-based probiotic in comparison to several strain present in the poultry probiotic market. When siderophores are produced overnight by the Bacilli growth, they will compete for iron uptake, chelating the iron. This leads to the blue dye color changing to green-yellow. This color change can be measured in a spectrophotometer as a drop-in absorbance when compared to an internal standard after 3 hours (delta T3-T0). The results show that the two Bacillus subtilis (DSM32325 and DSM32324) strains have a superior siderophore production with delta T3-T0 of -0,2149 and -0,1395 respectively compared to most of the Bacillus sp. strains present in the poultry probiotic market (from -0,0809 to +0,5515). In addition, the Bacillus amyloliquefaciens strain (DSM25840) also exhibits a higher siderophore production (delta T3-T0 of - 0,0776) than most of the comparative probiotics containing Bacillus subtilis (-0,0707 to +0,5515) and Bacillus velezensis strains tested in this assay (-0,061 to +0,0163). The capability of Chr. Hansen Bacillus triple-strains to produce superior siderophore activity represents an important property to compete for iron acquisition with pathogenic bacteria such as E.coli. By depriving them of this key element for their development and virulence properties, therefore they inhibit their growth (Harwood et al., 2018).

EFFECT OF SUPPLEMENTATION OF PHYTOBIOTICS ON THE GROWTH PERFORMANCE OF JAPANESE QUAIL

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Antibiotic growth promoters (AGP) in poultry feed has been banned in many countries due to their residues in products and development of antibiotic resistance in microbes. Nowadays, phytobiotics are commonly used as an alternative to antibiotics as natural growth promoters in the poultry feed. Hence, the present study was conducted to investigate the effect of herbal ingredients (phytobiotics) mixture (Ocimum tenuiflorum, Curcuma longa, Murraya koenigii, Trigonella foenum graecum, Allium sativum, Zingiber officinale, Piper nigrum, Foeniculum vulgare and Cuminum cyminum) on the production performance of Japanese quails in an organized Japanese quail farm, Chennai, India. A total of 360-day old Japanese quail chicks were randomly divided into four treatment group with three replicates of 30 chicks in each. Dietary treatments consisted of 0, 0.5, 0.75 and 1 % herbal ingredients mixture powder added to the basal feed. There was a significant difference ($P \le 0.01$) in body weight from 3 to 5 weeks of age between treatment groups. Japanese quail supplemented with 1 % herbal ingredient mixture had significantly ($P \le 0.01$) higher feed consumption and better feed conversion ratio. No significant difference in livability among treatment groups. The pre-slaughter live weight showed significant difference ($P \le 0.01$) among treatment and control groups; however eviscerated weight, ready- to- cook- yield showed non-significant difference among treatment and control groups. Supplementation of herbal ingredient mixture had non-significant effect on sensory attributes namely flavour, texture, juiciness, tenderness and overall acceptability. The present study concluded that supplementation of 1 % herbal ingredient mixture improved the growth performance of Japanese quail without affecting sensory attributes.

IN FIELD CONDITIONS, B-MANNANASE ALLOWS A FEED COST REDUCTION IN MEAT PRODUCING BROILER CHICKENS

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β-Mannans are antinutritional factors found in most feed ingredients. Those mannose polymers fibers are responsible of a wasteful innate response that reduce the productivity of poultry (1, 2). This property induces a significant and useless loss of energy. The study objective was to evaluate, in field conditions, the effect of 1,4- β -mannanase (enzyme) (HemicellTM HT Dry, Elanco Animal Health, Greenfield, IN, USA) in poultry. Fifty-one lots of broilers, raised in field conditions (including a total amount of 1°143°545 animals), were allocated to 2 treatment groups from week 36th, 2019 to week 48th, 2019. T1: Control (n=27), T2: as T1 less 60 kcal ME/kg + 330 g enzyme / ton of feed ie 52800 IU / ton of feed (n=24). For each lot, production parameters were collected: age at slaughter, BW, ADG, FCR, EPEF, Seizure at slaughtering, mortality. Moreover, 100% of the legs were scanned at the slaughterhouse for pododermatitis lesions scoring. Statistical analysis: a general linear model was used (using software JMP15) as Yi = μ + Ri + ε i with μ (Mean); Ri (feed effect); ε i (error). The criteria of success were "same performances between T1 and T2 with a 60 kcal ME sparing". On the overall production, no significance difference between T1 and T2 was observed for any parameters. The ADG was 55,16 vs 56,71 g/d for T1 and T2 respectively. As the volumes of feed consumption and animal growth were numerically improved in T2, FCR decreased (1,56 vs 1,54 respectively) and the total feed cost decreased by 6,4€/ton of broiler. Pododermatitis lesions decreased by 12,6% (41,3 vs 36,1%). When considering the "66% top breeders", T2 was significantly better than T1 (p<0,05) for "age at slaughter, BW, ADG, EPEF". The associated feed cost saving was 8,5 \in /ton of broiler. In this study, the use of a β -Mannanase in field conditions allowed to reduce feed costs by decreasing the energy expenses. Moreover, the decrease of pododermatitis lesion at slaughtering suggested health and welfare additional benefits for animals.

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FIELD EVALUATION OF THE EFFECT OF LIVE YEAST SUPPLEMENTATION ON BROILER PERFORMANCES

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Probiotics are defined as live micro-organisms which confer a health benefit on the host. One strain of yeast, Saccharomyces cerevisiae boulardii (SB), has been extensively studied for its probiotic effects (Czerucka et al., 2007). It has been used in human medicine for more than 40 years and is very well documented (Kelesidis et al., 2012). The objective of this trial is to study the effect of the supplementation of live yeast SB CNCM I -1079 (LY) on performance of broilers under field conditions. For this, over 2 consecutive years, the performances of a total of 16 broiler houses were followed from day of hatch to slaughter (35 days of age) on a commercial broiler farm. Each broiler house contained a total of 26000 broilers (Ross 308) at start (density: 20 birds/m²). Bedding material was wood shavings. Each year, 8 different broilers houses were included in the trial. Four of the houses received a standard 5- phase wheat-soy based broiler diet (T0) and the 4 other houses received the same diet but supplemented with LY at 1x10e9 CFU/kg feed (T1). Animals had free access to feed and water. Data on production parameters which include weekly body weight (BW) and average daily gain (ADG), feed conversion ratio (FCR) and liveability were recorded. Data were analyzed using a General Linear Model (SPSS 27.0, IBM), with treatment as fixed factor. BW at start was added as covariate for the analysis of the final BW. The year effect was tested and removed from the model since not significant. Supplementation with LY has resulted in significantly improved growth performances: +2.6% higher final BW (T0: 2.144 kg vs T1: 2.199kg; P<0.05) and ADG (T0: 60.0 g/day vs T1:61.6 q/day; P<0.05). FCR was numerically improved by 0.014 point. Besides, a significantly higher survival of + 0.9% could be recorded for the LY treated group (T0: 95.3% vs T1::96.2%; P<0.05). Results from this field evaluation indicate that the supplementation of broilers diets with LY has the potential to improve performances and survival under commercial conditions. This translated into an economic benefit for the farmer with a return on investment of 3:1.

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EFFECT OF CORN PARTICLE SIZE AND PELLET DIAMETER ON BROILER PERFORMANCE AND NUTRIENT DIGESTIBILITY FROM 1 TO 39 DAYS OF AGE

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The objective of this study was to evaluate the effect of corn particle size and pellet diameter on broiler performance and nutrient digestibility from hatch to 39 d of age. A total of 1,200 Cobb 500 male broilers were randomly assigned to 6 dietary treatments with 8 replicate and 25 birds/pen. The experiment consisted of a factorial arrangement of 3 corn particle sizes (750, 1150 and 1550 μ m) and 2 pellet diameters (3- and 4-mm). Diets were identical in nutrient and ingredient composition in all treatments, differing only in corn particle size and pellet diameter. Corn was ground with a hammermill equipped with 7.94-mm screen and a variable frequency drive to achieve target average particle sizes. Feed intake, body weight (BW), and mortality corrected feed conversion ratio (FCR) were determined on d 17, 27 and 39. Titanium dioxide was added as an indigestible marker (0.5%) during the finisher phase (27-39 d) and ileal digesta was collected from 4 birds per pen on d 39 to evaluate nutrient digestibility. Data were analyzed as a 3×2 factorial (corn particle size \times pellet diameter) and means separated using Tukey's HSD with statistical significance considered at $P \le 0.05$. Corn particle size did not influence BW (P > 0.05) at 39 d of age. However, broilers feed diets with 1150 μ m corn had lower feed intake (4629 vs. 4754 and 4656 g; P \leq 0.05) and lower FCR (1.51 vs. 1.53 and 1.53 g;g; $P \le 0.05$) than broilers fed diets with 750 and 1550 µm corn at 39 d of age. On d 17, broilers fed crumbles produced with 3 mm pellets had higher BW (773 vs. 754 g; $P \le 0.05$) and lower FCR (1.24 vs. 1.26 g:g; $P \le 0.05$) than broilers fed crumbles produced with 4 mm pellets. There was an interaction between particle size and pellet diameter on nitrogen (N)retention. Corn particle size did not influence N retention on broilers fed 4 mm pellets, however broilers fed diets with 3 mm pellets and corn particle size of 750 μ m had lower N retention (76 vs. 82 and 86%; P \leq 0.05) than broilers fed diets with 3 mm pellets and corn particle size of 1150 and 1550 µm, respectively. Broilers fed diets with corn particle size of 750 µm had lower IDE than broilers fed diets with 1150 and 1550 μ m (3018 vs. 3077 and 3157 kcal/kg; P \leq 0.05). Overall, reducing particle size of corn to 750 µm reduced N retention and IDE likely due insufficient gizzard stimulation and inadequate reverse peristalsis.

EFFECT OF SPLIT FEEDING SYSTEM ON NITROGEN RETENTION AND EMISSION OF LAYING HENS

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In our model experiment, the effect of split (two-phase) feeding system with different compositions and nutrient content of morning and afternoon diets on the nitrogen retention and emission of experimental animals was investigated. Nick Brown layers of 28 weeks of age were placed in metabolic cages and were randomly assigned to two experimental dietary treatments for 12 weeks: the split (n=24) and the conventional feeding program (n=24). The conventional diet had the same composition during the day while the morning diet of the split treatment had higher content of AMEn and crude protein and lower concentration of Ca compared to the afternoon diet.

In the case of split feeding, we observed lower (P<0.05) daily feed intake (108 vs. 114 g/hen/day), N-intake (2.51 vs. 2.80 g/hen/day) and significantly more favourable feed conversion ratio (1.83 vs. 1.93 kg/kg) than in the conventional feeding group. The egg production (%) and egg weight were similar in both treatment groups during the experiment. Based on the retention study performed at the end of the experiment, the N-retention of the animals in the two groups did not differ significantly. The hens in the split-feeding group excreted less N (1.49 and 1.6 g/hen/day) and the N-concentration of the excreta was lower (4.73 vs. 5.01 % in DM) than in the conventional group. Based on our result, split feeding technology may have a potential role in reducing the nitrogen emissions of layer flocks.

Supported by the ministry for innovation and technology from the source of the national research, development and innovation fund.

THE USAGE OF CHEMICALLY PRESERVED WHOLE MAIZE GRAINS USED IN PEKIN DUCK NUTRITION

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This study aimed to investigate the effects of a high-moisture whole maize grain (HM) addition in duck diets on their growth performance, selected internal organ parameters and gastrointestinal tract (GIT) measurements, and digesta pH values. In total, 300 29-d-old male Pekin ducks were randomly distributed to 3 dietary groups using 5 replicate pens per group. Birds had ad libitum access to water and feed for 21 d. The control pelleted diet was offered from 1 to 28 d of age, and after 29 d, the control diet was partially replaced by the addition of 5% (5HM) or 10%(10HM) chemically preserved HM. Body weight (BW) and feed intake (FI) were measured, and the body weight gain (BWG) and the feed conversion ratio (FCR) were calculated. Ten randomly chosen individual birds per treatment were stunned, sacrificed, and eviscerated to collect the materials for further analyses. The selected internal organ weight and lengths in relation to the BW were measured. All data were tested for normal distributions and homogeneity of variance using the Shapiro–Wilk test and Bartlett's test, respectively. Duncan's or Dunn's post-hoc test was used to determine the significance of differences between treatments at the significance level of p < 0.05. The implementation of 5HM or 10HM did not affect (p>0.05) the growth performance parameters, selected internal organ weights, or GIT segment weights and lengths. However, significant changes in terms of the gizzard (p=0.005), ileum (p=0.030), and cecal (p<0.001) diaesta pH were observed, especially in the case of the 10HM group, which exhibited the greatest increase in pH in the gizzard and cecal digesta and a decrease in ileal digesta pH. Additionally, the economical calculations clearly showed that the usage of both 5HM and 10HM may save up to 11.5 M \in in the European conditions. The implementation of whole wet maize may be used in waterfowl diets from 29 d of age. However, further investigation in terms of the microbial population and the impact of their activity on the pH value in the selected GIT segments, especially in the gizzard, as well as in the cecal digesta, is required. Additionally, chemical preservation can efficiently reduce the feed cost in duck nutrition.

EFFICACY OF A NEW MYCOTOXIN SOLUTION TO PREVENT THE ADVERSE EFFECTS OF MYCOTOXINS IN BROILERS

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The detrimental effects of mycotoxins in animal feed are well known. A new solution was developed to tackle the adverse effects of mycotoxins in animals by combining different strategies: from reducing bioavailability of mycotoxins to protection of organs, such as liver, supporting the immune system and preventing oxidative stress. To investigate the efficacy of this new mycotoxin solution, two trials were performed. A first study was conducted for 35 days on 96 male Ross 308 broilers. The trial was set up with 3 different treatment groups (8 replicate pens of 4 birds): (1) negative control (NC), (2) positive control (PC) (challenged with 800 ppb T-2 toxin), (3) positive control + 2 kg/T of mycotoxin solution (TOXFIN CARE, Kemin Europa NV) (MS). On day 14, the ileal villus to crypt ratio was numerically increased by the addition of the MS in contaminated diets (5.26 vs 4.54) compared to the PC. On day 35, the MS improved the liver color and relative liver weight of the birds (2.20 vs 2.65 %; P<0.05) receiving contaminated feed up to the level of the NC (2.33 %). Blood creatinine and malondialdehyde levels were also numerically improved by using the new MS in contaminated diets $(0.24 \text{ vs } 0.27 \text{ mg/dL} \text{ and } 18.5 \text{ vs } 19.3 \mu \text{g/L}, \text{ respectively})$ compared to the PC. A second trial was conducted, with same duration and assessing same treatments. The trial used 240 male Ross 308 broilers (8 replicate pens of 10 birds). Birds on all treatments (except the NC), received feed contaminated with 800 ppb T2-toxin, 450 ppb DON and 100 ppb Ochratoxin. At day 35, uric acid levels in the blood were numerically decreased by addition of the MS to the contaminated feed (7.04 vs 7.76 mg/dL) compared to the PC. A clear reduction in performance by the addition of mycotoxins was observed, as shown by the significant difference in daily weight gain (45.6 vs 40.4 g/bird) and feed intake (72.0 vs 66.9 g/bird) between the NC and PC (P<0.05). The new MS could avoid the performance losses in birds receiving the contaminated diet as the FCR of the PC was reduced (1.56 vs 1.70; P<0.05) to the level of the NC (1.64). In conclusion, the new mycotoxin solution showed beneficial effects on animal health together with significant improvements in zootechnical performance in broilers fed mycotoxin contaminated feed (T-2, DON and Ochratoxin).

MACRO ALGAE ULVA LAETEVIRENS AND SOLIERIA CHORDALIS CO-PRODUCTS IN BROILER DIETS: IN VITRO AND IN VIVO DIGESTIBILITY AND HEALTH RELATED EFFECTS

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Novel feed ingredients for the production of meat, milk and eggs are needed, which do not compete for currently used resources. Seaweed could be such a novel feed ingredient. This study aimed to determine nutrient digestibility and health-related effects of desalted seaweed co-products, and evaluate the effects of enzymatic hydrolysis (proteases Alcalase and Neutrase) on nutrient digestibility. A 2 \times 2 experimental design (Seaweed species×Enzymatic hydrolysis) plus added control was conducted. In total, 360 14-day old Ross308 male broilers were housed in 30 floor pens (0.96m2) with 12 birds per pen and 6 replicate pens per treatment. The basal diet (control) was diluted with 100 g/kg of one out of four seaweed products. Feed and water were available ad libitum and intake and bird weight were measured weekly. Faeces was collected qualitatively at pen level at d 26-27-28. At d 28, birds were euthanized and ileal digesta was collected. The villus height: crypt depth ratio (VL:CD) was analysed in duodenal tissue, IL13 and haptoglobin levels were analysed in blood plasma and jejunal content pH and gizzard weight were measured. Data were analysed using ANOVA with pen as experimental unit and significance stated at P=0.05. Body weight was not affected, but the feed conversion ratio (d14-21) was lower in birds fed untreated U. laetevirens (1.77) vs all other diets (1.84-1.94, P = < 0.001). For all nutrients, the apparent pre-caecal digestibility of the basal diet vs seaweed containing diets was higher (P<0.001), with a higher digestibility for U. laetevirens vs S. chordalis products. The enzymatic hydrolysis decreased digestibility of ash, nitrogen and multiple amino acids (P<0.05). Gizzard weight, jejunal pH and cytokine levels were not significantly affected by inclusion of seaweed products. Numerically, a 59% lower IL-13 level was observed in birds fed the untreated U. laetevirens diet vs the basal diet. Birds fed U. laetevirens vs S. chordalis diets had 11% shorter villi (P<0.001) and 10% lower VL:CD (P=0.006). Birds fed the treated vs untreated U. laetevirens diet had 8% deeper crypts, whereas the opposite was observed for birds fed S. chordalis diets (-4%; Seaweed×Enzyme effect, P=0.035). The seaweed digestibility coefficients indicate that these products interacted with the basal diet. Based on this experiment U. laetevirens is more suitable for inclusion in broiler diets, and the enzymatic treatment was not suitable for increasing digestibility.

BLACK SOLDIER FLY LARVAE FAT AFFECTS THE MICROECOSYSTEM OF THE BROILER CHICKEN GASTROINTESTINAL TRACT.

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This study aimed to determine the effect of Hermetia illucens larvae fat added to broiler chickens' diets as a partial (50%) or total replacement for soybean oil on a gastrointestinal tract (GIT)-selected microbial population and its activity. In total, 576 one-day-old female Ross 308 chicks were randomly allotted to 3 dietary treatments (16 replicates and 12 birds per replicate). Birds had ad libitum access to water and feed for 35 d. The following groups were applied: SO – 100% soybean oil, BSFL50 - a mixture of BSFL and soybean oils in a 50:50 ratio, BSFL100 -100% BSFL fat. The digesta samples from the crop, jejunum, and ceca were collected, frozen, and stored at -80°C for further analyses using fluorescent in situ hybridization. Gas chromatography was used to obtain short-chain fatty acid concentrations. Shapiro-Wilk and Bartlett's tests were used to determine normal distribution and homogeneity of variance, respectively. Duncan's or Dunn's posthoc test was used to determine differences between groups at the significance level of P<0.05. The application of BSFL fat in broilers' diets resulted in increased proliferation of potentially pathogenic bacterial populations in the crop. Furthermore, BSFL100 enhanced microbial activity via total SCFA production and lowered the pH in this segment. However, no detrimental effects were observed in terms of other GIT segments. The strongest impact on the reduction of select components of the microbial population in the cecum was observed with the BSFL50 treatment for potentially pathogenic bacteria such as Enterobacteriaceae, Bacteroides - Prevotella cluster, while commensal populations were also limited, i.e., Bacillus spp., C. leptum subgroup, and C. coccoides – Eubacterium rectale cluster. The present study revealed that BSFL fat led to adverse microbiota shifts in the chicken crop because of insufficient release of lauric acid. Nevertheless, the neutral effect of BSFL fat administration on the chicken jejunal microbiota and its activity or positive influence on cecal bacterial population content were noted.

This work was supported by National Science Center project no. 2020/39/B/NZ9/00237 titled "The role of Hermetia illucens larvae fat in poultry nutrition – from the nutritive value to the health status of broiler chickens".

EFFECTS OF A PHYTOGENIC ADDITIVE IN MYCOTOXINS MANAGEMENT IN COMPARISON WITH A KNOWN BINDER: AN APPROACH BY INCREASING THE TOLERANCE THRESHOLD OF FEED CONTAMINANTS.

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Feed contaminations by mycotoxins is still problematic nowadays. Mycotoxins have an impact on global performances in any animal production. Among more than 300 myctocoxins, aflatoxins, ochratoxin A, fumonisins, tricothecens and zearalanon are the most toxic ones. This trial conducted in an experimental facility for 21 days with a poly-contaminted feed aims to evaluate the efficiency of a phytogenic additive (clay-free) on zootechnical performances in comparison to a reference clay-based binder (including enzymes and phytogenics) available on the market. 240 one-day-old chicks with an average weight of 45.7 g are randomly divided into four groups. Each group comprises six replicates of 10 animals. The first group is the control group of which the feed is not contamited. For the 3 other groups, the feed is contaminated by way of experiment with three mycotoxins produced naturally by fungi at a rate of 1 ppm of aflatoxins, 50 ppm of fumonisins and 40 ppm of deoxynivalenol. Among these 3 groups, one is the positive control which only received the contaminated feed, one is the "binder" group and received the reference binder incorporated at 0,1% in the feed and the last is the trial group which received a phytogenic additive (a blend of plant extracts including Foeniculum vulgare, Rosmarinus officinalis and others) incorporated at 0,025% in the feed. At D21, zootechnical performances such as weight, feed conversion ratio (FCR) are calculated. On 5 birds per treatment, villi height / crypt depth ratio and relative weight of liver are measured. The data underwent an analysis of variance (one-factor ANOVA). The differences in the averages were compared by means of a Bonferroni test (P-value \leq 0.05). The results show that the group has a similar weight (764g) than the negative control group (747,7g) and a higher weight than the binder group (714,2g). The FCR of the trial and negative control groups are equal (1,57). The villi height / crypt depth ratio of the trial group is higher (10,03) than the other groups (negative control 8.34, positive control 8.64 and "binder" group 7.81). No difference between negative control, the binder group and the trial group was found regarding the relative weight of liver. The phytogenic additive, incorporated at 0,025% in a mycotoxin's poly-contaminated feed allowed birds to have similar and, in some case, better zootechnical performances than a reference binder, even though birds consumed mycotoxins.

THE BENEFICIAL EFFECT OF BACILLUS LICHENIFORMIS AND SALINOMYCIN MIXTURE ADDITION IN BROILER CHICKENS' DIET ON THEIR GROWTH PERFORMANCE AND GUT MICROBIAL POPULATION.

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The present study aimed to examine the effect of Bacillus licheniformis and salinomycin used as individual factors or in combination in broiler chicken diets on growth performance and microbiota populations. A total of 400 one-day-old female Ross 308 chicks were randomly distributed to 4 dietary groups (10 replicate pens, 10 birds each). Birds had ad libitum access to water and feed for 36 d. The following treatments were applied: NC-no additives; NC+SAL-salinomycin addition (60 mg/kg diet), NC+PRO-B. licheniformis preparation (1.6 \times 109 CFU/kg; 500 mg/kg diet), and NC+SAL+PRO—combination of salinomycin and B. licheniformis. The B. licheniformis (DSM 28710) preparation is recognized as safe by the ESFA and approved as a feed additive by the European Commission (UE, 2017/1904). The following variables were measured: body weight (BW) and feed intake (FI) on d 10, 22, and 36, and the following was also calculated: body weight gain (BWG) and feed conversion ratio (FCR). At the end of the experiment, 1 bird from each replicate was sacrificed and eviscerated to collect the crop, jejunal, and cecal digesta. The pH value of their content was measured immediately after slaughter. The remaining portion of the crop, jejunal, and cecal content was pooled based on two birds per bag and immediately frozen and stored at -80° C for nextgeneration sequencing analysis. Shapiro-Wilk and Bartlett's tests were used to determine the normal distribution and homogeneity of variances, respectively. Next, it was adopted to evaluate the homogeneity of variances. Dunn's test after Kruskal-Walli's test or Scheirer-Ray-Hare test was used to determine differences between treatments at the significance level of p<0.05. Probiotic addition resulted in improvement of BWG (1-10 d and 11-22 d) and FCR (11-22 d and 1-36 d). An interaction (p < 0.05) between experimental factors was noted in terms of lower pH values in the crop and ceca. Both factors lowered the alpha diversity and Enterobacteriaceae and promoted Bacillaceae communities in the jejunum. Interactions were also observed in terms of reducing Clostridiaceae in the ceca. The results of the study confirmed that the usage of B. licheniformis in broiler chicken diets has beneficial effects on growth performance. Furthermore, the usage of both factors resulted in modulation of selected microbiota populations through the whole broilers' GIT.

PREPARATION OF PULLETS: EFFECTS OF A LIGNOCELLULOSE SUPPLEMENTATION IN PRE-LAYING PHASE ON GUT MORPHOMETRY OF YOUNG LAYING HENS AT START OF LAY UNDER FIELD CONDITIONS

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A well-prepared pullet is the basis for high laying performance, a robust eggshell quality and stable bones. Young birds need to receive an appropriate fibre supply in their rearing phase for best development of their gastrointestinal tract to endure the intensive phase of egg production and to ensure good conditions at the onset of laying. In the present study the impact of two different qualities of fibre supplements on the intestinal development were evaluated and correlated with production performance. 50.000 cage housed Hisex White pullets were randomly allocated to two different treatments provided with two diets adjusted to identical fibre as well as protein levels. The diets differed in the quality of fibre ingredient, which was either wheat bran or a commercial wood derived insoluble but partly fermentable lignocellulose (LC) product (OptiCell®, Agromed Austria GmbH). In a three-phase feeding regime, age dependent requirements of fibre levels were considered since the trial lasted between week 8 until week 22 of age. Ad libitum supply of feed and water was ensured. The light regime followed the breeder recommendations. At 22 weeks of age, 10 birds were randomly selected for and collection of intestinal segments for necropsv macroscopic and histopathological analysis. The analysis of differences between treatments was performed by student's T-test. Results demonstrate a significantly higher villi and villus/crypt ratio, as well as a lower crypt depth in the jejunum of birds fed the LC rich diet compared to birds fed a diet with wheat bran as fiber source. This increase of resorptive capacity corresponds to a numerically improved maximum laying rate in the first three weeks after the trial end (79.4% vs. 67.8%) as well as an earlier onset of laying phase. The inclusion of insoluble and partially fermentable LC in the diets of growing and pre-posture pullets improved the development of the birds' intestinal tract. This allows for an efficient use of nutrients and consequently for a substantial improvement in eqg production in terms of an earlier start of the laying phase and optimized laying performance.

ENHANCEMENT OF INTESTINAL EPITHELIAL BARRIER FUNCTION BY A SYNERGISTIC IN-FEED TECHNOLOGY THROUGH UPREGULATION OF MUCIN AND TIGHT-JUNCTION PROTEINS IN BROILERS UNDER DIETARY CHALLENGE IN REAL FARMING CONDITIONS

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Dysfunction of the intestinal barrier is associated with increased microbial translocation and disease risk, impaired nutrient absorption, and reduced growth performance in broilers. Here, we evaluated the protective gut barrier effects of Lumance® (Innovad, Belgium; esterified butyrate, combined with plant extracts, essential oils, and other fatty acids): a) in-vitro, via an established permeability model (Transepithelial Electrical Resistance: TEER in IPEC-J2 cells) and, b) in-vivo, in a dietary-induced chronic inflammation model (high NSP diet: 60% Wheat + 5% rye without NSPase and coccidiostats), via the jejunal mRNA gene expression of mucins and tight-junction proteins in broilers. The impact on growth promotion was also monitored under two concentrations of Lumance® (1 or 2 kg/ton) in feed and a control (n=8 pens/treatment; n=30 birds/pen). Importantly, the pens were housed inside a commercial production unit of 55,000 broilers so that the experimental birds could get exposed to the same (real) farming conditions. Invitro, through the enhancement of TEER, Lumance® (1000 ppm) completely reverted LPS-induced barrier dysfunction at 12 hours post-challenge and increased TEER by 2-fold at 24 hours post-challenge (P<0.01). In-vivo, One broiler per pen was randomly selected on D28 and D35 for the determination of the intestinal expression of tight-junctions and the plasma level of FITC-d (the latter only at D28). ANOVA with Tukey post-hoc analysis revealed that Lumance® 1 and 2 kg/ton when compared to the control treatment, increased significantly the BW of broilers at D35 by 3% and 4%, respectively (P=0.040) and reduced the FCR (1.64, 1.62 and 1.57, respectively; P=0.01). FI was not affected by the treatments (P >0.05). A trend in reduction of FITC-d levels in plasma (P=0.098) was seen at D28 in birds receiving Lumance \mathbb{R} compared to the control. Lumance \mathbb{R} 1 and 2 kg/ton resulted in a profound increase of intestinal expression of Claudin-1 (approximately a 4- and 5-fold increase, respectively), Occludin and Mucin-2 (approximately a 2and 3-fold increase, respectively, for the latter two genes) compared to the control, both at D28 and D35 (P < 0.005 in all cases). In this study, broilers fed with an in-feed technology of plant extracts, fatty acids, and essential oils conferred a superior protective effect on gut barrier function, accompanied by enhanced growth performance. The positive results warrant further work for the elucidation of related mechanisms.
EFFECT OF PHYTOBIOTICS MIXTURE CONTAINING HOT PEPPER FRUIT, WHITE MUSTARD SEED, SOAPWORT ROOT, TURMERIC RHIZOME AND THYMOL ON SELECTED PRODUCTION PARAMETER IN CHICKEN BROILER

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In the era of reducing antibiotics (also used as antibiotic growth promoters), phytobiotics play an important role in poultry production1. A number of scientific studies have shown not only the beneficial effect of phytobiotics on the health of chicken broilers, but also a significant improvement in basic production parameters. The aim of this study was to evaluate the effect of phytobiotics mixture (AdiCox® AP) on Feed Conversion Ratio (FCR) and European Production Efficiency Factor (EPEF) in broiler chicken. Day-old Ross 308 broiler chickens were assigned to 2 groups; experimental group (EXP) where AdiCox® AP (100g/tone of commercial feed) was administered and a control group (CTRL) without the addition of complementary feed. Each group was repeated twice per cycle. The experiment consisted of the observation of 3 complete cycles with randomisation of the barns (commercial farm). FCR and EPEF were counted in two variations: a) exact slaughter time, b) standardised on day 40. Obtained data were statistically analyzed using 2-tailed Student's t test. Values of p < 0.05 were considered to be statistically significant. In this study, no significant differences were observed in the EPEF and FCR values of the groups for both variants of calculation. However, 16.5 points in EPEF difference was obtained: 385.5 as a mean for CTRL, and 402 for EXP, respectively. Lower FCR in the EXP group (1.53 EXP vs 1.595 CTRL) was also recorded. The results may clearly affect the economic aspect of broiler production (Day 40). Similar differences were observed for the exact slaughter time calculation. On the basis of present findings, it was concluded that supplementation of AdiCox® AP in broilers' diet improved their performance (FCR and EPEF). Therefore, considering the harmful effect of antibiotic growth promoter on consumer's health, AdiCox® AP can be recommended to use as growth promoter in broiler production.

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OPTIMIZE PHYTASE DOSE ACCORDING TO PHYTATE LEVELS IN BROILER FEED FORMULATION

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Phytic acid [inositol (1,2,3,4,5,6) hexakisphosphate] or phytate (salt of phytic acid) is the major (60-80%) phosphorus (P) storage in plant-based ingredients. However, because mono-gastric animals have limited capability of utilizing the phytate-bound P, exogenous phytase enzyme is commonly added to poultry diets. When first launched, phytase was commonly used at 500 FTU/kg with the main purpose of reducing the P excretion to the environment. According to literature, with more efficient and cost effective phytases available in the market, the phytase inclusion rate has recently increased to up to 5000 FTU/kg. However, in order to maximize phytate phosphorus digestion and minimize P excretion in the environment, optimal dose of phytase should be related to the level of the phytate substrate in the diet. Phytate content of major feed ingredients could be derived from literature or feed tables but this does not account for variability in different plant-based feedstuffs, not only due to plant type, but also depending for instance by the growing conditions and soil types and nutrient contents. This study reports the analyzed phytate-P content in major feed ingredients from different regions analyzed by Danisco Animal Nutrition (IFF) lab in the last 13 years. In total more than 1000 samples were analyzed. The data showed a large variation in phytate-P content for the same feed ingredients. For instance, the phytate-P content in corn samples was included in a range of 0.14 to 0.29% (288 samples). For SBM, phytate-P content was in a range of 0.29 to 0.54% (234 samples), and the range for wheat (101 samples) and barley (64 samples) was from 0.18 to 0.32% and 0.14 to 0.34% respectively. For ingredients with high phytate content, the variation was even bigger, for example, phytate-P content was in the range of 0.60 to 1.10% and 1.31 to 2.06% for wheat bran (68 samples) and rice bran (48 samples) respectively. Danisco Animal Nutrition (IFF) has developed a phytase dose optimization model, to help customers determine the optimal phytase dose based on ingredient prices and substrate levels. In order to help animal producers to maximize P digestion and therefore minimize P excretion in the environment, Danisco Animal Nutrition (IFF) has implemented in its laboratories a fast, reliable service to analyze phytate and calculate phytate phosphorus.

THE EFFECT OF XYLANASE AND FERMENTABLE XYLO-OLIGOSACCHARIDE SUPPLEMENTATION ON BROILER CHICKEN

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The use of locally available or alternative less expensive feed components such as industrial by-products is on the rise, however, the inclusion rate of such unconventional substances is often limited due to their indigestible non-starch polysaccharide content. A study was conducted to determine the effect of fibre, xylanase, xylo-oligosaccharides (XOS), and a combination of xylanase and xylooligosaccharides (STBIO) on chicken growth performance, energy, and nutrient availability, jejunum histomorphometry and caecal content of short-chain fatty acids (SCFA). The 35-day experiment was evaluated and approved by the Harper Adams University Research Ethics Committee. The experiment was conducted at the National Institute of Poultry Husbandry (UK) and performed on 1920 Ross 308 broiler chicks, reared in 96 pens and fed ab libitum. Experimental diets were split into two phases; starter (0-21d) and finisher (22-35d). The first of the basal diets contained 54% maize and in the second, 5% of the maize was replaced by wheat bran. The basal diets were split into four batches, one used as a control, and each of the others were supplemented either with xylanase (16000 BXU/kg), XOS or with the stimbiotic combination of xylanase and XOS (16000 BXU/kg) (AB Vista, UK). The data were analysed in GenStat (20th edition) by ANOVA using a 2 (basals) x 4 (additives) factorial design. Added STBIO improved feed conversion ratio (FCR) and increased weight gain (WG) from 21 to 35d and from 0 to 35d (P < 0.05). The inclusion of additional fibre from wheat bran had a negative effect on N and fat retention coefficients at 35d as well as N corrected apparent metabolisable energy and dry matter retention at 21 and 35d (P < 0.05). At 21d neutral detergent fibre (NDF) digestibility was increased when xylanase and STBIO were added to the diet (P < 0.001) and at day 35 the highest retention was noted when the diet was supplemented with wheat bran and xylanase or STBIO (P = 0.001). There was no dietary effect on jejunum histomorphometry (P > 0.05). The addition of wheat bran increased the quantity of caecal SCFA, as well as valeric and propionic acid at 35d old birds (P < 0.05). It can be concluded that the addition of STBIO in a maize-based diet could provide benefits in terms of fibre degradation, weight gain, and feed efficiency.

THE EFFECT OF CARVACROL IN BROILERS DEPENDS ON BLEND COMPOSITION OF THE PLANT EXTRACTS AND ON THE CHALLENGE ACUITY, INSIGHTS FROM A META-ANALYSIS

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Essential oil and aromatic plants appear to be interesting alternative to antibiotics growth factor based on some experiments while others did not confirm this effect. A meta-analysis focusing on the response of broilers growth performance to carvacrol was performed to better understand and optimise their effects. An exhaustive database was built based on dosage lower than 50 g/t carvacrol which was the most studied dose and acceptable in terms of price. It includes 23 publications and 52 different experiments that have studied different types of carvacrol products (ExtractCode): oregano essential oil (HEo), essential oil blends (HE), synthetic carvacrol (CS), and synthetic carvacrol with spice extracts added (CS+). Some of the experiments have included a positive control (C+) with antibiotic growth promoter, a negative control (C-) without antibiotic, and a carvacrol treatments (n=22). To test the hypothesis that carvacrol response was influenced by the challenge the bird experienced, the relative difference between them (C+/C-) was calculated. The supply of digestible lysine vs requirement (CodeLys) was also calculated and tested. Multiple linear regression models were used to study the response of broilers in terms of average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) expressed as a percentage of C- or not. The X variables were CarvacrolDose, C+/C-, ExtractCode, and CodeLys. The response to carvacrol was dependent on the type of product (ExtractCode x CarvacrolDose, P < 0.001). ADG increased with the addition of carvacrol more in the CS+ group (10%), followed by HEo (7.9%), HE (6.8%), and CS (5.5%). Similar results were obtained for ADFI (ExtractCode x CarvacrolDose, P = 0.013). The response to carvacrol also depends on the level of digestible lysine supply relative to requirement (CodeLys x DoseCarvacrol, P<0.001) showing a greater response at 90% of requirement excepted for CS+ for which the effect was independent of CodeLys. FCR decreased linearly with Carvacrol dose (P<0.001). The effect of C+/C- was only significant for ADG and showed that the response of carvacrol increased linearly as the C+ is higher than C- (DoseCarvacrol x C+/C-, P<0.001); adding 15 g/t of carvacrol resulted in an increase of 0.8, 2.5, and 4.8% of ADG when C+/C- was at 100, 105 and 110% respectively. These results confirm a positive effect of carvacrol on growth performance and modulating factors have been identified.

EFFECTS OF DIETARY SUPPLEMENTATION WITH GINSENG BY-PRODUCTS ON GROWTH AND BLOOD BIOCHEMICAL CHARACTERISTICS OF BROILERS

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Ginseng contains various bioactive substances and has been used as a medicinal plant for a long time. However, due to its high cost, only a few studies have investigated the effect of the application of ginseng by-products as feed additives in livestock. Consequently, the present study analyzed the effects of diet supplementation with ginseng by-products, including berries, stems, and leaves, on the growth performance, organ development, and blood biochemical characteristics of broilers. One hundred and twenty-one-day-old male broiler chicks (Ross 308) were randomly allocated to five groups: control (CON), 0.5% ginseng berry (GB1), 1.0% ginseng berry (GB2), 0.5% ginseng stems and leaves (GLS1), and 1.0% ginseng stems and leaves (GLS2). Ginseng berry extract was centrifuged to separate the seeds from berries, dried at 110°C for 72 h and then ground to powder. Ginseng stems and leaves were dried naturally and ground to powder. Both powders were mixed with the basal diet. Body weight gain and feed intake were measured weekly. Blood samples were collected from wing veins of five birds selected per treatment at 35 d. Data were analyzed using one-way Analysis of Variance using the GLM procedure in SAS v9.3, and Duncan's multiple range test, at p < 0.05. Body weight gain in groups fed with ginseng by-products during the grower period (1-21 d) was significantly higher (p<0.05) than that in the control group. Feed intake in the GLS2 group during the finisher period (22-35 d) was significantly higher (p < 0.05) than in the other groups; however, no significant differences in body weight gain or feed conversion ratio were observed among the groups. In addition, no significant differences were observed in growth performance among treatments throughout the period (1-35 d), and no significant differences were observed in serum biochemical profiles, excluding blood glucose. Glucose levels were significantly lower (p < 0.05) in the GLS groups than in the control group, and lower in the GB groups than in the control group, with no significant difference. In conclusion, dietary supplementation with ginseng byproducts could enhance early growth of broiler chickens and reduce blood glucose levels without any negative effects.

FUCUS ALGAE OF THE WHITE SEA IN FEEDING HENS

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Brown algae of the genus Fucus are a source of trace elements such as iodine and bromine in a bioavailable form, as well as a source a fucoidan, a polysaccharide that stimulates the growth of the number of Bifidobacterium in the intestine. Thus, Fucus algae can be used as a prebiotic and an anti-stress drug in poultry feeding. The aim of the study was to evaluate the effect of Fucus meal (0.2-0.8 mm fraction) in the feeding of laying hens. The experiment was conducted with laying hens (industrial cross "Hisex Brown"), aged 29-33 weeks. The egg-laying rate in the control and experimental groups at the beginning of the experiment was the same and amounted to 93.35 %. Fucus meal was obtained from dried brown algae collected on the coast of the White Sea, in the Belomorsky district of the Republic of Karelia in August, 2020. Fucus algae meal was supplied in the diet in the concentrations of 0.5 %, 1.0 %, and 1.5 %. Biochemical, zootechnical, and molecular genetic research methods were used to evaluate the effects of Fucus algae. The effect of the algae feed supplements on egg quality traits and egg weight was evaluated. In using the feeds containing Fucus algae meal no significant differences in live weight between the control and experimental groups were revealed. It should be noted that the productivity index of laying hens fed with additional algae in the amount of 0.5 % and 1.0 % was at the level of the control group. The egg-laying rate in the experimental group, which received 1.5 % of Fucus meal, was significantly lower than the control index by 1.2 %. Nevertheless, the eqg weight in the experimental groups was significantly higher than in the control one by 0.92-1.6 %. The differences in egg quality traits were also revealed, which confirms a positive effect of Fucus algae supplements. Thus, according to the results of research, it was found that the introduction of the large concentration of meal from brown algae of the genus Fucus does not significantly affect productivity indices of industrial laying hens. The positive effect of Fucus meal on the mass and egg quality traits was revealed.

COMPARATIVE EFFECT OF PARTIAL VITAMIN E REPLACEMENT WITH DRY GRAPE SEED EXTRACT (VITIS VINIFERA SPP. VINIFERA) ON BROILER PERFORMANCE AND ANTIOXIDANT STATUS UNDER STRESS CONDITIONS

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Polyphenols have demonstrated antioxidant activity, even more beneficial under stress conditions for broilers. As it contains high levels of polyphenols and proanthocyanidins, dry grape extract (Vitis Vinifera spp. Vinifera, Nuxafen®, Nuproxa Switzerland) is a promising alternative to synthetic antioxidants. The partial replacement with a 1:11 ratio of half of synthetic vitamin E (VE) with dry grape extract (DGE) and its effect on broiler zootechnical performance and antioxidant status was investigated. 900 Ross 308 broilers were randomly divided into 5 groups of 9 replicates of 20 males and until 42d. A basal starter diet (control - T1) was formulated with 22 % CP, 2901 kcal/kg ME and no added source of vitamin E. The other treatments were added on top as followed, T2: 50 mg/kg feed of VE, T3: 200 mg/kg VE, T4: 25 mg/kg VE + 2.27 mg/kg DGE (considered an equivalent of 50 VE with the 1:11 replacement ratio), T5: 100 mg/kg VE + 9.09 mg/kg DGE (considered an equivalent of 200 VE). Likewise, basal grower diet was 20.4 % CP and 3001 kcal/kg ME, and finisher 18.8 % CP and 3100 kcal/kg ME, no added vitamin E. The study was carried out under three stress factors: density (more than 40kg/m² at 42d), temperature 30°C from 11 to 6 pm during 29-42d and reused litter. Data were checked for normality and homogeneity of variance and analysed by one-way ANOVA followed by a Tukey post hoc, using SAS. Body weight, daily growth and litter scoring remained similar among treatments during all periods (p > 0.05). On the period 35-42d, when highest stress was applied, T1 had a higher feed intake than the other groups supplemented with antioxidants, which resulted in a higher FCR at 42d for the control group (1.56 ± 0.01) and proportionally lower FCR to antioxidant levels for the supplemented groups (T2: 1.55 ± 0.02 , T3: 1.53 ± 0.01 , T4: 1.55 ± 0.02 , T5: 1.52 ± 0.01 ; p < .0001). No differences in breast yield and meat exudation could be measured (p > 0.05) between the treatments, as well as meat colour. Total antioxidant capacity tended (p < 0.07) to be higher for T3 (1.14 ± 0.26 mmol/L) and T5 (1.17 ± 0.18), the two groups equivalent to 200 ppm of VE, compared with the control (1.11 ± 0.24) . As such, substituting 50% VE with a 1:11 ratio of DGE didn't affect zootechnical performances or carcass quality and seem to support the bird during stressful episodes. The antioxidant support of DGE compared to VE should be further investigated.

EFFECT OF FEEDING LOW PHYTATE MAIZE WITH VARYING DIETARY CONCENTRATIONS OF PHOSPHOROUS ON PERFORMANCE AND BONE BREAKING STRENGTH IN GRAMAPRIYA BIRDS DURING NURSERY PHASE

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High phytic acid present in maize is considered an anti-nutritional factor that sequesters important nutrients and limits their bioavailability. Some of the mutants of maize reported to have reduced phytic acid content. Through advanced breeding techniques, some of the hybrids were developed (FLPH-24). The experiment was conducted to assess the feeding value of low phytate maize (FLPH-24) with varying levels of dietary P concertation on performance, slaughter variables and bone breaking strength in Gramapriya birds during nursery phase. For the purpose, Gramapriya birds (day-old age; n=432; a rural variety) were randomly distributed to 6 dietary treatments having 12 replicates with 6 birds in each replicate. The phytate content of the low phytate maize was 41.4% lower than the normal maize. For conducting the feeding trial, three diets with normal maize with varying concentrations of P (0.25%, 0.33% and 0.40%) and three diets with low phytate maize with varying concentrations of P (0.25%, 0.33% and 0.40%) were computed and fed the experimental birds up to 6 weeks of age and recorded the performance parameters. Body weight gain (BWG) during first week did not vary significantly. However, better (P<0.03) feed conversion ratio (FCR) among the groups fed normal maize + 0.40% P and low phytate maize + 0.40% P compared to other groups. BWG during 3 and 6 weeks was higher (P<0.01) in groups fed low phytate maize (with different P content) compared to those groups fed normal maize. Similarly, the FCR during 3 weeks did not vary among different groups but during 6 weeks, the FCR was improved (P<0.02) significantly among groups fed normal maize or low phytate maize with P 0.33% compared to those groups fed normal maize + 0.40% P. Feeding low phytate maize did not affect slaughter variables in the present study. Bone breaking strength (compressive strength N/mm2) was higher (P < 0.02) among the groups fed low phytate maize + P 0.40% compared to other groups. Therefore, it is concluded that the feeding diets with low phytate maize is advantageous over the diets with normal maize as improved body weight gain, feed conversion ratio and higher bone breaking strength was recorded in the present study in Gramapriya birds.

BACILLUS VELEZENSIS PROBIOTIC, DEMONSTRATES IMPROVED PERFORMANCE OF TURKEY POULTS FED ON A WHEAT/SOY-BASED DIET

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Bacillus velezensis is a multi-strain probiotic. Bacillus velezensis strains have demonstrated to inhibit the growth of non-beneficial microbiota isolated from turkey gastrointestinal tracts. This study aimed to measure performance benefits in turkey Poults. 736 BUT10 male turkey poults were allocated to 2 dietary treatments with 16 replicate pens per treatment (23 birds/pen). The control diets were wheat based and fed over four phases; starter crumble (1-28 days), grower pellet (29-56 days), developer pellet (57-84 days) and finisher pellet (85-112 days). The basal diets were either fed un-supplemented or supplemented with a 3 strain Bacillus velezensis combination at 150.000 CFU/g feed. All diets were fed ad libitum. Body weight (BW) and feed intake (FI) were recorded on days 1, 28, 56, 84 and 112 and feed conversion ratios (FCR) and European Production Efficiency Factor (EPEF) were calculated. Results were compared using one-way ANOVA with significance level of P< 0.05. BW gain (kg) was similar for grower phase (4.79) for both groups. However, FCR was reduced by seven points (2.58 vs 2.51) in the group fed Bacillus velezensis. Similarly, from grower to finisher phase, BW was slightly better for Bacillus velezensis group (4.09 vs 4.39 kg), and FCR was ten points better (2.72 vs 2.62) compared to control group. Overall, this study demonstrated that Bacillus velezensis fed group showed a significantly improved FCR by 5 points (P < 0.05) compared to the control group and significantly improved BW gain by 3.1% (P < 0.05) compared to the control group. Finally, the results showed that EPEF was significantly increased 464 vs 486 (P < 0.05). It can be concluded that the utilization of Bacillus velezensis at 150.000 CFU/g feed in turkey diets, can improve performance parameters as well as provide an economical return.

THE RESPONSE OF BROILERS TO DIETARY ENERGY LEVEL IS DEPENDENT ON DIETARY CRUDE PROTEIN CONTENT

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Energy (E), followed by protein (CP), are the most expensive components in poultry diets. Variation of dietary CP and E supply may affect broiler performance and profitability, together with consequences on nutrient excretion, impacting the environment. Our purpose was to study the effect of a dietary E reduction in different CP diets. 936 Ross 308 male broilers were fed over 21-35 days of age with 12 treatments (6 replicates of 13 birds per treatment) corresponding to the combination of 3 levels of CP (CP-19, CP-18 and CP-17, in %) and 4 levels of E (E-3200, E-3100, E-3000 and E-2900, in kcal AMEn/kg) in a factorial design. Diets were based on corn, wheat, soya oil and soybean meal and contained adequate levels of indispensable amino acids (TD basis). Measurements of body weight (BW), daily gain (ADG), feed intake (FI), feed efficiency (FCR) and carcass guality (dressing percentage, breast meat, abdominal fat, etc.) were carried out. Firstly, the interaction between E & CP, tested by a 2-way ANOVA showed no significant interaction (p>0.05) on any of the studied indicators. Therefore, only the main effects of CP and E are reported. Compared to CP-19, CP-18 and CP-17 significantly increased FI (+4%), BW (+2%), ADG (+3%) but did not affect FCR. Abdominal fat was significantly higher (+5%) only in CP-18, and breast meat (+2%) only in CP-17, compared to CP-19. For E, only E-2900 differed significantly from E-3200 in FI (+3%), BW (-3%), ADG (-5%), carcass dressing (+1%) and abdominal fat (-7%). Consequently, daily AMEn intake decreased linearly with dietary E reduction (p<0.01). Secondly, the interaction was also tested with a linear regression model to study the strength of the response to E at various levels of CP. It was significant for only FCR (p=0.03), which was increased by 0.06, 0.03 and 0.04 points per 100 kcal AMEn decrease at CP-19, CP-18 and CP-17, respectively. A trend was also observed for FI, showing that the FCR interaction may be driven by FI. In conclusion, reducing CP increased FI but did not affect growth performance and feed efficiency. The response of FCR to E level was stronger with high dietary CP level. A minimal amount of E seems to be required (>2900 kcal/kg) to avoid negative effects on growth performance and carcass guality in growing broilers. The trial helps to better understand the effect of a reduction in both dietary CP and E to improve dietary efficiency, but the E system and broiler requirements might need to be re-evaluated.

VARIATION IN NUTRIENT PROFILE OF BLACK SOLDIER FLY LARVA MEAL FROM DIFFERENT SOURCES AND EFFECTS OF ITS DIETARY INCLUSION ON PERFORMANCE, SERUM BIOCHEMICAL PROFILE, IMMUNE RESPONSE AND CARCASS YIELDS IN BROILER CHICKEN

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A study was conducted to evaluate the variation in nutrient profile of Black soldier fly (Hermetia illucens) larva meal (BSFLM) from different sources and its feeding value in broiler chicken diet. Three samples (A, B and C) of BSFLM were procured from different production sources in India and analysed for proximate principles and amino acid, and fatty acid profile. A feeding experiment was conducted to evaluate one of the samples (B) of BSFLM in commercial broiler chicken (n=216) at 0, 2.5 and 5.0% in diet on iso-caloric and iso-nitrogenous basis from 1 to 42 days of age. The response of chicks was assessed in terms of performance, serum biochemical profile, cellular immune response (PHA-P response), dressing yields and weights of visceral, and lymphoid organs. The crude protein content ranged from 49.8 to 59.6% among the BSFLM samples, while the crude fat was in the range of 22.1 to 38.4% indicating great variation in the nutrient composition of the larva meal procured from different sources. The BSFLM samples were rich in various amino acids, some of which were higher in concentration than in soybean meal (SBM) (by 68% in lysine, 249% in methionine, 50% in threonine and 78% in valine), whereas the concentration of tryptophan was lower in BSFLM in comparison to SBM. The fatty acid profile of BSFLM indicated higher concentration of lauric acid (62% in sample B), whereas the unsaturated fatty acids were also in considerable concentration in sample C (oleic and linoleic acids). Body weight of broiler chickens was significantly (P<0.05) higher in the groups fed BSFLM in comparison to the control group during the early life (0-3 weeks). However, during the subsequent 4-6 weeks of age, the body weight was similar among the groups. Feed intake tended to increase with dietary inclusion of BSFLM, whereas FCR was largely unaffected, except during 3rd week, where the highest FCR was recorded with 5% BSFLM. The serum concentration of total protein, albumen and cholesterol, and the cellular immune response were not affected by dietary inclusion of BSFLM. Furthermore, the ready-to-cook and breast meat yields, weights of various visceral organs and abdominal fat content were also not affected. The study thus indicates that source of BSFLM considerably influenced its nutrient profile and BSFLM from a source could be safely included in the diet of commercial broiler chicken up to 5% with beneficial effects on performance during early life.

CANOLA MEAL AS A SUBSTITUTE FOR SOYBEAN MEAL IN REDUCED CRUDE PROTEIN BROILER DIETS

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Reducing dietary crude protein (CP) levels in broiler diets brings environmental and bird welfare benefits and reduces the industry's dependency on soybean meal (SBM). In Australia, production of canola is 2.7 million tonnes but only 40000 tonnes of soybeans. Therefore, canola meal (CM) is an attractive alternative to imported soybean meal. Hence, the present study evaluated the potential of replacing SBM with CM in moderately (190 g/kg) and severely (170 g/kg) reduced CP diets. A total of 144, Ross 308 off-sex male broilers were allocated to 24 cages with 6 birds per pen and 6 replicates for each treatment at day 15 post-hatch. Four experimental diets were formulated in a 2×2 factorial arrangement with two dietary crude protein concentrations (CP: 190 and 170 g/kg) and two levels of CM inclusions (0 and 150 g/kg). Growth performance, relative abdominal fat-pad weights and carcass traits were determined from 15 to 36 days post-hatch. The experimental data was analysed using two-way ANOVA in JMP® Pro 14.0 software package and probability level of less than 5% was considered statistically significant. Transition of dietary CP from 190 to 170 g/kg decreased weight gain by 7.15% (2054 versus 1907 g/kg, P < 0.001) and including 150 g/kg CM had no impact on weight gain. A treatment interaction (P = 0.002) was observed in FCR where inclusion of 150 g/kg of CM did not influence FCR in broiler chickens offered diets with 190 g/kg of dietary CP but compromised FCR was observed in broiler chickens offered diets with 170 g/kg of dietary CP (1.477 versus 1.537). In diets without CM inclusion, reducing dietary CP from 190 to 170 g/kg did not compromise feed efficiency. However, dietary CP reduction and CM inclusions increased relative-fat pad weights by 38.5% (7.09 versus 9.82 g/kg; P < 0.001) and 18.2% (7.75 versus 9.16 g/kg, P = 0.021), respectively. Dietary treatments did not influence the carcass traits. In conclusion, replacing SBM with CM in the 190 g/kg CP diet and replacing SBM with non-bound amino acids in the 170 g/kg CP diet did not compromise FCR.

Keywords: broiler chickens, canola meal, non-bound amino acids, reduced-crude protein diets, soybean meal.

Acknowledgements: This study is funded by the Australian Research Council Discovery Early Career Researcher Award offered to Dr. Sonia Liu and the Australian Government Research and Training Program (RTP) provided a scholarship for the Ph.D. candidature of Shemil Macelline

APPLICATION OF NEAR-INFRARED TECHNOLOGY TO PREDICT SPECIFIC MAIZE APPARENT METABOLISABLE ENERGY ON ADULT ROOSTERS

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Each year in France, maize harvest starts in September and special attention is given to characterise its nutritional value. Harvest guality for poultry feeding is assessed by measuring and predicting apparent metabolisable energy (AME). This value is usually obtained by performing in vivo digestibility trials on adult roosters and by updating predictive equations based on maize analytical data. Near-Infrared (NIR) analysis is a fast and cheap alternative to predict maize AME. The aim of this study was to develop a NIR calibration for rooster AME on maize. Multicereals calibrations weren't an acceptable solution because of cereal landings obtained using this process. A total of 74 maizes were studied through digestibility trials conducted from 2011 to 2019 according to the protocol of Bourdillon and al. (1990). NIR maize spectra were made using a MPA (Multi Purpose Analyzer, Bruker Optics SARL). Calibrations were developed to predict AME from spectra according to in vivo trials. Two calibrations were studied, on whole maize and ground maize, their performances were equivalent. Using whole maize calibration allows to avoid grinding purchase and additional analytical time. This specific calibration has several advantages compared to in vivo trials: it is economical (no additional analytical cost), considerably time saving, approximately 6% more accurate than the equation-based method to predict maize AME according to the Root Mean Square Error of Cross Validation (RMSECV): 31 versus 33 kcal/kg and approximately 7% more specific than the equation-based method according to correlation coefficient 0,43 versus 0,39. In conclusion, development of this calibration improves the ability of a continuous reassessment of nutritional value of maize even more precise and the possibility to observe new trends during harvest earlier thanks to NIR technology. It opens prospects such as real-time maize nutritional quality assessment in feed plants.

ASSESSMENT OF POSTBIOTIC METABOLITES OF LACTOBACILLUS PLANTARUM ON IMMUNE RESPONSE AND GUT HEALTH IN BROILER CHICKEN IN COMPARISON TO THE ANTIBIOTIC GROWTH PROMOTER

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The live microbial feed additives termed the probiotics have extensively been researched to substitute antibiotic growth promoters in broiler industry, but recent studies claimed that these beneficial organisms could acquire and transfer the resistance genes to gut pathogens. Postbiotics, the metabolic byproducts of probiotics can perform a similar function without any fear of resistance genes and its study in chicken are much limited. Postbiotic metabolites of laboratory isolated probiotic Lactobacillus plantarum (LGFCP4) were collected and stored at 4°C. Following ethical approval, a biological experiment was undertaken for 42 days with 300 day old Caribro-Vishal broiler chicken, in 6 groups, each consisting 5 replicates with 10 chicks each, reared in experimental pens with ad libitum feed and water. The treatment birds included basal diet (control), basal diet +chlortetracycline, basal diet +un-inoculated media, basal diet +0.2% postbiotics, basal diet +0.4% postbiotics and basal diet +0.6% postbiotics. The immune response (log10 titre), caecal microbes (log10 cfu/g), jejunal histomorphometry (µm) and jejunal antioxidant activity (Trolox, mM) was studied at 7, 14, 21 and 42 days of chicken. The assessment of cell mediated (day 28) and humoral immune response (day 14 and 21 post vaccination to New castle disease) of birds showed significantly higher response (p < 0.05) in antibiotic and all of postbiotic fed birds (0.2-0.6%) than other birds. Enumeration of total microbes in caecal contents showed significantly (p < 0.01) higher counts in postbiotic fed birds at 0.4 and 0.6% compared to other treatment birds at day 7 and day 21 of study. Significantly (p < 0.05) higher lactobacillus counts were noticed in postbiotic fed birds (0.4, 0.6%) throughout the study and caecal gram negative counts showed significantly (P<0.05) lower colonies only at day 21 of study in postbiotic groups (0.4, 0.6%) compared to other treatments. Broilers fed with antibiotic and postbiotics (0.4, 0.6%) had significantly longer villi and less deeper crypts (p<0.05) compared to control throughout the experiment. Also the jejunal antioxidant concentration was higher (p < 0.01) in birds fed with antibiotic and postbiotics (0.4, 0.6%) at day 7 and in postbiotic supplemented birds at 0.6% at day 14 compared to the other diets. The study indicated that postbiotics at 0.4 and 0.6% improved the immune response and gut health in broiler chicken similar to the antibiotic growth promoter.

GROWTH PERFORMANCE RESPONSE OF BROILER CHICKENS FED DIETS CONTAINING DEFATTED MICROALGAE MEAL UP TO 22 DAYS OF AGE IN PARTIAL SUBSTITUTION OF SOYBEAN MEAL

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Defatted microalgae meal could be considered as a sustainable alternative protein source to the widespread use of soybean meal in poultry diets. The aim of this study was to investigate the effects of the partial substitution of soybean with increasing dosages of microalgae meal (Spirulina spp.) up to 22 d of age on the growth performance of broiler chickens. A total of 1,000 one-d-old Ross 308 male chicks were divided into 4 experimental groups (10 replicate pens/group with 25 birds each) fed either a commercial soybean-based diet throughout the trial (CON) or the same diet including 5, 10 or 15% of microalgae meal up to 22 d (M5, M10, and M15, respectively). Then, all groups received the same commercial soybeanbased diet up to the slaughter age (47 d). All diets were isoenergetic and isonitrogenous, and formulated according to a 4-phase feeding program (starter 0-14 d; grower I 15-22 d; grower II 23-29 d; and finisher 30-47 d). Productive parameters were recorded on a pen basis at housing (0 d), at 22 d, and at slaughter. Data were analyzed through one-way ANOVA and Tukey post-hoc test considering the dietary treatment as independent variable. At 22 d, body weight was linearly reduced and feed conversion ratio significantly worsened as the dietary inclusion of microalgae meal increased (931 vs. 850 vs. 709 vs. 462 g, and 1.539 vs. 1.656 vs. 1.783 vs. 2.312 for CON, M5, M10, and M15, respectively; P<0.001). CON and M5 group presented similar feed intake from 0 to 22 d, which was significantly higher if compared to that of M10 and M15 (1.367 and 1.333 vs. 1.184 vs. 0.964 g/bird, respectively; P<0.001). At 47 d, CON and M5 groups exhibited comparable body weight, while M10 and M15 showed lower values (3,455 vs. 3,446 vs. 3,221 vs. 2,802 g, respectively; P<0.001). No significant difference in feed conversion ratio was observed in the overall period of trial (0-47 d). Similarly, mortality was not substantially affected by the treatments either at 22 or 47 d. Overall, these results indicate that the dietary administration of microalgae meal during the first 22 d significantly impaired growth performance of broilers. However, by re-feeding a commercial soybean-based diet up to slaughter (23-47 d), broilers receiving 5% of microalgae meal up to 22 d achieved similar body weight and feed efficiency to those fed conventional soybean-based diets in all feeding phases.

EFFECTS OF PHYTOGENIC FEED ADDITIVE ON BROILER PERFORMANCE AND ILEAL & CECAL MICROBIOME.

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The goal of our study was to assess the potential use of phytogenic feed additive (PFA), which is normally applied as poultry red mite repellent, as a non-antibiotic growth promoter in broilers. The tested PFA, RIDofMITE® (PATENT CO. DOO), consisted of essential oils blend that predominately originated from Thymus vulgaris and Origanum vulgare, was dosed at 500g/ton of feed during the whole fattening period of 42 days. The trial was performed on a farm with 800 broilers (Cobb 500 line) separated into 2 groups. Both groups consisted of 8 pens with 50 broilers each. For the body weight (BW) of the broilers and feed conversion ratio (FCR), each pen was the experimental unit. The BW of the broilers was measured by pen on days 1., 14., 30. and 42. At the end of the trial, the mid ileum, and cecal fecal samples (FS) of 2 birds per pen were taken for microbiological, and rectal FS from one bird per pen was taken for parasitological analysis at the slaughter line. The total number of E. coli, Enterobacteriaceae, Clostridium perfringens, and Lactobacillus spp. was determined in each ileal and cecal FS, while the number of positive and negative samples on Eimeria sp. oocyst was determined in rectal FS with the MacMaster method. The normal distribution of BW and microbiological data was tested with the Shapiro - Wilk test. The data were normally distributed (Shapiro - Wilk test, p>0,05), and results of BW were compared using the two-way ANOVA with repeated measures in one factor followed by Tukey's test, while FCR data were compared using the t-test for independent samples. While the frequency of positive and negative samples on oocysts between the two groups was analyzed using Fisher's exact test. The average weight (AW) of broilers in the trial group was 6, 7, and 28 g more than in the control group on 14., 30, and 42 days of the trial, respectively. However, the statistical difference in AW was not detected during the trial. Similarly, the statistical difference in FCR was not detected between the trial group (1.81) and the control group (1.84). Statistically significantly different (p < 0.05), the lower, number of E. coli and C. perfringens was detected in cecal FS of the trial group in comparison with the control group. The trial group had 18,75% of samples positive to oocyst presence in rectal FS, whereas the control group had 31.25% of positive samples. The results of this study suggest that investigated PFA has a beneficial impact on the gut health of poultry.

DO THE EMULSIFIER AND MULTI-CARBOHYDRASE ENZYME SUPPLEMENTATION INFLUENCE NUTRIENT UTILIZATION AND AMEN OF MAIZE-RAPESEED MEAL BASED BROILER DIETS?

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Intensive broiler production under sustainable conditions of development enforces greater feed conversion, as well as diminish of nutrients environmental pollution. Successful usage of enzymes in poultry diets is well known, however, no data are available in literature about use of emsulsifiers and carbohydrase in birds. Presumably, improved fat digestibility decreases fat content in digesta and, consequently, enhance carbohydrate availability for microbial enzymes. The objective was to evaluate possible synergic effect of emulsifiers and carbohydrases on nutrient utilization and AMEN of maize-rapeseed meal-based broiler diets. The 384 male broiler chicks assigned to 4 dietary treatments (12 replications, 8 individuals) were used to obtain the effect of emulsifier and multi-carbohydrase enzyme supplementation on nutrient utilization and AMEN in maize-rapeseed meal-based broiler diets. The control diet consists of sovbean, maize and rapeseed meal with soybean and palm oil. The experimental groups consisted of emulsifier (EMU), enzyme (ENZ), and both (EMU+ENZ) addition. The ileum (n=10) digesta samples were possessed due to determination of nutrient digestibility. Statistically significant differences in AMEN values occurred only on the 28th day among EMU and EMU+ENZ groups. The highest apparent pre-caecal digestibility (APD) of crude protein (CP), as well as total tract neutral detergent fibre (NDF) degradation, occurred in EMU+ENZ treatment (P<0.05). Obtained results indicate the additive effect of simultaneous emulsifier and carbohydrase usage have beneficial effects on nutrient digestibility and the degradation of nutrients in a maize-rapeseed mealbased broiler diets.

EFFECTS OF REPLACING SOYBEAN MEAL IN BROILER DIETS

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Soybean meal is an ideal source of protein in broiler diets. A broiler diet composed of alternative protein components is expected to be of lesser quality and could reduce birds' performance. However, the use as feed is controversially discussed by the public. Finding an equivalent alternative to soy as a protein source is difficult. Thus, the aim of the study was to test whether this is possible with a combination of several protein components as well as supplementation with synthetic amino acids. Growth performance, litter quality and foot pad dermatitis were assessed, fed three different diets. The starter diet was identical for all treatments, fed from 1 to 10 d of age. For the grower (11 to 28 d of age) and finisher (29 to 37 d of age) diet, soybean meal was used only in the control diet (C), while it was completely replaced in the experimental diets without (W) and without plus (WP). In the WP finisher diet, the amino acids arginine and isoleucine were added. The diets were formulated to contain 19 and 18% CP in the grower and finisher diet, respectively and were isoenergetic. The 5400 Ross 308 broilers were kept in compartments of 20 m2 with 270 birds each and were fed ad libitum. Data were subjected to ANOVA using SAS 9.4 and were analyzed considering diet as fixed effect. For multiple comparisons of the Least Square means the Tukey-Kramer option was used. Statistical significance was set to p < 0.05. Final BW was greater with C than with W, with WP in between (2.19, 2.11 and 2.14 kg, respectively). After the growing phase, the broilers with W and WP were exactly the same weight, 3% lighter than with C. However, the broilers with WP were able to catch up in the finisher phase, as shown by the daily gains between 29 and 37 d of age (84.9 g with C, 80.7 g with W and 83.7 g with WP, p < 0.05). Feed efficiency was similar in all treatments during 37 days (1.57 with C, 1.59 with W and 1.55 with WP, p > 0.05). Litter quality was best with WP, with only 28% of encrustation compared to C and W with 50 and 43% of encrustation, respectively. The better litter quality with WP led to a lower percentage of broilers with foot pad dermatitis. The results show that with alternative protein sources, the amino acid profile is very important. From the point of view of ecology, economy, availability and product quality, the choice of alternative protein sources needs to be clarified. From an animal welfare perspective, the substitution of soybean meal is optimal.

CORRELATION BETWEEN PROCESSING AND BROMATOLOGICAL QUALITY INDEXES OF SOYBEAN MEAL AND ENERGETIC VALUES FOR BROILERS

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In this experiment, the quality of soybean meals of different origins was evaluated for the indexes of ureatic activity (UA), protein solubility in KOH (SKOH) and protein dispersibility (PD) and the correlations of metabolizable energy levels of soybean meals. We evaluated the quality of soybean meals of four origins and determined the processing quality indexes: UA, SKOH and PD. After this, bromatological levels were obtained as dry matter (DM), mineral matter (MM), crude protein (CP), ether extract (EE), crude fiber (CF) and apparent metabolizable energy (AME) and apparent metabolizable energy corrected by nitrogen balance (AMEn) of the soybean meals. A correlation was estimated between the bromatological (DM, MM, CF, CP, EE) and processing quality indexes (UA, SKOH and PD) and AME and AMEn levels. The highest value of crude protein was observed with the highest SKOH values. The highest CP values in soybean meals were positively correlated with PD (r = 0.5687; P < 0.0215) and SKOH indexes (r = 0.8065; P < 0.01) and negatively correlated to UA indexes (r = -0.5532; P < 0.0262). The EE values of sovbean meal were negatively correlated to CF levels (r = -0.6305; P < 0.01). On the other hand, no correlation was observed to EE and MM levels of soybean meals with processing quality indexes: UA, PD e SKOH and to energy data (AME and AMEn). UA had no significant correlation to PD levels and AME e AMEn data. But, a negative correlation between UA and SKOH (r = -0.6317; P <0.01) were observed. Soybean meal PD values in this study were positively correlated to EMAn (r = 0.6311; P < 0.01) in a 40% soybean meal metabolic assay measured for broilers from 17 to 21 days of age. And no significant correlation was observed between PD and AME and between PD and SKOH of soybean meals. SKOH was no significant correlated to AME and AMEn. AME and AMEn for broilers were positively correlated (r = 0.7851; P < 0.0215), and this can be explained by the nitrogen balance as a correction factor. In this study, the positive correlations between the bromatological data and processing quality indexes and AME and AMEn values for broilers. This is an indication of the importance of soybean meal processing methods and its nutritional quality for broilers.

APPARENT METABOLIZABLE ENERGY AND RETAINED NUTRIENTS IN BROILER CHICKENS FED SINGLE OR MIXTURE OF FEED INGREDIENTS-BASED DIETS WITH OR WITHOUT AN EIMERIA CHALLENGE

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Feed ingredients are inherently unique in terms of anti-nutritional factors and indigestible components making nutrient digestibility vary between feedstuffs. Understanding the undigested dietary fractions in excreta can provide insight in developing strategies to maximize feed efficiency. However, various stressors such as coccidiosis, can inevitably reduce nutrient digestive and absorptive capacity caused by inflammation and damage to the intestinal tract, leading to reduced performance, increased mortality and major economic losses for poultry producers. For this reason, it would be necessary to characterize the effect of Eimeria challenge on apparent retention (AR) of components and AME content of major feed ingredients fed to broiler chickens. A total of 840 male d-old Ross x Ross 708 chicks were placed in 84 cages (10 birds/cage) and allocated to either a nitrogen-free diet (NFD), or one of 6 test diets based on a single or mixture of feed ingredients as the sole source of AA (n=12). Test diets were: 1) corn, 2) wheat, 3) soybean meal (SBM), 4) pork meal (PM), 5) corn, SBM, and PM (CSP), and 6) wheat, SBM, and PM (WSP). On d 10, birds in 6 cages/diet were orally gavaged with 1 mL of E. acervulina and E. maxima mixture while the other 6 cages with sham. Excreta samples were collected from d 12 to 14. All test diets contained 0.3% titanium dioxide as the indigestible marker and nutrient digestibility was determined by substitution method. There was a treatment and challenge interaction effect (P < 0.05) seen in the retention of calcium and phosphorus such that Eimeria decreased the retention in NFD, corn, and PM diets. The effect of challenge (P < 0.05) was seen in the retention of crude fat (CF) such that retention decreased in challenged birds across all dietary treatments compared to nonchallenged birds. The AMEn values for the key ingredients without or with Eimeria were corn (2966.7 vs. 2961.9), wheat (3103.2 vs. 3518.04), SBM (2452.5 vs. 2386.1), and PM (2830.42 vs. 3803.66) respectively. A challenge effect (P < 0.05) was seen on AMEn values such that challenge decreased retention for corn and SBM, and increased for wheat and PM. The results of the present study demonstrated that treatment and challenge can significantly influence energy and nutrient digestibility of different feed ingredients. These differences warrant considerations in feed formulation for broiler chickens exposed to an Eimeria infection.

Key words: broilers, Eimeria, AME

PEPPERMINT OIL AS AN EFFECTIVE PHYTOGENIC FOR SUSTAINABLE BROILER PRODUCTIONS

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This study was conducted to investigate effects of dietary peppermint oil supplementations on growth performance, economic return, meat quality, and serum biochemistry of broiler chicks. A total of 384 1-day-old, unsexed Ross 308 broiler chicks, were assigned to 4 dietary treatments that included peppermint oil at concentrations of 0, 50, 100, or 150 mg/kg. Each treatment had 12 replicate pens with 8 birds. The experiments lasted for 35 days of age. The peppermint essential oil contained 40.62% menthol. Body weight (BW), BW gain and feed intake linearly increased with the increase in dietary peppermint oil (P < 0.001) and in turn, caused linear improvements (P < 0.01) in feed conversion values and, therefore, a higher net return was observed. Furthermore, increasing peppermint oil improved (P < 0.01) cook-loss and drip loss percentages of breast and leg muscles. Serum cholesterol, aspartate transaminase and alanine aminotransferase significantly decreased by peppermint oil supplementations compared with the control group. Interestingly, the relative weight of abdominal fat was decreased and dressing was increased without any side effects on internal organs by supplemental peppermint oil. Overall, based on the observed results peppermint oil can be used as an effective novel nutritional bio-agent up to 150 mg/kg for sustainable meat production and health of broiler chicks.

Keywords: Broiler, economics, meat quality, production, serum biochemical

APPLICATION OF PHYTOGENIC FOR SUSTAINABLE EGG PRODUCTION IN LAYING HENS DURING THE LATE LAYING PERIOD

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Improved chickens' performance and sustainable egg production can be accomplished by phytogenic as feed supplements. This study investigated the dietary effects of Moringaoleifera leaves (MOL) supplementation on egg quality, laying performance, excreta ammonia concentrations and serum biochemistry of laying chickens during the late laying period. A total of 240 64-week-old Hy-Line Brown hens were assigned to four treatment diets including MOL at 0, 3, 6 or 9 g/kg, respectively, for eight weeks. The treatments had twelve replicates with five hens each. The results revealed that incremental dietary MOL significantly increased (p < 0.01) egg weight, production and mass through 64–68, 68–72 and 64–72 weeks of age. Simultaneously, feed conversion ratio was significantly improved (p < 0.01) with MOL supplementation compared with the control. Haugh units and the thickness of eqashells significantly improved as a response to diets supplemented with 3, 6 and 9 g/kg MOL leaves at 72 weeks of age. The economic efficiency of hens fed diets supplemented with MOL was greater (P<0.01) than that of hens fed the control diet. Interestingly, excreta ammonia concentrations, serum cholesterol, aspartate transaminase and alanine aminotransferase significantly decreased with MOL leaves supplementation compared to the control group. In conclusion, introducing MOL supplementation at 3, 6 and 9 g/kg increased egg production, eggshell quality, Haugh units, and decreased serum cholesterol, trialycerides, excreta ammonia concentrations besides serum liver enzymes, uric acid and creatinine. Overall, based on the observed results, MOL supplementation was very promising as an effective feed additive in laying hens' diet during the late laying period.

Keywords: ammonia, egg quality, laying hens, Moringa, production, serum biochemical

26th World's Poultry Congress, abstracts selected in 2022

General physiology

Selected short communications

CENTRAL TAURINE INDUCES HYPOTHERMIA THROUGH MODIFYING MONOAMINERGIC PATHWAYS AND AMINO ACID METABOLISM IN NEONATAL CHICKS

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Brain monoamines and amino acids have been reported to regulate body temperature. Our recent findings showed that central injection of taurine induced hypothermia in neonatal chicks through gamma-aminobutyric acid A receptor. The aim of this study was to examine the involvement of brain monoamines and amino acids in connection with taurine-dependent hypothermia in chicks. In Experiment 5-days-old male Julia layer chicks (n = 10) were subjected 1. to intracerebroventricular (ICV) injection with saline or taurine (5 μ mol/10 μ L) under control thermoneutral temperature. In Experiment 2, 5-days-old male Julia layer chicks (n = 10) were ICV injected with saline, taurine (5 μ mol/10 μ L), fusaric acid (FA, dopamine hydroxylase inhibitor: 558 nmol), or taurine with FA. In Experiment 3, 5-days-old male Julia layer chicks (n = 10) were ICV injected with saline, taurine $(5 \mu mol/10 \mu L)$, para-chlorophenylalanine (PCPA, tryptophan hydroxylase inhibitor: 400 nmol), or taurine with PCPA. Rectal temperature was recorded at 0, 30 and 60 min after the ICV injections. Chicks were properly anesthetized by isoflurane before collection of the brain. Brain monoamines and amino acids in Experiment 1 were analyzed using HPLC. Experimental data were subjected to a Thompson rejection test to eliminate outliers (P<0.01) and the remaining data were used for statistical analysis by the Stat View version 5.0. The results showed that central taurine significantly increased diencephalic tryptophan (the precursor of serotonin (5-HT), 5-HT and its metabolite 5-hydroxyindoleacetic acid concentrations. Moreover, central taurine significantly decreased diencephalic concentration of tyrosine (the precursor of L-DOPA, which converts to dopamine and norepinephrine (NE)). However, the NE concentration in the brain stem and its metabolite 3-methoxy-4-hydroxyphenylglycol in both the brain stem and diencephalon were significantly increased after 30 min following central injection of taurine. Brain histidine (the precursor of histamine) concentration was significantly increased while, glutamate, proline, arginine, ornithine and isoleucine concentrations were decreased after 30 min following central injection of taurine. We further found that FA and PCPA attenuated taurine induced hypothermia. These results indicate that NE and 5-HT mediate taurine-induced hypothermia. Furthermore, central injection of taurine might regulate amino acid metabolism to contribute to hypothermia.

DIURNAL FLUCTUATIONS IN THE TISSUE DISTRIBUTION OF CALCIUM AND PHOSPHORUS TRANSPORTER GENES IN LAYING HENS.

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Calcium (Ca) and phosphorus (P) are essential minerals needed for the development of strong eggshells and skeletal integrity in laying hens. Understanding how these minerals move between tissues during egg formation would provide insight into possible nutritional and genetic strategies that could improve bird welfare and saleable eggs. This research evaluated expression of Ca and P transporters across tissues during egg formation. At 32 weeks of age, 72 commercial laying hens were euthanized, and their ileum, kidney, and shell gland (SG) were collected at 1, 3, 4, 6, 7, 8, 12, 15, 18, 21, 23, and 24-hours post oviposition (HPOP) (n=6 hens per time point). Levels of mRNA were determined using reverse transcription-quantitative PCR. Data were analyzed by one-way ANOVA, and means were compared using Fisher's Least Significant Difference test when ANOVA indicated significance ($P \le 0.05$). Sodium/calcium exchanger 1 (NCX1), which is responsible for Ca entry into the cell, was not affected by HPOP in the ileum or kidney (P>0.05). However, HPOP significantly influenced its expression in the SG, with an increase from 6 to 12 HPOP ($P \le 0.05$). Calbindin 1 (CALB1) acts as a chaperone protein once Ca enters the cell. CALB1 expression in the ileum showed a close-to-significant time dependence on HPOP (P=0.06), with elevated levels at 7 and 24 HPOP which were greater than 15 HPOP ($P \le 0.05$). The ileal expression patterns of CALB1 are likely representative of Ca uptake by the intestine. The SG had reduced expression of CALB1 during the period when there was no eqg in the SG (3 to 4 HPOP) and a four-fold increase expression during eggshell calcification (8 to 15 HPOP) ($P \le 0.05$). Thus, during egg calcification, CALB1 and NCX1 appear to act together to provide Ca to the eggshell. Additionally, during egg calcification, bone is broken down to provide Ca for the shell in a process also releasing P that is transported by inorganic phosphate transporter 1 (PiT-1). The kidney showed peak expression of PiT-1 at 15 HPOP, with an overall elevated expression during 12 to 23 HPOP ($P \le 0.05$), which is likely related to P excretion. Expression of PiT-1 in the SG was noted later at 18 and 21 HPOP $(P \le 0.05)$. The later peak in PiT-1 expression in the SG occurs during a time when P accumulates in the palisade layers of the shell and cuticle. Taken together, these results illustrate how Ca and P transporters enable the hen to dynamically utilize and excrete Ca and P during eggshell formation.

CHARACTERIZATION OF BROILER CHICKENS INDIVIDUAL MYOFIBRILLAR PROTEINS SYNTHESIS RATE

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INTRODUCTION: Fractional Synthesis Rate (FSR) of tissue protein is proposed as an early biomarker of growth and health status. Muscle protein turnover studies in broiler chickens are not common and have been limited to the assessment of mixed muscle protein FSR. Our novel approach for estimating individual proteins FSR involves the use of heavy water (D2O), which labels free amino acids during de novo synthesis and transamination, in combination with proteomics, to allow simultaneous assessment of isotopic enrichment at the peptide level from multiple proteins. The present study has characterized the FSR of the three most abundant proteins in broiler chicken breast muscle during an immune challenge and in untreated controls. METHODOLOGY: 24 male Ross 308 broiler chickens were housed across 6 different pens. Birds were administered an oral bolus of 10 g D2O/kg body weight at 21 days of age. Chickens from 3 pens received a 2mg/kg bolus of E.Coli lipopolysaccharide (LPS) or saline solution intravenously one hour after D2O dosage. One bird per pen was blood sampled and culled 24h, 48h and 96h post D2O administration, breast muscle samples were removed and snap frozen immediately. Blood was collected in tubes coated with EDTA and plasma was obtained by centrifugation. Deuterium enrichment in body water was quantified by FTIR analysis of plasma. 200 mg of breast muscle were homogenized and proteins were extracted. Proteins were trypsin-digested and the resulting peptides analysed on a Q-Exactive LCMS. Raw data was analysed using Maxguant and Skyline software. Peptide isotopic enrichment and protein FSR were calculated using an R routine. Statistical analysis (t-test) was performed using Microsoft Excel.RESULTS: A total of 42 actin, 124 myosin heavy chain (MYHC) and 18 myosin light chain (MYLC) peptides were assessed from the control group while 51 actin, 126 MYHC and 14 MYLC peptides were assessed from the LPS group. Mean actin FSR decreased significantly from 21.15 % day-1 (\pm 6.28) to 16.36 % day-1 (\pm 6.11) during the immune challenge. Mean MYHC FSR decreased significantly from 35.99 % day-1 (± 11.26) to 28.12 % day-1 (± 9.10) during the immune challenge. Mean MYLC FSR decreased significantly from 31.61 % day-1 (± 9.40) to 23.44 % day-1 (\pm 5.48) during the immune challenge. CONCLUSION: New data on male broiler chicken individual myofibrillar protein FSR has been obtained. Broiler chicken myofibrillar protein FSR decreases significantly during an immune challenge.

DIETARY ANTIBIOTICS INFLUENCE THE EXPRESSION OF GENES ASSOCIATED WITH BROILER INTESTINAL HEALTH AND FUNCTION IN THE FACE OF A NECROTIC ENTERITIS CHALLENGE

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A shift in consumer preference and legislative requirements have led to an increase in the number of antibiotic-free broiler production systems, necessitating the development of alternative strategies that mitigate effects of enteric diseases such as necrotic enteritis (NE). Therefore, the objective of this study was to evaluate effects of subclinical NE on gene expression associated with intestinal health and function in the absence and presence of subtherapeutic levels of bacitracin methylene disalicylate (BMD). Day-old male broilers were randomly assigned to one of three treatments (n=5 pens/treatment): unchallenged without dietary BMD, challenged without dietary BMD, and challenged with dietary BMD. Birds in challenged groups were orally gavaged with coccidia oocytes on post hatch day (D) 14 followed by oral doses of Clostridium perfringens between D19-D21. On D21, D22, D24, and D28, jejunal mucosa samples were collected from one bird per pen and expression of genes associated with barrier function, inflammatory status, and nutrient transport was determined. Data were analyzed by ANOVA followed by Fishers Least Significant Difference test. On D21, birds in the challenged group had reduced expression of the barrier function genes OCLN, ZO1, and MUC2 (P≤0.05), and BMD partially (MUC2) or fully (ZO1) restored expression to levels in non-challenged birds. IFNa and IL1 β were also decreased on D21 by NE challenge ($P \le 0.05$) and elevated back to non-challenged levels by BMD. On D21, jejunal mRNA for glucose transporters GLUT1 and GLUT2 was downregulated in response to NE ($P \le 0.05$); BMD increased GLUT1 mRNA during NE but had no effect on GLUT2 mRNA. Levels of GLUT8 were decreased on D22 only in challenged birds fed BMD. While expression of CAT1, CAT2, LAT1, and y+LAT2 amino acid transporters was not influenced by NE regardless of BMD supplementation, levels of ANPEP, which is necessary for breakdown of dietary proteins prior to intestinal amino acid uptake, were reduced on D21 in birds challenged with NE in the absence and presence of BMD. These results suggest that NE compromises intestinal health and function by reducing expression of select barrier proteins, inflammatory mediators, glucose transporters, and an enzyme necessary for digestion of dietary protein. Effects that were reversed by BMD represent targets for alternative strategies aimed at reducing impacts of enteric disease on broiler production efficiency.

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Physiology of perinatal development

Selected short communications

EFFECTS OF PRE-INCUBATION STORAGE DURATION AND NON-VENTILATION INCUBATION PROCEDURE ON SASSO BREEDER EGG EMBRYONIC PHYSIOLOGY AND POST-HATCH CHICK PERFORMANCE

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It's well known that chick embryonic development and post-hatch growth is retarded by long- term pre-incubation storage. In contrast, non-ventilation has been reported to accelerate embryonic growth and hatching time. It is not clear if the use of non-ventilation can compensate for the negative effects of long-term pre-incubation storage of hatching eggs. This study investigated the effects and possible interactions of storage and non-ventilation during incubation for eggs from Sasso broiler breeder flock on pre- and post-hatch incubation results. A total of 1, 260 Sasso eggs from a 58-weeks old broiler breeder flock were individually numbered, weighed and stored for 7 d or for 18 d in a climatecontrolled room (16 °C, 75 % RH). After storage, eggs were weighed, and randomly assigned equally into two incubators. One of the incubators (V) was ventilated for the entire incubation and the second was non-ventilated (NV) for the first 12 d. At d 18, the eggs were weighed, candled and fertile eggs were transferred from the turning trays to hatching baskets. During the last 3 days of incubation, hatching eggs were checked individually every 3 h for hatching events and hatchability of fertile eggs. After pull out at d 21.5, post-hatch performances were determined until 1 week of age. The Generalised Linear Model Procedure was used to analyse the effect of storage duration and nonventilation on measured parameters.Results showed that, embryo weights from eggs in NV incubator was significantly higher (p < 0.05) in both stored eggs compared to those from eggs in ventilated incubator, but embryos from eggs stored for 18 d were smaller (p < p0.05) than those from eggs stored for 7 d. Hatchability was higher (p < 0.0001) in NV incubator compared to V incubator in both 7 d and 18 d stored eggs and an interaction was found between incubation ventilation and storage duration on both hatchability and embryonic mortality (p < 0.0001). Chick weights from NV incubator at 7 d post-hatch was greater (p = 0.0009) than those from V incubator. Serum T3 and T4 concentrations were significantly higher (p < 0.05) in NV compare to V group at internal pipping stage. It was concluded that the effect of long-term pre-incubation storage on embryonic physiology and post-hatch growth interacted significantly with incubation ventilation and that non-ventilation can compensate for the negative effects of storage on some hatching and post-hatch performances.

DYNAMICS OF ENERGY RESOURCES (GLYCOGEN AND CREATINE) PRE AND POST HATCH IN BROILER EMBRYOS

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During incubation, the broiler embryo relies on egg components to supply energy for development and growth. This study aims to examine the levels of 2 high-value energy sources (glycogen and creatine) in four key tissues: liver, Yolk Sac (YS) tissue, Breast Muscle (BM) and Pipping Muscle (PM), along with expression of related genes. Accordingly, embryos (Cobb500) were sampled during incubation (E11, E13, E15, E17, E19), at hatch and at chick placement. Liver, YS tissue, BM per and PΜ were analyzed (6 replicates age) for creatine and glycogen content and for expression of genes involved in gluconeogenesis (FBP1), glycogen and creatine synthesis (GYS2; AGAT; GAMT). Results show that glycogen is accumulated in all four tissues reaching a peak at E17 and E19, then significantly decreasing at hatch and at placement (36h post hatch). Exemplified by data from the liver: glycogen concentration (mg/g) increased by 2.85-fold from E11 (27.98) to E17 (79.78), then decreased by 27-fold reaching a low level of 2.89 at placement. The expression levels of GYS2 and FBP1 genes followed glycogen dynamics. As for creatine, its concentration (mg/g) increased significantly from E11 to hatch in the liver (from 0.03 to 0.19) and YS (from 0.13 to 0.17). Then, at placement, decreased by 2-fold (to 0.09) in the liver and by 1.5-fold (to 0.11) in the YS. Analysis of AGAT and GAMT genes show decreased expression towards hatch in the YS tissue and increased expression in the liver. This demonstrates a shift in functionality between the YS tissue and liver towards hatch. According to our findings, glycogen storage is mainly in the YS tissue and creatine storage is mainly in the PM and BM. Our findings highlight the energetic limitations of hatchlings up to their exposure to first feed at chick placement. Additionally, our findings suggest that in order to attain better quality hatchlings, a promotion to their energy resources during the peri hatch period is needed.

Keywords: Incubation, Creatine, Glycogen, In-ovo feeding

STUDYING THE IMPACT OF LIGHT DURING INCUBATION AS A POTENTIAL CANDIDATE TO INFLUENCE THE HATCHING TRAITS AND SUBSEQUENT POST HATCH GROWTH PERFORMANCE OF BROILERS

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The chicken embryo starts responding to light from the second day of incubation, hence, just like temperature, moisture, turning, and ventilation, light also has a significant role in embryonic growth and development. Providing light during incubation can help the embryo efficiently adjust to its pre and post-natal environment. Yet, such a wonderful aspect remained neglected due to the possible interference of heat generated from light sources with incubator temperature. However, the advent of some new light sources, especially, the LEDs (Light Emitting Diodes) in variable designs have changed the dynamics of light particularly no heat generation. Light from such sources can be utilized to modulate embryonic growth as well as post-hatch broiler performance. Keeping in view such a scenario, a study was planned to evaluate the effect of LED-based light during incubation on hatching traits and post-hatch performance of broilers. A total of 2250 eggs of three different commercial broiler strains were incubated under 0 (zero), 12 (twelve), and 24 (twenty four) hours lighting regimes to study the hatching traits in terms of egg moisture loss during incubation, early, mid and late embryonic mortality patterns, hatch window, hatch of fertile, and overall hatchability, post-hatch stress response in terms of heterophil/lymphocyte ratio, physical asymmetry, feather score, and gait score as well as subsequent broiler growth performance in terms of feed intake, body weight gain and FCR. A two-way factorial analysis was performed while significant means were separated through Duncan's Multiple Range test using SAS 9.1 software. Improved hatch window in terms of hatch synchronization, the higher hatch of fertile with reduced embryonic mortality each at early, mid and late stage with enhanced overall hatchability and superior chick quality were observed in response to12 hours light stimulation during incubation. Likewise, a significant improvement was observed in stress response as well as growth performance of broilers in response to light stimulation, especially for 12 hours of stimulation. Considering the appreciable results of the present study, it is recommended to adopt 12 hours of light stimulation during incubation as a prerequisite factor in order to achieve improved hatching results, superior chick quality, hence, improved broiler economics.

THERMAL MANIPULATION DURING INCUBATION OF BROILERS AFFECTS THE GENE EXPRESSION OF TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS IN THE BRAIN.

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For the last two decades, broiler production has rapidly increased in (sub)tropical areas. However, this has also increased concerns about heat stress due to the simple ventilation systems employed in these countries. Broilers can suffer from heat stress due to these factors combined with their own inherent heat production, resulting in production losses. Thermal programming during incubation can be used as a possible strategy to induce heat tolerance. Regardless of the many investigations already conducted, further knowledge of thermosensitivity is needed. Therefore, an experiment has been conducted in which three incubation treatments were used: Control (C, machine temperature 37.6°C), treatment 1 (T1, machine temperature 37.6°C with 8h peaks from embryonic day (ED) 15-ED20 of 38.6°C), and treatment 2 (T2, machine temperature 37.6°C with 8h peaks from ED15-ED20 of 39.6°C). After each heat peak, 12 embryos per treatment were decapitated for brain harvesting. The mRNA level of 22 target genes was determined by a 48 x 48 dynamic array integrated fluidic circuits (Fluidigm Corporation). All genes were selected based on potentially being thermosensitive. Cycle Threshold (CT) values were standardized against a general pool of samples and normalized using the the combination of reference genes EF1A1, YWHAZ, and GADPH. A linear regression model was constructed for each gene and multiple comparisons were performed using R version 4.0.0. Eight genes showed a significant treatment effect, either as a main effect or within a certain embryonic day. For the latter, both TRPA1 (heat sensing) and TRPM8 (cold sensing) showed increased levels of T1 and T2 compared to C on ED15, an effect seen again at IP for T2. The contradicting increase of both heat and cold sensing TRPs as reaction to a heat stimulus at ED15, can be described as an uncoordinated immediate response. For T2, the heat sensing TRPA1 had a persistent increased expression. T2 seems to induce acclimatization, a result seen previously by increased heat tolerance at later life for similar T2 treatments. Additionally, this study demonstrated for the first time, the existence of 22 target genes between ED14 until hatch. All 22 target genes showed expression at ED14, making thermal programs at ED14 or later justifiable as temperature inputs could thus be detected by the embryos.

26th World's Poultry Congress, abstracts selected in 2022

Physiology

Posters

STRUCTURAL MODIFICATIONS OF THE OUTER AND INNER LAYERS OF THE CHORIOALLANTOIC MEMBRANE DURING CHICKEN EMBRYONIC DEVELOPMENT

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In oviparous species, the egg contains protective systems and provides energy and nutrients that support the development of the embryo until hatching. During embryonic development, extraembryonic structures are vital to assimilate egg nutrients, to protect the embryo, to ensure gaseous exchange and to accumulate metabolic waste. The chorioallantoic membrane (CAM), which develops in close contact with the inner eggshell from day 5 of incubation, is a highly vascularized structure. The CAM is located between the eggshell membranes (ESM, outer side) and the allantoic fluid that collects embryonic waste (inner side). The CAM was reported to be involved in mineral transport from the eggshell to the embryo for mineralization of its skeleton, in acid-base homeostasis, and in ion and H2O reabsorption from the allantoic fluid. To decipher the kinetics of maturation of its physiological functions, we compared the structure of the inner and outer surfaces of the CAM by electron microscopy. ESM and the eggshell were analyzed in parallel to better appreciate the impact of CAM activity on its surrounding structures. Eggs from Ross 308 broiler hens were incubated for 11 or 15 days (ED11, early vs ED15, late stage of CAM development). At each time point, eggs were broken, and the eggshell, ESM and the CAM were further processed for analysis by scanning electron microscopy. Images were obtained of the cross-section and the inner side of the eggshell, eggshell membranes (ESM, outer and inner sides) and CAM (outer and inner sides). Results showed that the inner part of the eggshell is weakened during embryonic development while the mammillary knobs appear to be tightly attached to the external membrane of the ESM at ED15. The CAM also undergoes major structural changes with some villous cells that emerge from its outer surface to reach the eqgshell membrane and the eggshell. This study is currently being complemented by transcriptomic analysis of the CAM to identify the molecular markers associated with changes in CAM structure and activity during this developmental period. Altogether, we believe that these approaches will lead to better understanding of CAM functions that are necessary for embryonic skeletal mineralization and its overall development.

INFLUENCE OF THE PROTECTIVE LAYER ON THE HATCHABILITY OF PEKIN DUCKS FROM EGGS STORED FOR A LONG TIME AT DIFFERENT TEMPERATURES

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Egg washing is the recommended method of sanitizing duck eggs, but removing the mucin laver may reduce the hatchability of long-stored eggs. The aim of the study was to examine using silicon or chitosan preparation that recreates the surface shell-layer of the washed egg depending in length (LS) and temperature (TS) storing. Hatching eggs from 31-33 (young flock, YF) and 64-67 weeks old (old flock, OF) parental flocks of Cherry Valley Pekin ducks (farm Gardawice, Poland) were delivered to Lab weekly in batches of 500 eggs by 4 weeks. The eqgs were randomly divided into 3 groups, weighed and dipped in 1%aqueous solutions (T 37-39° C): silicone (Dergall) or chitosan (Chitozan D) preparation (both ICBPharma Jaworzno, Poland) which cleaned and disinfected and simultaneously created a layer on the eqgshell surface. The control group was only disinfected by ozonation. Next, the eggs were placed in four climatic chambers (Iglotech Kraków, Poland) and stored at 8, 12, 18 or 22° C. These procedures were repeated for each weekly batch eggs (LS was 7, 14, 21 and 28 days). The eggs from all groups were set into the incubators at the same time (the temperature incubation gradually decreasing from 37.8 to 36.2°C between 1. and 28. incubation day (E); RH 60-65%). The all unhatched eggs were breakout-analyzed. The results were statistically analyzed with using three-way ANOVA and Tukey post hoc test. Hatchability was depended on LS and TS (P<0,001) and also the age of the reproductive flock (P<0.05), while using a silicone or chitosan preparations did not affect (P > 0.05). Higher sensitivity to the increase of LS and TS was found in OF than YF. The highest hatchability (93.2%) was obtained for eggs from the FY stored by 7 days at 8°C and the lowest (12.2%) for OF stored by 28 days at 22°C. However, satisfactory hatch results (> 80%) were also obtained when eggs were stored for 7 days, for YF regardless ST but for OF only 8-12°C. The relationship between hatchability from set eggs and TS and LT can be described by the multivariate regression formula: HATCH = 1.433 - (0.0326TS) - (0.0239LS), for N=96 and R2=0.691. The decrease of hatchability in both flocks was mainly caused by a rapid rise of anembryonic eggs (unfertilized and died during stored) to level 75.0 and 81.7% of set eggs. To conclude, the hatching Pekin ducks' eggs should be stored at a temperature of 8-12°C and covering eggshells with a silicone or chitosan layer doesn't affect the hatchability. The study was financed by European Funds, the project no POIR.01.01-00-1010/17 by the National Centre for Research and Development, Poland.

EFFECT OF PHYTOBIOTICS MIXTURE CONTAINING HOT PEPPER FRUIT, WHITE MUSTARD SEED, SOAPWORT ROOT, TURMERIC RHIZOME AND THYMOL ON MIR-26A-5P, MIR-30A-5P AND MIR-99A IN A MUSCLE TISSUE OF CHICKEN BROILER

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Introduction and objectives: Phytobiotics seem to be the key additives in poultry nutrition in terms of today's customer requirements. Previous studies related to the single components of this mixture suggested that they may affect physiological and pathological processes in different tissues. MiRNA have been shown to be a key player also in muscle physiology.1 The influence of a number of phytobiotics on the expression of selected miRNAs has been repeatedly demonstrated2. The aim of this study was to evaluate the effect of phytobiotics mixture (AdiCox® AP) on selected miRNA previously described as those related to crucial processes for muscle tissue health. Experimental design: Birds were kept for 35 days in single cages and were fed with commercial feed with addition of AdCox®AP in the amount of: 60 or 100g per ton of complete feed. Muscle samples were taken from pectoris major. Five selected miRNAs related to muscle physiological and pathological processes were tested for each dose using RT-gPCR3. Calculation of the relative miRNA expression using the $\Delta\Delta$ Ct method was applied. Obtained data were statistically analyzed using 2-tailed Student's t test. Values of p<0.05 were considered to be statistically significant. Results and discussion: Among selected miRNAs 3 were statistically significant for 2 experimental groups. For both doses 2 miRNAs demonstrated lower expression (miR-26a-5p, miR-30a-5p) and in a group with dose 60g/tone one (miR-99a) presented higher expression when compared to control. These miRNAs were previously described as involved in muscle cell proliferation/differentiation, myogenesis, oxidative stress. inflammation, muscle hypertrophy and regeneration. Conclusion: AdiCox® AP has changed expression of 3 selected miRNAs responsible for physiological and pathological processes in broiler's skeletal muscle. This data supports the hypothesis that AdiCox® AP has an impact on muscle tissue.

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STRUCTURAL MODIFICATIONS OF THE CHORIOALLANTOIC MEMBRANE AND THE INNER EGGSHELL DURING CHICKEN EMBRYONIC DEVELOPMENT

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In oviparous species, the egg contains protective systems and provides energy and nutrients that support the development of the embryo until hatching. During embryonic development, extraembryonic structures are vital to assimilate eqg nutrients, to protect the embryo, to ensure gaseous exchange and to accumulate metabolic waste. The chorioallantoic membrane (CAM), which develops in close contact with the inner eggshell from day 5 of incubation, is a highly vascularized structure. The CAM is located between the eggshell membranes (ESM, outer side) and the allantoic fluid that collects embryonic waste (inner side). The CAM was reported to be involved in decalcification of the inner eggshell and mineral transport from the eggshell to the embryo (for mineralization of its skeleton), in acid-base homeostasis, and in ion and H 2 O reabsorption from the allantoic fluid. To decipher the kinetics of its structural maturation, we compared the structure of the inner and outer surfaces of the CAM by electron microscopy. In parallel, ESM and the eggshell were analyzed to better appreciate the impact of CAM development on its surrounding structures. Eggs from Ross 308 broiler hens were incubated for 11 or 15 days (ED11 vs ED15, early and mature stages of CAM development, respectively). At each time point, eggs were broken, and the eggshell, ESM and the CAM were gently separated for analysis by scanning electron microscopy. Images corresponding to the cross-section and the inner side of the eggshell, eggshell membranes (ESM, outer and inner sides) and CAM (outer and inner sides) were obtained. At ED15, results showed that the inner side of the eggshell is degraded (decalcification) during embryonic development while the mammillary knobs of the eggshell remain tightly attached to the external membrane of the ESM. The CAM also undergoes major structural changes with some villous cells that emerge from its outer surface to reach the eggshell membrane and the eggshell. The molecular components associated with eggshell decalcification and mineral transport from the eggshell to the embryo via CAM's vasculature remain essentially unknown. This study has been recently complemented by a transcriptomic analysis of the CAM (RNA sequencing) at these two developmental stages. Altogether, we believe that the combined findings of this work will lead to better understanding of CAM functions that are vital for embryonic development and skeletal mineralization during incubation.

BREEDERS AGE AND INTERMITTENT THERMAL MANIPULATION DURING INCUBATION: EFFECT ON HATCHING PROCESS AND POST HATCH PERFORMANCE OF SASSO BROILERS IN TROPICAL CLIMATE

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Many studies have shown that thermal manipulations during the embryonic period (TME) has positives effects on thermotolerance and productive performance of heat-stressed broilers. This effect could be influenced by breeders age. Objectives: The aim of this study was to investigate the effects of breeders' age and TME on hatching performances and early post-hatch performances of Sasso broilers under tropical climate. Methods: 800 Sasso hatching eggs from young breeders (Y: aged of 45 weeks) and old breeders (O: 62 weeks of age) were incubated until day 7, when they were divided into 2 sub-groups for each breeder age: the control group(C) was incubated at standard incubation conditions (37.8°C, 60% RH) and T group was subjected to TME (T°=38.5°C, RH=65%, E10-18, 6h/day). Between 450 and 510 h of incubation, eggs were checked for hatching event. Chicks from each incubation sub-groups (YC, YT, OC, OT) were raised for one week. Hatchability, chick's temperature and feed conversion ratio (FCR) were determined. Statistics software GraphPad Prism 8 was used for data analysis, which was made using two-way Anova and Tukey's test. Results: TME reduced (p<0.05) incubation duration, feed consumption and improved (p<0.05) FCR. Old breeders' eggs had the highest (p<0.05) hatching weight and FCR compared to young breeders. There is an interaction between breeders' age and TME on hatching weight, chick's temperature, feed consumption and weight gain (p<0.05). Conclusion: The effects of thermal manipulation are influenced by breeder's age. TME applied on young breeder's eggs allows for the best productive performances of broilers.

Key words: Thermal manipulation, Breeders age, Hatching process, early Posthatch performances, slow growth broilers. 26th World's Poultry Congress, abstracts selected in 2022

Food safety

Selected short communications

LONGITUDINAL STUDY REVEALS PERSISTENCE OF MULTIDRUG RESISTANT ESCHERICHIA COLI AND HORIZONTAL TRANSFER OF ANTIMICROBIAL RESISTANCE GENES IN THE ANTIBIOTIC FREE POULTRY PRODUCTION PYRAMID

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Antimicrobial resistance (AMR) has decreased in French broiler production for the last 10 years, linked to lower antibiotics use. Nonetheless, animal gut and the breeding environment are still considered as reservoirs of AMR bacteria. The aim of this study was to characterize the AMR dynamic of transmission and persistence in an experimental facility simulating the broiler production pyramid (WPC eposter 380).

Multiresistant E. coli isolated from feces (n=121) of chickens from five families followed over three generations including two sibling batches, one for reproduction and one for meat production, and from each of the two breeding environment samples (n=31) were subjected to whole genome sequencing (Illumina technology). A subset of isolates (n=36) were also sequenced using Oxford Nanopore MinION technology. The 152 E. coli isolates mainly belonged to the phylogroup A (55.9 %) and to the phylogroup B1 (36.2%). A very strong association was observed between phylogroups and batches, with most phylogroup A isolates (96.5 %) coming from the reproduction batch, and most phylogroup B1 isolates (90.9 %) coming from the meat production batch. The major sequence types ST2701 (A), ST93 (A), ST162 (B1), and ST 453 (B1) were shared between families and persisted through generations, and in the environment. The detected AMR genes in E. coli isolates showed a weak diversity, with several genes (i.e. strA, aadA, sul, dfrA, tet(A), blaTEM1) being shared by diverse ST and even phylogroups. However, MinION-based analysis showed that they were carried by independent plasmids, i.e. pIncFIB and pIncHI1 in ST2701, pIncBOKZ in ST93, pIncI1 in ST453. Exchange of AMR genes between ST was nonetheless observed at the level of mobile genetic elements (Insertion Sequences, integrons, transposons).

Our results indicate that the persistence of specific ST in the breeding environment contributes to the transmission of AMR to animals of the successive batches without direct vertical transmission (mother to offspring) between generations. Rearrangements observed in the genetic environment of AMR genes also indicate occasional horizontal transfers within the breeding facility.

CAMPYLOBACTER SPP. IN CHICKEN LIVERS IN SPAIN.

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Campylobacter is the most common bacterial cause of human gastroenteritis worldwide. The largest contributors of human campylobacteriosis in developed countries are poultry and poultry products. The current European legislation regulates levels of Campylobacter on the neck skin from broiler carcasses at the slaughterhouse. However, there have been outbreaks of campylobacteriosis attributed to undercooked chicken livers, which can be contaminated with Campylobacter on both the inside tissues and the outer surfaces. There is a lack of information on the occurrence of Campylobacter in chicken livers in Spain. Therefore, the aim of this study was to determine the prevalence and levels of C. jejuni and C. coli in chicken livers by sampling 56 flocks from two slaughterhouses in Catalonia, northeastern Spain. Three carcasses per flock were collected randomly during the evisceration of the animals, analyzing in total 168 liver and ceca samples. Prevalence of Campylobacter thermophilic species from cecal contents, liver surface and inner tissue were compared by chi-square test for independence. Overall, Campylobacter prevalence was 54,8% in cecal samples, whilst in the liver surface and the internal tissue was 71,4% and 35,1%, respectively. Out of the 92 Campylobacter-positive cecal samples, 45,7% were C. coli-positive, 48,9% were C. jejuni-positive and the remaining 5,4% coinfections with both bacteria were identified. Among the 120 Campylobacter-positive samples of the external surface of the liver, 40,0% were positive to C. coli, 45,8% were positive to C. jejuni and in the remaining 14,2% we found coinfections with both bacteria. Finally, out of the 59 Campylobacter-positive samples of internal tissue liver, a 37,3% were C. coli-positive, a 52,5% were C. jejuni-positive, whilst in the remaining 10,2% of samples we detected coinfections with both bacteria. However, no relation was found between Campylobacter species identified and type of the sample (P>0.05). Coinfections were more prevalent in the livers than in the ceca. The data highlights chicken livers as a potential source of human campylobacteriosis, not only due to the Campylobacter prevalence but particularly because of the bacterial load, which was $>10^3$ CFU/liver or $>10^3$ CFU/g respectively, in 40,1% of the samples of surface liver and in 6,6% of the internal tissue samples. Further research is needed to determine the potential risk of campylobacteriosis due to consumption of chicken livers.

LABORATORY MODEL TO SIMULATE SURVIVAL OF SALMONELLA SPP. IN FAT AND FEED RESIDUES FOUND IN POULTRY HOUSES

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Survival of Salmonella spp. in organic matter has an important influence on recontamination with Salmonella in poultry farming. Therefore, removal of all organic matter is an essential part of the cleaning and disinfection (C&D) process. Especially feeding pipes are hard to clean and seem to be a hot spot for Salmonella persistence between production cycles. Although various reasons for Salmonella persistence in poultry houses are known, information on the survival ability of Salmonella in residues in feeding pipes are limited. The aim of the present study was to contribute to a more detailed understanding of the cause of re-infections in poultry flocks due to survival of Salmonella in feed related substrates. Results from this study could serve for optimization and adaptation of C&D measures for Salmonella positive poultry houses. In order to gain an overview about the ability of Salmonella serovars in fat residues of broiler feed, data under laboratory conditions were generated from 10/07 to 11/21 2021. Different serovars (S. Enteritidis, S. Infantis and S. Typhimurium) isolated from poultry farms in North-West Germany were used for the laboratory experiments. Four types of matrices (phosphate buffer saline, fat, feed, fat-feed mixture) were applied to evaluate Salmonella survival during a simulated production cycle consisting of a 7-day service period (20-35°C) and a 35-day production cycle (35-21°C). To evaluate the growth and survival status of the Salmonella microbiological examination for Salmonella species occurred at five defined time points (-7, 0, 4, 7, 35 days). All samples were invested quantitatively and qualitatively for Salmonella according to ISO 6579-1:2017. In all matrices the bacterial count of Salmonella decreased at the end of the artificially contamination of production cycle. The highest Salmonella count was observed in phosphate buffer saline matrix at day 0 $(6.80\pm0.11 \log 10)$ cfu/q) and did not extremely decline during the production cycle. The lowest Salmonella count was found in fat matrix at day 4 for S. Typhimurium and at day 7 for S. Enteritidis (0 log10 cfu/g). The present study highlights the diverse ability of Salmonella serovars to survive in feed-related matrices. Further investigations are required to determine different survival abilities of Salmonella spp in feeding pipes. Considering the survival ability of Salmonella spp. serovars in feed residues when cleaning poultry houses is highly recommended.

SALMONELLA PREVALENCE AND PERSISTENCE IN INDUSTRIALIZED POULTRY SLAUGHTERHOUSES

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Salmonella contamination sources and transmission routes were studied in five poultry slaughterhouses. Samples from the slaughter and cutting line after cleaning and disinfection were collected, as well as neck skin samples and thighs during slaughter of the first flock.

In total, 680 swab and water samples were taken from the slaughter line before slaughter. In all slaughterhouses Salmonella was notwithstanding cleaning and disinfection still isolated from the slaughter line before start of activities. The prevalence of Salmonella in the plucking area was 10.4% (38/365) (hanging area: 5.0%, scalding tank: 5.8%, plucking machine: 17.0%); in the evisceration room, 1.5% (2/138); and in the cutting area, 2.0% (3/149). No Salmonella (0/28) was found in samples from the chilling line. On neck skin samples taken from the various lines, Salmonella prevalence was 16.1% (48/299) after plucking, 16.0% (48/300) after evisceration, 23.3% (70/300) after chilling; on thighs, prevalence was 10.0% (24/240). Nine Salmonella serotypes were identified of which S. Infantis was the most common serovar (53.8%), especially in slaughterhouse A. Two contamination causes were identified; first, although all flocks had an official Salmonella negative status, this was in one case incorrect and led to an enormous contamination of the neck skins of the flock and the slaughterline (i.e. cooling water). Second, molecular typing revealed cross-contamination from flocks slaughtered one day prior to sampling. Salmonella was apparently not always eliminated by the cleaning and disinfection process and able to contaminate the carcasses of the first slaughtered flock.

In conclusion, the results of this study provided practical insights for poultry production to further improve their Salmonella control, eg. Salmonella status determination closer to the slaughter date, to adapt cleaning and disinfection protocols especially for critical machinery and better hygienic designed equipment.

VACCINATION OF DAY-OLD BROILERS WITH A S. TYPHIMURIUM LIVE VACCINE TO CONTROL SALMONELLA IN EUROPE: A CASE REPORT BASED ON INTEGRATED RISK MANAGEMENT.

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Across Europe, the top Salmonella serovars responsible for human infections of poultry meat origin include group B and group C. Beyond that, out of all serotyped Salmonella isolates from food and animal sources reported by EU member states, 70% originated from broilers. In addition, prevalence of Salmonella Infantis (SI) is on the rise in Europe. SI is known for its increasing multi-drug resistance profile therefore represents an important threat to public health being the 4th most reported serovar causing human salmonellosis in Europe (EFSA and EDC, 2021). In this context, it's critical for the poultry industry to adopt targeted interventions at the live production stage so that less Salmonella reaches the processing plant and ultimately the consumer. The objective of this field study was to assess the protecting effect of single oral immunization of broilers via drinking water with a Salmonella Typhimurium live attenuated vaccine (AviPro[™] Salmonella Vac T, Nal2/Rif9/Rtt), as part of an integrated risk management intervention, to reduce Salmonella prevalence under the official monitoring program. A large-scale field trial including 14 broiler farms, 212 vaccinated flocks and more than 5 million vaccinated broilers has been performed across Europe as part of the Elanco Salmonella 360 program to assess vaccine efficacy to control SI and Salmonella Java (SJ) positivity under commercial conditions, flock after flock. Elanco Salmonella 360 risk management intervention consists of four consecutive steps: a) Sizing the problem; b) Identifying critical control points; c) Mitigating the risk through vaccination and d) Measuring success. Although general prophylaxis (e.g. biosecurity, cleaning and disinfection...) is key to keep the Salmonella infection pressure low in broiler production, vaccination of one-day-old broilers when used systematically has shown to be a relevant tool to reduce positivity rate in environmental samples in those farms with high infection pressure and precedents of SI or SJ. An average reduction of the positive rate has been obtained from 91% in non-vaccinated flocks to 25% after vaccination during at least 3 consecutive broiler flocks. A 100% reduction of the positivity rate was obtained at 5 broiler complexes after having orally vaccinated all flocks belonging to the complex at first day of age during 5 consecutive production cycles.

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PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERNS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM INDUSTRIAL EGGS IN ARDABIL CITY, IRAN

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Staphylococcus aureus is one of the main causes of food-born illnesses. Eggs are considered as major sources for pathogenic bacteria like S. aureus. The purpose of this study was to determine the prevalence and antibiotic resistance patterns of S. aureus on the shell and the contents of industrial eggs in Ardabil city, northwest of Iran. Fifty industrial eggs were collected randomly from poultry farms in different parts of Ardabil city, northwestern Iran during 2019. In terms of Staphylococcus contamination, both shell and egg contents were examined using bacterial cultures and biochemical tests. Then antibiotic resistance rate of isolates was determined using agar disk diffusion method. In this study, 18% of the egg shell samples were infected with S. aureus. also, 4% of contents samples were infected with S. aureus. The highest of antibiotic resistance profile of S. aureus isolates from shell and contents samples was recorded for ampicillin (81.8%), followed by methicillin (72.7%), tetracycline (63.7%), erythromycin (54.5%), streptomycin (45.5%), ciprofloxacin (45.5%), chloramphenicol (36.4%), vancomycin (27.3%) cephalothin (18.2%) and gentamicin (9.1%). The results of this study indicated that lack of attention to hygienic points in storage of eggs can lead to the transmission of S. aureus. Also, due to the high antibiotic resistance of isolates, excessive and incorrect use of antibiotics must be prevented in poultry industry.

Key words: Antibiotic resistance, Staphylococcus aureus, industrial egg, ardabil

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Marketing and economy

Selected short communications

COST-BENEFIT ANALYSIS FOR EGGS COST-BENEFIT ANALYSIS FOR EGGS PRODUCERS PRODUCERS

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Introduction: The supply and variety of feed additives for laying hen feeds is increasing from yearly, which is why growers are debating whether the increase in cost is worthwhile in terms of added cost versus the expected increase in revenue as a result of improved performance. Aim: To examine the cost of various variables in laying hen feeds compared to the benefit of improving the performance by those variables and their financial gain. This is an effective and useful tool for farmers and nutritionists giving them an opportunity to achieve optimal economic decisions. Materials & methods: The model examines the interaction in the following current flock parameters: Feed price, average egg weight, average daily feed consumption, hen-day production, percentage of cracked eggs and percentage of dirty eggs, and changes in these parameters on a monthly basis. Any cost modification due to feed prices, average daily feed consumption and/or a variation in income as a result of a change in the hen-day production, egg weight, percentage of cracked eggs and percentage of dirty eggs are taken into consideration. The model calculates a current flock revenue versus monthly expenses, with reference to the current layer flock performance, as well as taking into account upcoming fluctuations in feed variables cost against the expected additional revenue. The calculated balance is per layer hen for the coming month. For example: (prices in \in) Eqgs price: XL= 0.1202, L= 0.0548, M= 0.0484, S= 0.0373, Cracked & Dirty eggs = 0.028. Current Flock Data: Feed Price = 324 €/ton, Average Eggs Weight =64-gram, Average Daily Feed Consumption = 120 gram/day/hen, Hen-Day Production = 90%, Percentage of Cracked Eggs = 2% and Percentage of Dirty Eggs=2%. If the price of the feed increases by $7 \in /$ ton as a result of adding an additive to the feed, it will cost the farmer 0.026 € per month per hen. In order to offset this added cost, hen's performance needs to be improved. Egg weight must increase by 0.4 gram, or average daily feed consumption must decrease by 2.5 gram. The model can combine the parameters on demand. Conclusion: The model examines the different interactions between the above-discussed parameters and allows the farmer the opportunity to make an optimal judgment concerning the contribution of feed additives or other decisions related to the interaction of parameters.

LAYING HENS - HUSBANDRY SYSTEMS ON DIFFERENT WELFARE LEVELS – AN ECONOMIC COMPARISON

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Due to stricter animal protection's regulations worldwide, Germany seeks to get its pioneering role in this area. One of the most important milestones, among others is the abolition of cage farming for laying hens. However, the question remains: is everything on track? Most of the current farming systems show some weaknesses which are not yet adequately covered. Towards a better future, together with farmers and consultants, the Thünen Institute developed criteria for objective evaluation of the current housing systems in a comparative way. In the first step, biological and technical production figures were compared for each farming system in order to figure out the causes of the discrepancies in total costs. The assessment of the production costs in the investigated housing systems was carried out on the basis of branch evaluations at farm level, which provided valuable information. The evaluations showed that none of the farming systems currently being used can be completely convincing. Although barn husbandry enables low production costs (7.5 ct./egg), however, the animals suffer limitations regarding space availability and exercise space outside. Providing a veranda could help in this context, but at the same time it might impair the ventilation system. With free-range system, animals are given the opportunity to use the outdoor area, but often stay close to the barn to escape birds of prey, especially when there are no shelters. This can lead to high nitrogen emissions and infection risks. Furthermore, if free-range farming has to be temporarily banned due to an outbreak of avian flu, the conditions in the free range-barn are the same as in the barn husbandry without outdoor access. In principle, this problem also arises with mobile barns; however, the damage caused by predators is even worse. Moreover, it can also be difficult to ensure the mobility of the barns and thus sufficient nutrient distribution in winter and rainy conditions. The production costs are more than twice as high as for barn husbandry, mainly because of the high labour input. In terms of direct costs (especially feed), smaller flocks can also incur cost disadvantages if rebates can only be obtained for higher purchase charges. The process of optimizing current husbandry systems has started.

KEYWORDS: laying hens, animal welfare levels, free range, cost comparison

THE KNOWLEDGE AND ATTITUDE OF PEOPLE ON ALLEGED ROLE OF POULTRY PRODUCTS IN SPREAD OF COVID-19, ITS ECONOMIC REPERCUSSIONS ON INDIAN POULTRY INDUSTRY AND ROAD AHEAD

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As WHO declared Covid-19 as pandemic, a global lockdown ensued, which restricted major economic activities. The panic among people was all time high, which helped spread rumours faster than the pandemic itself. The alleged role of poultry products in Covid-19 spread is one such example. It caused drastic downfall in poultry demand way before the lockdown, causing collapse of Indian poultry market with a loss of \$ 3.06 billion, even worse than Avian influenza epidemic of 2006. With an intent to assess the knowledge and attitude of people from all backgrounds towards alleged role of chicken in Covid-19 spread, we conducted a google form-based survey with multiple choice and checkbox questions. 78% respondents were graduate or above. 41% were not directly associated with poultry industry. 36.2% of them believe that animals and poultry can get infected with Covid-19, based on social media inputs (56%). Yet, 69% respondents denied possibility of Covid-19 spread to humas through egg and meat with 75% further denying to witness any such case and 57% told it was a myth. 70.6% respondents consumed and 63% convinced others to eat poultry products during pandemic and agreed to advise eggs to Covid-19 patients to boost immunity (75%). 65% of respondent agreed that Covid-19 affected poultry industry economically, albeit it bounced back by convincing consumers with awareness drives (51.5%) and scientific discussions (55%). On a positive note, many agreed that one can be trained to spot such rumours and deal with them by nourishing scientific temperament (36%), refraining to propagate potential misinformation (55%), consulting concerned experts and fact-check (45%). Only 26% respondents think that government helped the industry financially. Many respondents opined that, loan waiver (40%), provision of capital (41%), direct cash transfer (26%) and subsidy on raw material (36.4%) would have been of great relief to farmers. State can also control possible damage by creating awareness (66%), framing strong legislations (53%) and improving surveillance and cyber security (46.4%). To conclude, the role of poultry products to spread Covid-19 in humans is a myth and yet to have a scientific base, however, it has already affected Indian poultry industry. This survey gives insights into the changing attitude of people towards the issue and highlights the necessity to train deal more maturely in future. mankind to with such issues the Key words: Covid-19; Poultry; India; Economics

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Hygiene

Selected short communications

EFFECTS OF DOWN-TIME AND LITTER MANAGEMENT PRACTICES ON CROSS-CONTAMINATION OF BROILER FLOCKS WITH CAMPYLOBACTER JEJUNI

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Campylobacter is an important foodborne pathogen affecting the poultry industry and causes over 96 million cases per year worldwide. This organism is highly associated with poultry products, therefore efforts to control Campylobacter during production are necessary. Because broiler farms typically reuse litter for multiple flocks, there is potential for cross-contamination with Campylobacter from one flock to another. The objective of this experiment was to determine if reusing litter leads to Campylobacter cross-contamination, and to determine the effects of common litter treatments on this potential phenomenon. Two flocks of birds were raised in 25 pens up to 42 days for this experiment. For the first flock, birds were inoculated with 3 marker strains of Campylobacter jejuni (ciprofloxacin resistant) on day 7 and allowed to naturally contaminate fresh litter by fecal shedding. One set of 5 pens was left uninoculated to serve as a negative control. After flock 1 was terminated, a down-time of 19 days was implemented, and prior to placement of the second flock, litter treatments were applied. Treatments consisted of 5 treatments with 5 pens each. The treatments included uninoculated, fresh litter; untreated reused litter; composted reused litter; reused litter treated with sodium bisulfate; and reused litter composted and treated with sodium bisulfate. The second flock of birds were then placed on the treated, reused litter. For both flocks of birds, on days 7, 14, 21, 28, and 42, litter samples from each pen were collected by boot swab and 5 ceca samples per pen were collected. Samples were enriched and analyzed for C. jejuni using the 3M Molecular Detection System, and positive samples were confirmed by streaking onto Campy Cefex plates supplemented with 1mg/L ciprofloxacin. For flock 1, overall C. jejuni prevalence in ceca samples was 0% at day 7 (before inoculation) and reached 94.4% by day 42. Prevalence in boot swabs was also 0% at day 7 and reached 100% by day 42. For flock 2, no C. jejuni was detected in any samples. These results indicated that reusing litter is not a potential source for C. jejuni cross-contamination when a down-time of 19 days is applied between flocks. Further research is needed to determine the minimum down-time necessary to eliminate this risk.

THE ROLE OF SALMONELLA RECONTAMINATION ON SOCKS SWAB SAMPLES IN THE COMMERCIAL BROILER FARMS OF THAILAND

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Salmonella infections are a major public health concern worldwide. The main reservoir of Salmonella is the intestinal tract of food-producing animals such as poultry, with Salmonella are widely distributed and persistent in the environment through biofilm formation. Methods for its control include effective cleaning and disinfection (C&D) of poultry houses and equipment as well as biosecurity procedures during broiler production. However, the presence of Salmonella recontamination, the reoccurrence of Salmonella contamination in poultry house and equipment after completion of the C&D process, there is little evidence to support recontamination as a major factor. The objective of this study was to examine the role of recontamination of the broiler houses and equipment by using sock swabs to sample litter and gauze swabs to sample equipment in commercial broiler farms in Thailand. The study was conducted from October 2019 to October 2020 in Northeastern regions. In total, seventy-four gauze swab samples were sampled to include live darkling beetles, floor of service room, feeder and drinker system, hopper, bin, house walls, rodent bait box, wire rope, brooding barrier, electric pipeline, cooling pad, ventilation, shoes and weighing scale in the period of post C&D and three days prior to slaughter which are thirty-five days apart, compared with the socks swab sampling the floor litter of the houses at three days prior to slaughter from one commercial broiler farms established according to the Thai Agricultural Standard on Good Agricultural Practices for Salmonella spp. examination follow with ISO 6579-1:2017. Comparison of Salmonella results for post C&D samples and samples collected three days prior to slaughter were conducted. Then the relative risk of Salmonella recontamination samples with serovars linkage in socks swab samples were analyzed statistically using Pearson's method (P < 0.05). The result showed the relative risk of chi-square recontamination by Salmonella on socks swab is 2.9870 with statistical significance (p<0.05). The conclusion of this study showed the Salmonella recontamination play an important role to Salmonella positive with serovars linkage founded by socks swab. Effective C&D could prevent Salmonella from remaining and spreading to other areas with biosecurity procedures from post C&D period until slaughter being crucial during broiler production.

BACTERIAL CONTAMINATION OF NIPPLE WATERING SYSTEMS IN EMPTIED BROILER HOUSES

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The quality of drinking water is important for broiler health and welfare. Contamination of the water supply systems with pathogenic organisms may result in zoonosis and epizootics. No studies have been conducted in Hong Kong to investigate drinking water contamination in broiler farms. In this study, we aimed to investigate the change of the bacterial burden of nipple watering systems in emptied houses between flocks. Twenty-four out of 29 broiler farms, representing 83% of all licensed premises in the territory, participated in this study. Water samples were collected from drinking nipples at proximal, medial, and distal positions to the reservoir tanks at the finishing period (T1) and immediately before the introduction of a new flock (T2). The houses remained empty for seven days (i.e. between T1 and T2). Total bacterial count (TBC) and total coliform count (TCC) were determined by heterotrophic plate count and membrane filtration, respectively. At T1, the ventilator blades of poultry houses were additionally sampled. Tracheal swabs were collected from finisher broilers. At T1, both the median TBC and TCC were less than 1 colony-forming unit (CFU) and 987 CFU per mL, which were acceptable according to the American Public Health Association guideline. Immediately before introducing a new flock, the bacterial load of TBC and TCC increased by four-fold (t=-3.207, P=0.003). Tracheal colonization of finisher broilers with Escherichia coli was associated with an increased risk for isolating the organism from the ventilator blades (Odds ratio=3.2, 95% CI 1.6-8.7) The ventilators and water nipples are reservoirs for coliforms. Prolonged static water flow of the drinking pipe systems may have contributed to the overgrowth of aerobic bacteria. Water quality of nipple systems should be monitored at the end of the emptying phase. Increasing the water flow for a few hours before introducing a new flock is advised.

Authors' contributions

JH and OS provided intellectual input and performed data analysis. DY and RT supervised the project, collected field specimens and liaised with stakeholders. KLS and GL were responsible for laboratory testing, data curation and assisted field visits. This project was funded by Agriculture, Fisheries and Conservation Department of the Hong Kong Special Administrative Region (AFCD-SADF-0010).

IMPACT OF THE VACCINES ADMINISTRATION QUALITY ON THE IMMUNIZATION STRATEGY AGAINST CHICKEN ANEMIA VIRUS

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The administration of poultry vaccines via drinking water is a long-established and widely used vaccination route. Although it looks easy at first glance, this route of administration requires great care in order to obtain satisfactory results. The objective of this field study was to evaluate the impact of the vaccines administration quality on the immunization level of breeders vaccinated with different vaccination strategies against Chicken anemia virus (CAV). This evaluation took place in a broiler breeder rearing farm facing challenges to achieve optimal seroconversion with the implemented vaccination strategy against CAV. In the first part of this study, four breeder flocks (group A) were vaccinated with the regular program with Nobilis® CAV P4 (MSD) via subcutaneously at 6 weeks of age and AviPro® Thymovac (Elanco Animal Health) orally at 13 weeks of age. A quality control vaccination audit was performed and revealed poor water quality and several deficiencies in the drinking water vaccination procedure. Vaccination crew was trained to improve the quality of the water and the drinking water vaccination process. In the second part of the study, the same vaccination crew administered orally AviPro® Thymovac to four breeder flocks (group B) at 13 weeks of age. Vaccination audits revealed corrected measures to improve drinking water quality and vaccines administration quality were successfully implemented. CAV serological response of the two groups was assessed with Idexx® ELISA kits at 6 weeks after AviPro® Thymovac vaccination. Serological results of group A showed presence of seronegative breeders and heterogeneity of the CAV antibody response with coefficients of variation (CV) higher than 65% in the four vaccinated flocks. Interestingly, flocks of the group B received only one CAV vaccine but all of them presented 100% seroconversion, very high homogeneity of the CAV antibody response (CV lower than 20%) and significantly higher mean antibody titers than group A. The improvements in vaccination effectiveness evidenced that single administration of AviPro® Thymovac induces strong immunization in breeders. These findings highlight the importance of correct procedures used for poultry vaccination; inappropriate administration can neutralize or decrease the immunological properties of vaccines resulting in minimal or no immunization and a waste of time and money for the poultry operation.

IN VITRO SURVIVAL OF LIVE SALMONELLA ENTERITIDIS AND SALMONELLA TYPHIMURIUM VACCINE STRAINS ON MATRICES RELEVANT TO POULTRY HOUSE ENVIRONMENTS

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Live, attenuated vaccines against Salmonella Enteritidis and S. Typhimurium are widely used in laying hens and breeding flocks, due to their ability to induce both humoral and local cellular immune responses in the intestinal tract. Live vaccines are shed by immunized birds for a short period of time after vaccination, and persistence of the vaccine strains in the environment is undesirable. Earlier studies have indicated that the live vaccine strains Sm24/Rif12/Ssg (AviPro™ Salmonella Vac E, Elanco Animal Health) and Nal2/Rif9/Rtt (AviPro[™] Salmonella Vac T, Elanco Animal Health) have a limited survival time in the environment compared to wild Salmonella strains, which can persist for type several months. The aim of this study was to determine in more detail the survival properties of the above-mentioned vaccine strains on a range of relevant matrices. These included different types of bedding (rice husks, straw and wood shavings) and solid galvanised surfaces (wood, steel, polystyrene and concrete). Faeces from Specific Pathogen Free hens and a Salmonella-free free-range laying flock, spiked with 103-104 cfu of the live vaccines, were added to the matrices and held at 10°C and 20°C. At different time points, faeces were collected and suspended in Buffered Peptone Water supplemented with antibiotics that are selective for the vaccines (rifampicin + streptomycin for strain Sm24/Rif12/Ssq; rifampicin + nalidixic acid for strain Nal2/Rif9/Rtt). A portion of the broths were plated out directly onto antibiotic-supplemented selective agars (Brilliant Green Agar for strain Sm24/Rif12/Ssg and Xylose-Lysine-Deoxycholate Agar for strain Nal2/Rif9/Rtt) to obtain a quantitative result. The remaining broths were incubated at 37°C for 18-22 hrs. Where no colonies were obtained from direct plating, enriched broths were plated onto selective agars to detect low levels of vaccine. The source of the faeces had a major effect on vaccine survival, with the competitive flora in the faeces apparently having a considerable impact on the survival of the vaccine strains. In samples containing faeces from SPF birds, there was a much slower decline in recoverable levels of vaccine compared to faeces from the free-range flock, for which a >99% reduction in detectable numbers was observed within six days. Full results of survival of the two vaccine strains on all seven matrices with statistical analyses and discussion are presented.

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Hygiene and pathology

Posters

MODE OF VACCINATION IMPROVES WELFARE OF POULTRY

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Introduction

Most of the vaccines because of its mode of application, can cause stress. Disadvantage of in ovo delivery is related to the age of the parent flock, time of incubation when it is applied is expensive and therefore not always affordable for smaller producers. Therefore, it is not used for ND, POX, MD, IBD or IB control. An alternative offers ultrasound nebulisation. Particles (3-5µ) of vaccine water suspension reach the deep respiratory system thus increasing the adsorption surface, enabling immunity to develop much earlier than after standard aerosol. Material and methods. In small trial groups of 20 chickens, an ultrasonic nebulizer was used to deliver water suspension of virus vaccines to newly hatched chickens. The vaccine used were against MD (HVT), ND (La Sota, QV4 or Ulster 2C), IBD (228E, Winterfield 2512), IB (IB 4/91), or simultaneous avian POX+ HVT. One dose per bird was given for 5, 10, or 30 seconds to prove the influence on resulting immunity and health. In field trial total of 307.955 light hens were simultaneously vaccinated i/m CVI988 and 24 hours later with HVT (nebulization). Control flocks of 307.955 birds were vaccinated only with CVI988. Chickens vaccinated against POX + HVT were revaccinated at age of 28 days with the AE+POX vaccine using a standard sprayer. Challenge infection for ND was performed with Texas GB (for QV4 and U2C) or Herts 33 (La Sota). For IBD a "hot"strain was used, and for POX an analogous wild strain was used. Immunity level and its duration were determined by standard serology. Controls were also included. Results. No adverse effect of nebulization could be seen. Better yield (body weight at day 28 and 35) was recorded in chickens vaccinated against IB, IBD, or POX while other groups did not differ significantly. The viremia for MD (HVT) appears after only 48 hours, and for ND, IB, IBD last much longer compared to standard or in ovo vaccination. Simultaneous vaccination against MD resulted in a reduction of mortality to 0.23% compared to 4,98 in the CVI 988 group up to 18 weeks of age. Primed with the POX vaccine chicken is revaccinated at age of 10-12 weeks with the AE+POX using a simple sprayer. Conclusion. Nebulization is safe, avoids stress to vaccinated poultry. Affordable for small enterprises, save labor and improve welfare, especially important when birds are caught to be injected. The device is adaptable to the size of the operation, and offers inexpensive control of threatening diseases like MD, ND, POX, or even avian influenza.

EFFICACY OF A NEW IMMUNE-COMPLEX IBDV VACCINE AGAINST AN EXPERIMENTAL INFECTION WITH THE DD1 VVIBDV STRAIN IN BROILER CHICKEN.

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Introduction: GUMBOHATCH® is a new immune-complex vaccine against Infectious Bursal Disease (IBD) developed by HIPRA. This new immune-complex vaccine has introduced a different formulation (IgY egg origin) and control parameters to ensure the complete coating of the vaccine virus and the maintenance of the maximum potency, even in the presence of high levels of maternal antibodies (MDAs). The intended use of the vaccine is to protect chicks as soon as the MDA levels start to drop.DD1 strain is a very virulent infectious bursal disease virus (vvIBDV) strain isolated in 2016 from broiler chickens in the Volgograd region of Russia. The objective of this trial was to assess the efficacy of GUMBOHATCH® in broiler chickens after an experimental challenge with the DD1 strain. Experimental design and description of material and methods: a randomized trial to assess the efficacy of GUMBOHATCH® vaccine was performed in broiler chickens. Forty animals were included in the study, either vaccinated with GUMBOHATCH® or mock vaccinated with PBS. The administration of the vaccine was carried out subcutaneously to 1-day-old chicks. Each chick was administered with one dose of the vaccine. The trial involved a challenge with the DD1 strain at 28 days of age. Some birds were necropsied before challenge in order to check the vaccine virus replication. The rest of the animals were challenged and necropsied at day 5 after infection. To assess the protection several variables were studied. The primary variable was the presence of external oedema in the Bursae of Fabricius (BF) after challenge. Secondary variables were macroscopic lesions on the BF and the relative weight of the BF and spleen. Serological response and presence of viral RNA at the bursa of Fabricius before and after infection were also monitored. All continuous variables were described and compared between groups, using the Mann-Whitney test or t-tests depending on the data. Proportions were compared using the Fisher exact test. Results: seroconversion to IBDV was observed in GUMBOHATCH® vaccinated birds before challenge. Vaccinated animals showed a reduction in the macroscopic lesions, including oedema of the bursa of Fabricius, compared to mock-vaccinated animals. Moreover, splenomegaly was only observed in the mock-vaccinated and infected aroup. Conclusions: The results of this study demonstrate the efficacy of GUMBOHATCH® vaccine in broiler chickens against an infection with the DD1 strain.

SENSITIVITY AND RESISTANCE TO NARASIN-NICARBAZIN OF EIMERIA SPP. ISOLATED FROM BROILERS IN EUROPE ANNO 2020-21.

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Narasin-nicarbazin (NN) is a combination anticoccidial for controlling Eimeria in chickens. The product has demonstrated reliable control of coccidiosis in broilers since its introduction but after years of routine use, the sensitivity of broiler field Eimeria populations seems to decline. The latest publications on anticoccidial sensitivity test (AST) monitoring in Europe refer to the period 1996-2002 showing a high level of resistance to most coccidiostats (Peek & Landman, 2006). Unlike anticoccidials, vaccines do not evoke resistance. Omitting anticoccidials from the feed and use coccidiosis vaccines instead may restore sensitivity in resistant Eimeria strains (Chapman & Jeffers, 2015). The present study surveyed the current sensitivity of broiler Eimeria strains to the NN combination. The strains were collected from fecal samples of 3 to 4-week-old broiler flocks in 10 European countries between November 2020 and April 2021. These included 23 flocks that routinely used coccidiostats and 4 flocks that had opted for preventive vaccination as the exclusive coccidiosis control method. All samples were passaged on chickens to prepare the inocula for use in AST trials. E. acervulina was the predominant species. Each inoculum was administered by oral gavage to 2 groups of 30 broilers that were either left untreated or received in-feed treatment with NN, at the age of 13 days. At 6, 7- and 8-days post inoculation, the chicks' weight gain, their coccidiosis lesion score and the number of excreted oocysts were determined. The protection provided by the anticoccidial was evaluated by using statistics and the anticoccidial index criteria of Chapman & Shirley (1989). Untreated, uninoculated birds were used as negative controls. Isolates from farms using in-feed anticoccidials showed poor sensitivity to NN, with resistance and partial resistance observed in respectively 83% and 17% of them. By contrast, all isolates from the coccidiosis vaccinated farms demonstrated good sensitivity, no resistance found. This concurs with the studies of Peek & Landman, demonstrating that farms that routinely use coccidiosis vaccination have a high level of sensitivity to anticoccidials. As resistance to anticoccidials increases, even to combination compounds, new strategies must be considered for sustainable coccidiosis control. Chapman H. & M. Shirley. 1989. Res Vet Sci. 46, 114-117; Chapman H. & T. Jeffers. 2015. Poult. Sci. 94, 943-946; Peek H. & W. Landman. 2006. Avian Dis. 50, 434-439.

DIFFERENCES IN CAMPYLOBACTER GROWTH IN A NOVEL MEDIUM IN FLASKS INCUBATED AEROBICALLY OR ANAEROBICALLY

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Campylobacter are major foodborne pathogens associated with poultry products. These bacteria require carbon dioxide (CO2) for growth and are usually cultured in containers incubated in artificially produced, microaerobic atmospheres. The objective of this research was to examine the growth of the pathogen in a novel medium in containers incubated aerobically or anaerobically. A medium composed of beef extract, tryptose, soluble starch, sodium bicarbonate, sodium lactate, and agar was prepared then inoculated with log 4.0 cfu/ml of Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, or Campylobacter lari. Media was transferred to flasks with vented caps, vented caps covered with Parafilm, or plugsealed caps; then incubated at 37C for 48 h in aerobic (21% 02, 0.04% CO2, 78% N2) or anaerobic (10% H2, 5% CO2, 85% N2) atmospheres. Campylobacter were then enumerated, and significant differences in the number of cfu recovered were determined using GraphPad InStat statistical software. Results indicated that Campylobacter growth was dependent on type of flask closure and incubation atmosphere. There was no significant (p < 0.05) difference the growth of different Campylobacter species incubated in flasks with the same closures when incubated in the same atmosphere. Although, no Campylobacter were recovered from vented flasks incubated aerobically, 4-5 log cfu Campylobacter/ml were recovered from vented flasks incubated anaerobically. Also, significantly more Campylobacter (log 7-8 log cfu Campylobacter/ml) were recovered from Parafilm-covered flasks and plugged flasks than from vented flasks incubated aerobically or anaerobically. Other studies have shown that relative CO2concentrations in the flasks were vented<Parafilm<plugged; therefore, the growth of the pathogen in the flasks was probably related to the CO2 concentration in the flasks. Atmospheric exchange between vented flasks and aerobic atmospheres produced low CO2 concentrations that could not support Campylobacter growth; however, exchanges between vented flasks and anaerobic atmospheres produced CO2 concentrations in flasks that could support Campylobacter growth. Conclusions indicate that Campylobacter can be grown in the novel medium in sealed containers without the production of artificial, microaerophilic atmospheres. These findings demonstrate that this procedure can be used as a more economical, simplified procedure for growth of Campylobacter.

ISOQUINOLINE ALKALOIDS ALONE IMPROVE PERFORMANCE OF COCCIDIOSIS VACCINATED BROILERS MORE EFFECTIVELY THAN IN COMBINATION WITH SAPONINS

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Objective: The aim of this study, carried out with coccidiosis vaccinated broilers, was to assess the individual and combined effects of two feed additives that in practice are often successfully combined with a coccidiosis vaccine: isoquinoline alkaloids and saponins. Coccidiosis vaccinations are becoming increasingly popular in practical broiler production due to efforts to reduce the use of chemical and ionoophoric coccidiostats. Material & Methods: For a study duration of 49 days, 880 1-day-old female Cobb 500 broilers were allocated to 5 treatments with 8 replicates of 22 broilers each. The treatments were: NC (Negative Control); VA (Coccidiosis vaccination (1x) with Fortegra, MSD); IQ (VA + Isoquinoline alkaloids from 60 ppm of Sangrovit® Extra, Phytobiotics Futterzusatzstoffe GmbH, Germany); SA (VA + 500 ppm of a combination product containing saponins from Quillaja saponaria and Yucca schidigera); IQSA (VA + IQ + SA). Performance was measured by feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and mortality. Data analysis was done by ANOVA with SAS. Significance level was set at p<0.05. Results: From Day 1-21, the groups SA and IQSA had a significantly lower (p<0.05) FI than the NC (1,216 g, 1,245 g and 1,314 g, respectively), but this effect did not persist throughout the remaining trial. Overall BWG (Day 1-49) of the group IQ was significantly higher (p<0.05) compared with SA (2,859 g vs. 2,706 g), while the other treatments were intermediate with no significant differences. IO had a significantly lower FCR than SA and IOSA (1.75; 1.82 and 1.81, respectively) (p < 0.05). VA also had a significantly lower FCR than SA (1.77) (p<0.05). The FCR of the other treatments did not differ (p>0.05). There were no significant differences in mortality between the treatments (p>0.05). Conclusion: Combining a coccidiosis vaccine and isoquinoline alkaloids yielded the best performance regarding BWG and FCR. Both parameters differed significantly from the treatment combining the vaccine and saponins. Furthermore, the sole supplementation of saponins led to a significantly higher FCR than the vaccinated treatment with no additive. While the BWG of a combination of isoquinoline alkaloids + saponins (IOSA) did not differ significantly from a supplementation of isoguinoline alkaloids alone, the FCR of IQSA was significantly higher, indicating that the sole supplementation of isoquinoline alkaloids was most beneficial for performance and feed efficiency.

EFFECT OF CO-INFECTION OF LOW PATHOGENIC AVIAN INFLUENZA H9N2 VIRUS AND AVIAN PATHOGENIC E. COLI ON H9N2 VACCINATED COMMERCIAL BROILER CHICKENS.

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For decades, low pathogenic avian influenza virus (LPAIV) subtype H9N2 has been endemic and still in many Middle Eastern countries including Egypt. The majority of losses associated with the H9N2 virus come from complicated and mixed infections in commercial broilers especially E-Coli infection. Furthermore, the role of H9N2 vaccination in worsening these losses was not evaluated before. In this work, 688,065 Arbor acres broiler chickens from the same breeder company were distributed equally in four stations, where two pens were vaccinated against LPAIV of subtype H9N2 virus, and the other two pens served as a non-vaccinated control. All were placed in the same station under the same management conditions. Twenty birds from each pen were moved to Biosafety level -3 chicken isolators (BSL-3) on days 21 and 28 of life and challenged with LPAIV-H9N2 or E-coli. Seroconversion for H9N2 was evaluated before and after the challenge. The recorded results revealed a significant decrease in clinical manifestations, and virus shedding in terms of virus amounts and the number of shedders in vaccinated birds compared to non-vaccinated birds. However, no significant differences were found between the vaccinated and non-vaccinated flocks in mortality rates. In groups co-infected with E. coli and the wild strain of LPAIV of subtype H9N2, mortality rates were higher than those in groups challenged with LPAIV of subtype H9N2 alone. In conclusion, use of the LPAIV H9N2 vaccine can minimize the losses and risks after co-infection with E-coli and AIV-H9N2 virus. Vaccination with LPAIV-H9N2 has considerably impacted the health status, amount of virus shed, and mortality of challenged commercial broilers.

CLINICAL AND SUB-CLINICAL CHICKEN COCCIDIOSIS IN DHAKA, BANGLADESH: PREVALENCE, PATHOLOGY AND RISK FACTORS

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Introduction with objectives: Coccidiosis is an important protozoal disease caused by Eimeria spp. and has the greatest economic impact in poultry industry in Bangladesh. Coccidiosis is mainly controlled by the application of anticoccidial feed additives and chemotherapeutic agents in Bangladesh, but it may contribute to drug resistance. To adapt alternative approach and effective control program, it is essential to have adequate epidemiological data on chicken coccidiosis in farm conditions, that is so far, limited in Bangladesh. Therefore, this study was conducted to determine the prevalence and pathology of clinical and sub-clinical coccidiosis in chickens in relation to age and type of chickens at Dhaka division of Bangladesh. Methodology: A total of 384 samples from three age groups of chickens of 26 layer farms, 26 sonali farms, 18 broiler farms and 34 native chickens were collected from Dhaka division. Questionnaire survey, necropsy and oocyst identification were done to detect the prevalence and pathology of chicken coccidiosis. Results: The prevalence of chicken coccidiosis were 18.46%, 20%, 23.85% and 17.65% in layer, broiler, sonali and backyard chickens, respectively with an overall rate of 20.57%. Among the positive farms, clinical and sub-clinical coccidiosis was 63.16% and 36.84%, respectively. Out of them 15.38%, 16.67% and 19.23% of layer, broiler and sonali farms, respectively were infected with clinical coccidiosis. On the other hand, 7.69%, 11.11% and 11.5 4% layer, broiler and sonali chicken farms, respectively were infected with sub-clinical coccidiosis. The overall prevalence of coccidiosis was highest in poultry of >3-6 weeks of age, followed by >6 weeks and <3 weeks of age group, respectively. Prevalence of cecal coccidiosis, intestinal coccidiosis and mixed infection were found highest in sonali (18.46%), broiler (8.89%) and native chickens (5.88%), respectively. Clinical signs of coccidiosis were mostly bloody feces, emaciation, drowsiness and poor weight gain. In case of cecal coccidiosis, the necropsy findings included ceca full with blood, cecal core formation inside the cecal blind pouch and complication with ND or IBD. In case of other intestinal coccidiosis, whitish plaques in middle intestine, small petechia and sero-mucoid exudates in duodenum and jejunum were observed. Conclusions: This study will add important information about chicken coccidiosis in Bangladesh, which will help to design appropriate control strategies.

EFFECT OF COCCIDIOSIS VACCINE CHALLENGE ON HEALTH, GROWTH PERFORMANCE AND PLASMA VITAMIN ABSORPTION OF BROILER CHICKENS FED OPTIMUM DIETARY VITAMINS (OVN)

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In this study the effect of coccidiosis vaccine challenge on health, growth performance and plasma vitamin absorption of broiler chicks with optimum dietary levels of vitamins (OVNTM) were evaluated. A total of 840 fourteen-day-old male broiler Cobb 500 chicks were allocated to two treatments: control and challenged group. On day 14, birds in challenged group were given a 30-fold dose of coccidiosis vaccine while the others in the control group were given the same amount of sterile water. All birds were provided with the diet containing optimum dietary levels of vitamins (OVNTM). The trial lasted from 14 to 28 days with 12 replicate pens for each treatment and 35 chicks in each pen. Coccidiosis lesion scores and plasma vitamin absorptions were determined at 7-day post-challenge. Results showed that, coccidiosis vaccine challenge resulted in higher (P < 0.05) coccidiosis lesion scores, reduced (P<0.05) growth performance and plasma absorptions of vitamin A, a-tocopherol and 25-OH-D3. Birds fed OVN-diet in the control group performed superior to the Cobb 500 standard, while the performance was similar to the Cobb 500 standard when the birds were challenged with coccidiosis vaccine. There was a strong positive correlation (R = 0.976, P<0.001) between i-check quick-test a-tocopherol and HPLC analytical a-tocopherol. The present results suggest that the use of OVNTM-diet may exhibit beneficial effect on growth performance.

PROTECTION CONFERRED BY A LIVE SALMONELLA ENTERITIDIS VACCINE AGAINST A PATHOGENIC SALMONELLA PULLORUM STRAIN IN YELLOW-FEATHERED CHICKENS

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Pullorum disease is an acute and systemic pathogenic condition caused by Salmonella Pullorum, which is characterized for high mortality rates in young chickens, being an epidemic disease that seriously endangers the development of poultry breeding industry in China and many other parts of the world. Our laboratory evaluated the safety and efficacy of the commercial live Salmonella Enteritidis vaccine strain Sm24/Rif12/Ssq (AviPro[™] Salmonella Vac E) using yellow-feathered chickens as experimental animals, orally challenged with the standard S. Pullorum strain CVCC533 (infectious dose of 5×106 CFU), to provide reference for the clinical application of this live vaccine to mitigate the risk against pullorum disease. The study assessed the protective effect against S. Pullorum infection conferred by the tested live vaccine, using three different immunization schemes: single dose (on day 2 of age) with challenge on day 3 of age; two doses (on day 2, week 6) with challenge on week 7 of age; 3 doses (on day 2, week 6 and week 16) and challenged on week 17 of age. After early challenge at day 3 of age, vaccinated birds have shown only 40% mortality, while the control group did show 70% accumulated mortality rate, thus leading to significant statistical differences. These experiments under controlled conditions demonstrated that the tested vaccine was able to reduce mortality by 30%. One vaccination (on day 2) could reduce the mortality caused by S. Pullorum infection with significant statistical differences versus the control group, thus minimizing the clinical signs and reducing the course of disease. After two vaccinations, reduction in excretion of the challenge strain was observed from 40% to 25% at 3 days post-challenge. Vaccination alone is not enough to provide complete protection to the flock; it is therefore recommended to apply the vaccine according to the label indication in association with complementary measures under the frame of an integrated risk management program to fully reduce the morbidity and mortality in chicks.

Keywords: Salmonella Pullorum; chickens; mortality; efficacy

FIELD EVALUATION OF COLD CHAIN MANAGEMENT PERFORMANCE

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Cold chain management still represents a significant challenge of the poultry operation because play an extremely important role to ensure the livability of poultry vaccines. Inappropriate cold chain, temperature monitoring equipment or maintenance of cold chain equipment can negatively affect the vaccines immunogenicity resulting on lack of protection of the vaccinated flock. A parent stock company located in the Southwest of Brazil was facing vaccination failures evidenced during serological monitoring after the administration of temperaturesensitive live vaccines. The most important finding of the vaccination audits was inaccurate temperature monitoring of the refrigerators used for storage of the vaccines. Purpose of this study was to evaluate the cold chain performance during vaccines storage as potential cause of lack of vaccines efficacy. Temperature data loggers were installed inside the refrigerators used for storing vaccines in six different breeder rearing farms of this company. Highly sensitive temperature data loggers of the Hobbo® brand were used to record the temperature every 5 minutes during two consecutive months. Temperature analysis was performed after the measurement period with special attention to the temperatures outside the recommended range (2°C to 8°C) for more than 30 consecutive minutes. During the study period, 100% of the loggers recorded temperatures above and below the recommended range. In-deep temperature analysis identified too high temperatures as most important deviation of some refrigerators and too low temperatures with consequent risk of frozen vaccines in other refrigerators. Some potential factors identified to contribute to weaknesses of the cold chain were quality of refrigerators, improper use of refrigerators, power interruption, equipment breakage, lack of trained personnel on cold chain management as well as inaccurate monitoring and recording of the refrigerator temperatures. Findings were presented to the vaccination crew and a comprehensive training was performed to show the importance of the cold chain and temperature recording. This allowed corrections in internal company processes and investments in refrigerators. Cold chain management is critical to ensure the quality, safety and efficacy of a vaccination program. Once the potency of a vaccine is lost, it cannot be restored and the vaccine will no longer provide immunization against the target disease.

ISOLATION AND MOLECULAR CHARACTERIZATION OF FOWL ADENOVIRUS STRAINS FROM BROILER CHICKENS WITH INCLUSION BODY HEPATITIS IN ARGENTINA

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Inclusion body hepatitis (IBH) is an acute disease caused by fowl aviadenoviruses (FAdV) and can result in significant economic effects to the global poultry industry. In recent years, an increased incidence of IBH in different parts of the world led towards an increased awareness about the importance to identify the most prevalent serotypes. Rise of IBH cases in broiler flocks occurred in different regions of Argentina during 2020 and 2021 causing substantial economic losses to the broiler industry. The objective of the present study was to isolate and characterize FAdVs associated from the clinical cases of IBH. A total of 60 liver samples were collected from 25 broiler farms experiencing IBH outbreaks and located in the Entre Ríos Province from Argentina. Clinical signs from affected broiler flocks included increased lethargy, ruffled feathers, inappetence, diarrhea, poor uniformity and high acute mortality. Gross lesions included pale and enlarged and swollen kidneys, mottled and enlarged liver and petechia in muscle. Tissues with microscopic lesions consisting of necrosis of hepatocytes associated with basophilic intranuclear inclusion bodies were submitted for isolation in chicken embryo liver cells culture, confirmation by PCR and sequencing of the FAdV hexon gene. Cytopathic effect was observed in cell cultures and presence of FAdV was confirmed by PCR from all investigated samples. Phylogenetic analysis of the hexon gene showed that isolated FAdVs belonged to FAdV 8b and 11. These findings correlate with adenoviruses isolated from IBH cases in other countries as well. Our findings revealed the specific serotypes of FAdV responsible for the reported IBH pathological manifestations and support strategic control with autogenous vaccines including the isolated serotypes.

INVESTIGATION OF MYCOPLASMA GALLISEPTICUM AND MYCOPLASMA SYNOVIAE PREVALENCE IN COMMERCIAL LAYER AND LAYER BREEDER FLOCKS IN GREECE.

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Mycoplasma gallisepticum (MG) and synoviae (MS) are important pathogens for poultry production causing respiratory disease, while MS is also related to arthritis and egg guality problems. The scope of our survey was to investigate the prevalence of Mycoplasma gallisepticum and Mycoplasma synoviae infection in commercial layer and layer breeder flocks in Greece and their relation to increased flock mortality. Sixty-one different flocks were sampled (4 layer breeder flocks, 14 layer pullet and 43 commercial layer flocks) during the period 2016-2017. Flocks appeared to have a normal or increased mortality status. Ten serum samples were collected from each flock to be tested using an Enzyme-Linked Immunosorbent Assay (Zoetis Elisa Proflok MG and Zoetis Proflok MS kits). When samples were negative, a second sampling was performed 3 to 6 weeks after the first one to evaluate seroconversion occurrence None of the selected flocks was vaccinated against MG or MS; therefore, the presence of positive antibody titers demonstrated exposure to mycoplasma. According to the OIE terrestrial manual, 2018, an infection frequency of 2 out of 10 samples collected from each flock is required to characterize a flock as 'infected'. None of the 4 layer breeder flocks tested positive for MG while a high prevalence was observed for commercial layers (86%) and for rearing pullets (64%). Concerning MS, all commercial layer flocks and rearing pullet flocks were positive, while no infection was reported to the layer breeder flocks. Furthermore, 78.94% and 94% of flocks displaying a normal mortality and 73.81% and 92.85 of those with increased mortality were positive to MG and MS infection, respectively. The association between MG and MS infection and increased mortality was tested using a Chi-square test and proven statistically significant (p<0.05). No statistically significant difference was recorded between mycoplasma infection (MG or MS) and increased or normal flock mortality. In conclusion, our study reported a high prevalence of Mycoplasma gallisepticum and Mycoplasma synoviae in commercial layer and rearing pullet flocks while no exposure to the pathogens was observed for the tested layer breeder flocks. Mycoplasma infection was not always related to increased flock mortality. REFERENCE: OIE terrestrial Manual, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 8th Edition, 2018, Volume, chapter 3.3.5, Avian mycoplasmosis

IMPACT EVALUATION OF COCCIDIOSTAT EXTENDED USE ON INTESTINAL INTEGRITY IN ITALIAN BROILER INDUSTRY

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Coccidiosis is one of the most important disease of poultry worldwide, with possible major implications for bird health and welfare. The damages caused by primary infestation can be followed by intestinal inflammatory reaction, with destructive effects on cellular lining and normal flora disruption. Coccidiostats are considered a good option for coccidiosis control, as they prevent the clinical damages, allowing the coccidial subclinical development, resulting in broiler birds' immunity.

A total of 1.402 Italian broiler birds with diet containing a coccidiostat Narasin + Nicarbazin (NN) (Maxiban[™] – Elanco Animal Health©, 2500 Innovation Way, Greenfield, IN 46140) during 2015-2019, were examined to compare and evaluate the coccidiostat possible impact on overall Intestinal Integrity, when used from 0 to 21 days (Group A) or extended till 28 days (Group B). All the birds were examined through Health Tracking System (HTSi®), a necropsy-based tool that goes beyond simply lesion scoring; it's a broiler health platform for data capture, processing and analysis. HTSi® Intestinal Integrity parameter (I2) allows cumulative assessment of all considered enteric categories. Scores range from 0 to 100, based on a weighted average of all intestinal parameters, with the most weight given to those lesions which have the greatest impact on the birds' intestinal health and therefore performance. Score decreases based on degree of loss of I2. All collected data were analysed using SAS© GLM procedure (means compared using t Test).

Results showed a better overall I2 in birds fed with NN till 28 days (average score 92,1) vs the still good ones with NN in their diet till day 21 (90,3) (p < .0001). Both the analysed populations showed a normal distribution of their elements with 91 as median score for Group A and 93 for Group B. The dispersion of the two examined target populations, investigated with Coefficient of Variation demonstrated 6,16 for Group A and 5,48 for Group B. In score range 90-100 were distributed 71% of the Group B Intestinal integrity scores vs the 60% for the ones in Group A. Looking at the lowest scores detected (70-80) only 1% of group B scores were distributed in that range vs the 5% detected for group A.

In conclusion the extended use of NN, beside the coccidia infestation control through the infestation peak, can let the birds to maintain and improve overall Intestinal Integrity, with important benefits in terms of general health, welfare and performance.

THE EFFICACY OF MATERNALLY DELIVERED IMMUNOGLOBULIN (Y) AGAINST SOME GUT PATHOGENS IN CHICKENS

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The widespread application of antibiotics results in development of resistance in animal products. Thus, the need to alternatives is increased. This study was designed for testing the efficacy of the locally prepared egg yolk immunoglobulin (Iq) Y for the prophylaxis and treatment of broiler chickens from Escherichia coli (E. coli), Salmonella enteritidis (S. Enteritidis), and Clostridium perfringens (C. perfringens). Two hundred, one-day-old broiler chicks were divided into 10 equal groups, each containing 20 birds, and they were kept for 5 weeks experimental period. Group (1) was kept as control negative (not-challenged and not-immunized with IgY). In a prophylactic trial, chicks in groups (2), (5), and (8) were immunized with anti IgY for E. coli, S. Enteritidis, and C. perfringens, respectively and then challenged with the homologues bacterial strain. In the therapeutic trials, each chick in groups (3), (6), and (9) was challenged with E. coli, S. Enteritidis, and C. perfringens respectively. Once the specific signs were appeared, challenged birds were divided into groups (4), (7), and (10) and they immunized with the anti IgY for E. coli, S. Enteritidis, and C. perfringens, respectively. Signs, mortalities, and post-mortem lesions of birds were recorded daily. The productive performance parameters including body weight, cumulative body weight gain, feed intake, feed conversion rate, and specific growth rate were measured weekly. Serum samples were collected weekly for detection of the immune response. Liver, heart, and intestine were collected on the week 5 of age for bacterial re-isolation and histopathological examination. The data were submitted to ANOVA. The results indicated that oral administration of the anti IgY either for the prophylactic or therapeutic purpose was efficient in terms of reduction of the clinical picture, significant ($P \leq 0.05$) enhancement of the performance parameters and the immune response, and reduction of the bacterial re-isolation and the histopathological alterations when compared with non-immunized groups. However, the prophylactic application of anti IgY in broiler chickens was more efficient than the therapeutic one.

In conclusion, immunization of broiler chickens with the specific anti IgY is promising strategy for the prevention and treatment of E. coli, S. Enteritidis, and C. perfringens.

CLINICAL, IMMUNOLOGICAL, AND BACTERIOLOGICAL EVALUATION OF STABILIZED NON-VIABLE LACTOBACILLUS FOR THE TREATMENT OF ESCHERICHIA COLI IN CHICKENS

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Application of antibiotics to control some important septicaemic diseases of chickens' faces some constrains such as antibiotic resistance. Accordingly, searching for alternatives is the must. This work was designed to evaluate the efficacy of a compound containing stabilized non-viable Lactobacillus against Escherichia coli (E. coli) challenge in broiler chickens. A total of 400, day-old broiler chicks were randomly divided into 4 equal groups (1-4) consisting of 100; each assigned into 2 equal replicates (50 each). Chickens in the 1st group were received the dry form of the compound at doses of 1 kg and 0.5 kg /ton feed for the starter and grower, and the finisher rations; respectively. However, chickens in the 2nd group were given the aqueous form of the compound in a dose of 4 ml/liter of the drinking water during the first 3 days of life and at a day before and after each vaccination. Both treatments regimens were administered to chickens in the 3rd group. Each bird in the 1st, 2nd and 3rd groups was challenged with E. coli (078) at 7-days-old. Group (4) was kept as non-treated but challenged control. All groups were kept for 5 weeks for recording signs, mortalities, and lesions. The body weight, feed intake, and the feed conversion rate were estimated on weekly basis. The antibody titers against Newcastle disease virus vaccine were measured weekly using Enzyme Linked Immunosorbent Assay. The bursa of Fabricus was weight every week and compared with the body weight to calculate the bursa/body weight ratio. At the end of the study, the total intestinal bacterial count was estimated. The results indicated that treatment of E. coli challenged broiler chickens with stabilized, non-viable Lactobacillus compound induced significant $(P \le 0.05)$ amelioration of the clinical disease picture, enhancement of the performance parameters, boosting of the haemagglutination inhibition antibody titers, improvement of bursa/body weight ratio, and reduction of the intestinal bacterial count in comparison with challenged non-treated group.

In conclusion, the tested stabilized, non-viable Lactobacillus compound either in dry and/or aqueous form is recommended for improving the health, performance and general body conditions, and immunity of the E. coli challenged broiler chickens.

LONG-TERM SEROLOGICAL STUDY OF BROILER BREEDERS VACCINATED AGAINST CHICKEN ANEMIA VIRUS

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Chicken infectious anemia (CIA) is an immunosuppressive viral disease of chickens causing an enormous economic impact to the poultry industry. The disease produces anemia and death in young chickens and immunosuppression in chickens older than 3 weeks of age. Due to the highly resistance and ubiquitous nature in the environment of CIA virus (CIAV), the eradication is not an option. Only administration of efficient vaccines to future breeding flocks allows the control of the disease by avoiding the transmission of the virus to the progeny and by providing a sufficient maternal immunity to protect them during the first weeks of life. The aim of this study was to evaluate under field conditions the serological response generated by a commercial live CIAV vaccine containing the low attenuated Cux-1 strain (AviPro® Thymovac, Elanco Animal Health). Vaccination was performed via drinking water to 16.000 pullet broiler breeders at 9 weeks of age. They were located in two identical houses (each 8.000 places) in the same poultry farm based in the South-East area from France. 20 blood samples were collected from breeders in each house at 9, 15, 23, 31, 40, 49, 57 and 63 weeks of age, 20 samples of sera of day-old chickens hatched from fertile eggs of both vaccinated breeder flocks at 57 and 61 weeks of age were collected to measure maternally derived antibody titers. All samples were tested for CIAV-specific antibodies with Idexx® ELISA. According to Idexx® guidelines, ELISA S/N values lower than 0,2 are considered highly protective as correlates with virus neutralization antibody titer log2 higher than 11. Results showed robust and highly homogeneous serological response in both breeder flocks presenting CIAV ELISA S/N values lower than 0,2 and a coefficient of variation below 40% during the complete observation period. Progeny originated from vaccinated breeders at the end of cycle presented high maternal immunity with CIAV ELISA S/N values lower than 0,2. Based on this study, vaccination with the low attenuated Cux-1 strain via drinking water provides extremely high and uniform CIAV humoral immune response to broiler breeders enabling them to transfer protective maternally derived antibodies to the progeny. The method of administration is convenient for the breeding staff with a limited invasiveness for the animals.
EFFICACY OF FLURALANER FOR THE TREATMENT OF NATURAL ALLOPSOROPTOIDES GALLI INFESTATIONS IN LAYING HENS

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Introduction

The proliferation of ectoparasites has caused significant economic losses in confined poultry production. Allopsoroptoides galli (A. galli; Analgoidea: Psoroptoididae), a new mite species, has been found with increasing frequency in commercial farms in Brazil and cause intense economic losses. The present study aimed to evaluate the acaricidal efficacy of fluralaner (Exzolt®) to control A. galli in a natural hen infestation in Brazil. Materials and Methods. In this study, a total of 34 white hens, 56 weeks of age, were purchased from a commercial hen farm in the city of Bastos-SP, Brazi, where A. galli infestations had previously been identified and the animals presented mite-induced skin lesions. Thirty-four hens naturally infested with A. galli mites were evaluated and included in this blinded and randomized trial. Each hen represented an experimental unit. The study consisted of two experimental groups of 17 hens each: the treated group received the fluralaner formulation (Exzolt[®]) in drinking water at a dose of 0.05 mL/kgbody weight, twice, 7 days apart (day 0 and day 7); the control group received no treatment. The efficacy evaluations were carried out by mite counts before treatment (day -1) and post-treatment on days 7, 14, 21, 28, 44, 56, and 70. Statistical analysis of infestation data for both groups was performed using the Student's t-test, considering the probability level of 5%. The Ethics Committee previously approved the procedures for Animal Experiments (CETEA/IB) under Protocol No. 165/19. Results and discussion. During the evaluation, was noticed that in the fluralaner-treated group, the mite count assessed by skin scraping showed a significant decrease (p < 0.05) from the first day of evaluation (day 7) after the first dose of Exzolt® up to day 70. In the treated group the mite population continued to decline thereafter. On day 14, the reduction reached 59.2% and exceeded 80% from day 21 (80.7%). The maximum efficacy value was seen on day 56 with 98.8%. The number of mites in the control group remained high throughout the study. The results obtained in this study showed that Exzolt® (Fluralaner) was effective in the treatment and control of the mite Allopsoroptoides galli in naturally infested laying hens.

Funding: The study was funded by MSD Animal health. OSTIC ID: 2021-ms-5602.

YEAST MANNAN RICH FRACTION MODULATES REACTIVE OXYGEN SPECIES AND KEY METABOLIC PROTEINS IN ANTIBIOTIC RESISTANT E. COLI

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Mannose-rich fraction (MRF) isolated from Saccharomyces cerevisiae has been studied for its role in ameliorating animal intestinal health. Previous research with MRF reported lower prevalence of antibiotic resistant Escherichia coli in the caeca of broilers (Parker et al. 2019) and greater susceptibility of antibiotic resistant E. coli in vitro through modulation of bacterial respiration (Smith et al. 2020). It was hypothesised that MRF influenced antibiotic susceptibility via generation of reactive oxygen species (ROS), thereby promoting metabolic perturbations. In this study, we examine ROS and key intermediates of central metabolic pathways in the presence of MRF combined with antibiotic treatment. The Fluorometric Intracellular ROS Assay Kit (Sigma) was used for detection of intracellular ROS. Proteomic analysis was carried out using reverse-phased capillary high-pressure liquid chromatography coupled with the Thermo Orbitrap Fusion Tribrid Mass Spectrometer (Thermo Scientific). Quantitative label-free data analysis was performed using Progenesis QI for Proteomics (version 2.0). Statistical analyses of results were performed using Minitab statistical software package version 16 (Coventry UK) and EXCEL. The results showed that ROS production in resistant E. coli was $53.99 \pm 9.53\%$ greater with exogenous supplementation of MRF combined with antibiotic treatment increased as compared to the control culture conditions $(p \le .05)$. Analysis of relative peptide abundance showed greater expression of several key TCA-cycle proteins, such as ARCA, ICD, GLTA, ACNA, and ACNB, potentiating functional metabolic activity ($p \leq .05$). The results demonstrated that supplementation with MRF in combination with antibiotic treatment modulated metabolic function, via hydroxyl radical formation in resistant E. coli ($p \leq .05$). This analysis reveals a potential novel strategy to augment efficiency of antibiotics and is the first example of prebiotic yeast MRF supplementation leading to greater ROS generation in E. coli and proteomic change in bacterial metabolism. Given the increased spread of antibiotic resistant strains of bacteria there is a need to not only reduce our reliance on antibiotic use but also to find solutions to combat the issue of antimicrobial resistance. By increasing our knowledge of the interaction between prebiotics such as MRF and resistant microorganisms it may be possible to strategically reduce antibiotic use naturally whilst promoting animal health.

THE IMPACT OF LIVE YEAST SUPPLEMENTATION ON THE PERFORMANCE AND SURVIVAL OF BROILER CHICKENS SUBMITTED TO APEC CHALLENGE

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Avian pathogenic Escherichia coli (APEC) causes severe respiratory and systemic disease in chickens: colibacillosis. APEC constitutes an important threat to the poultry industry. Colibacillosis causes losses due to early mortality, condemnation of carcasses and reduced productivity (Alber et al. 2019). The aim of the study was to investigate the impact live yeast supplementation (LY) has on the performance and survival of broilers.

For this trial, 440 one-day old male cobb chicks were divided into 4 groups which were fed with Control diets or diets with LY added at the rate of 109 CFU/kg feed until 42days old. At day 14,15&16, two of the groups were exposed to an intestinal challenge with APEC (108 CFU/bird). During the whole trial feed (3-phase pelleted diet) and drinking water were supplied ad libitum. Body weight (BW) of the birds was recorded weekly pen-wise (10 replicates / treatment). The cumulative feed intake (FI) was determined per feeding phase, as well as the average daily gain (ADG) and average daily feed intake (ADFI). Data were analyzed using a General Linear Model with two fixed factors (diet & challenge) and their interaction (SPSS Statistics 24.0, IBM). APEC challenge significantly impacted the performances: reduced growth (BW: No Challenge: 2683 vs APEC: 2547 g) and lower ADFI (-3.9%) resulting in a significant degradation of the Feed Conversion Ratio (FCR) by +0.02 units for challenged birds. Mortality was numerically higher for the birds challenged with APEC (2.3 times higher). As a confirmation of the successfully applied APEC challenge, the European Production Index (EPI) was significantly lower for challenged birds (EPI=364) compared to the non-challenged ones (EPI=404). Independently of the challenge, birds in LY groups had a significantly higher final BW compared to Control (Control: 2585g vs LY :2645g). Groups receiving LY had the lowest mortality rate (1.82% vs. 7.27% for animals not receiving LY). EPI was significantly improved by 26 units for the LY birds.

The results from this study demonstrate adding LY to broiler diets improves their performances and survival, whether or not birds are subjected to a challenge.

Alber, A.; Morris, K. M.; Bryson, K.J.; Sutton, K.M.; Monson, Melissa S.; Chintoan-Uta, C. et al. (2019): Avian Pathogenic Escherichia coli (APEC) Strain-Dependent Immunomodulation of Respiratory Granulocytes and Mononuclear Phagocytes in CSF1R-Reporter Transgenic Chickens. In Frontiers in immunology 10, p. 3055.

ASSOCIATION BETWEEN THE PREVALENCE OF E. ACERVULINA AND E. MAXIMA IN BROILER FLOCKS IN THE FIELD.

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Diagnosis of Eimeria maxima is perceived to be difficult, but taking into account the impact of on performance and intestinal health, it is crucial to take into account this species. Using Aviapp®, the Huvepharma® health monitoring platform, prevalence of E. maxima in absence and presence of E. acervulina was investigated in field conditions.

Lesion scoring data logged in Aviapp® (Huvepharma®) from 2018-2020 were taken into account. To exclude impact of extreme observations, was filtered to range between 15-42 days (lesion scoring data of 5,279 flocks; 38,007 birds). To assess the effect of presence of E. acervulina on prevalence of E. maxima, a generalised linear mixed effects model was used. Age and presence of E. acervulina as well as their interaction were used as fixed effects. To adjust for farms differences, a random effect for farm was included. The level of significance was set at 0.05. It can be concluded that there is a much higher prevalence of E. maxima lesions in case E. acervulina lesions are present. At 30 days E. maxima is present in 25.4% of the birds if E. acervulina lesions are not present. When E. acervulina lesions are present in birds of 30 days of age, the prevalence of E. maxima increases to 36.3%. The relation between the presence of E. acervulina and the occurrence of severe E. maxima (scores \geq 2) is even higher: at 30 days prevalence of severe E. maxima is 4.5% in absence of E. acervulina but if E. acervulina is present, prevalence of severe E. maxima almost doubles (8.7%). This effect is seen as from 20 days of age and is considerable higher in older birds. Statistical analysis of data from the Aviapp® platform demonstrates a clear association between presence of E. acervulina and E. maxima lesions showing that the prevalence of E. maxima scores is higher when E. acervulina lesions are present.

The chance of finding severe E. maxima can be 2 times higher at critical ages when E. acervulina is present. The presence of E. acervulina is an indication of insufficient coccidiosis control in general and can be used as a potential detector for other species such as E. maxima which are more difficult to diagnose.

EVALUATION OF THE EFFICACY OF TWO COCCIDIOSTAT COMBINATION PRODUCTS, IN THE CONTROL OF COCCIDIOSIS IN BROILERS IN FIELD CONDITIONS

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The objective of this study was to compare the efficacy of nicarbazin/monensin (Monimax \mathbb{R} ; Huvepharma \mathbb{R}) and nicarbazin/narasin (Maxiban \mathbb{R} ; Elanco \mathbb{R}), both used at the highest registered dose (50/50 ppm). On one European farm (203,200 Ross 708 broilers), each house of eight was divided in half and in every house, half of the birds received nicarbazin/monensin at 50/50 ppm from D0-D38 and the other half received nicarbazin/narasin at 50/50 ppm from D0-D38. From D39 to D41, all birds received blank feed. Daily weight gain (DWG; corrected to 38 days), feed conversion ratio (FCR; corrected to 2500g), European production efficiency factor (EPEF), intestinal lesion scoring at 21, 25 and 28 days and foot pad lesions (score A-no lesions; B-some lesions; C-severe lesions) at thinning and at end of trial, were compared. Total mean lesion score (TMLS; sum of the mean lesion score of Eimeria acervulina, E. maxima and E. tenella for the examined birds) for the groups receiving Monimax® are lower compared to the groups receiving nicarbazin/narasin for the different ages (21, 25 and 28 days of age). TMLS was analysed by a linear mixed model. Age and program were fixed effects and house was taken as a random effect. The significance level was set at 0.05. The interaction between age and program was also evaluated but was not significant as assessed by a likelihood ratio test (p-value = 0.24). The average difference over the ages between the two programs was 0.436 (p-value 0.03). Nicarbazin/monensin had higher DWG (64.0 vs 62.4g/day), lower FCR (1.54 vs 1.59) and higher EPEF (403 vs 382) in comparison to nicarbazin/narasin. At thinning and at slaughter, a higher percentage of birds with foot pad scores A were found in the nicarbazin/monensin group (72.4% at thinning, 62.8% at slaughter) in comparison with nicarbazin/narasin (68.9% at thinning, 47.4% at slaughter). Performance outcomes, FCR, DWG, and EPEF were evaluated by means of a t-test with a bonferroni correction. Average differences are 0.05 for FCR (P=0.07), 1.6g for DWG (P=0.02) and 21 points for EPEF (P=0.009). In this study, the birds receiving nicarbazin/monensin outperformed nicarbazin/narasin on the different parameters evaluated: lower coccidiosis lesion scores; better performance; lower foot pad lesions. Better coccidiosis control is known to result in better performance and potentially also in improved litter quality, which in turn is directly linked to foot pad lesions.

EVALUATION OF THE EFFICACY OF TWO COCCIDIOSTAT COMBINATION PRODUCTS, IN THE CONTROL OF COCCIDIOSIS AND DYSBACTERIOSIS IN BROILERS IN FIELD CONDITIONS

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Coccidiosis and dysbacteriosis control of Belgian farms on 2 different coccidiostat shuttle programs (17 farms nicarbazin+narasin-salinomycin; 17 farms nicarbazin+monensin-salinomycin) were evaluated.

Nicarbazin+narasin (Maxiban®, Elanco®) or nicarbazin+monensin (Monimax®, Huvepharma®) was given until 18 days and salinomycin (Sacox®, Huvepharma®) until 44 days. The Total Mean Lesion Score (TMLS) is the sum of MLS per Eimeria species and the (MLS) or Mean Lesion Score is the average score per Eimeria species (total score per Eimeria species divided by the number of birds scored). The dysbacteriosis score is a calculated score based upon the presence or absence of following parameters: gut ballooning, abnormal content*, tonus of the intestinal tract*, thickness of the intestinal tract*, inflammation*, undigested feed. The parameters indicated with * are scored twice, once in the cranial part of the intestine (before Meckel's diverticulum) and once in the caudal part of the intestine (after Meckel's diverticulum). If the parameter is abnormal it gets a score 1 when absent it is scored 0. All scores are added together and divided by the number of birds which were scored. Average coccidiosis and dysbacteriosis pressure were calculated by dividing the area under the curve by the age range of observations (AUC). Stratified bootstrap sampling (n=5000) was performed and 95% Confidence Interval (CI) calculated. Non-overlapping intervals are considered to be significant on the 0.05 level. The average AUC for the nicarbazin+monensinsalinomycin group was significantly lower (P-value < 0.05) for the E. maxima lesions (95%CI: [0.10-0.20] vs [0.20-0.28]), TMLS (95%CI: [0.57-0.73] vs [0.77-0.98]) and dysbacteriosis score (95%CI: [0.82-0.92] vs [1.07-1.16]). The average AUC for E. acervulina was lower but not significantly better for nicarbazin+monensin-salinomycin. The E. tenella lesions were very low and not different for both groups.

This comparison demonstrates a better control for Monimax®-Sacox® compared with Maxiban®-Sacox. Using the approach described, nicarbazin+monensin-salinomycin shows superior control over nicarbazin+narasin-salinomycin with respect to E. maxima, the total coccidiosis control and dysbacteriosis.

EVALUATION OF THE EFFICACY OF A NEWLY REGISTERED COCCIDIOSTAT FOR THE CONTROL OF COCCIDIOSIS IN BROILERS IN FLOOR PEN CONDITIONS

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Recently a new coccidiostat product was registered for use in broilers in EU. Efficacy of amprolium (Coxam®; Huvepharma® at a dose of 125ppm in feed from day 0 to day 35, was evaluated in broilers in floor pen conditions. Mortality, intestinal lesion scoring (at 6 dpi), oocyst excretion (OPG at 6, 14 and 21 dpi) average daily gain (ADG), feed conversion ratio (FCR) and European production efficiency factor (EPEF) were compared to an infected untreated control (IUC) and an uninfected untreated control (UUC) group. Each group consisted of 14 replicate floor pens (31 Ross 308 males/pen). All birds, except from UUC, were challenged at 14 days of age with a European Eimeria isolate (E. acervulina, E. maxima, E. tenella) administered on the feed. Challenge was successful, as it resulted in significant higher mortality, coccidiosis lesion scores, OPG and FCR and lower ADG in IUC compared to UUC. The group receiving Coxam had an overall significantly lower mortality (0.5% vs 6.7%), higher ADG (57.9 g vs 53.1 g) and lower FCR (1.63 vs 1.72) compared with the IUC resulting in a significantly better EPEF (349 vs 283) with p-value < 0.001. On 6 dpi birds receiving Coxam had a significantly lower score for all Eimeria species compared with the IUC. No lesions for E. acervulina, E. maxima and E. tenella were seen in the Coxam treated birds, similar to the UUC. Moreover, at 6 dpi the birds receiving Coxam had also a significantly lower OPG count compared with IUC (p < 0.001). No significant differences were seen on the other time points. Supplementation with Coxam was able to prevent the development of coccidiosis lesions 6 dpi whereas oocyst excretion was reduced but not completely prevented. As a result, broilers supplemented with Coxam had a significantly better performance demonstrating the efficacy of the product. The fact that development of lesions is prevented but oocyst excretion not completely is beneficial for immunity development, similar to leaking principle of ionophores.

TRANSITION FROM CEO ILT TO VECTORED HVT-ILT VACCINES IN COMMERCIAL LAYERS: LESSONS LEARNED

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Infectious laryngotracheitis (ILT) is a highly contagious acute respiratory disease of chickens that causes severe production losses, increased mortality, decreased egg production, weight loss, and predisposition to infection with other respiratory diseases. Control of ILT is mainly achieved by vaccination with conventionally attenuated live virus vaccines that are characterized by their suboptimal attenuation and frequent reversion to virulence.

A fourteen million commercial layer complex located in the United States used live attenuated chicken embryo origin (CEO) vaccines for approximately 15 years. Although CEO vaccines provided good protection against clinical signs and egg production losses, over time this multi-age complex faced complicated respiratory reactions. Such respiratory reactions during the grow-out period were associated with the difficulty of placing the ILT CEO vaccine properly spaced among other live respiratory vaccines, causing negative effects on body weight gain, body weight uniformity and bird health.

The objective of this case report is to present information related to the transitioning from an only - CEO ILT vaccine program to a recombinant vaccine program (rHVT-ILT and rHVT-ND-ILT) alone and in combination with a TCO (Tissue Culture Origin) vaccine. The new program allowed for a better distribution of respiratory vaccines during the grow out. Furthermore, improvements in key performance parameters, such as egg production and livability, were observed.

Vaccination quality control using Viral Flex Sequencing and a glycoprotein I-specific ELISA test were also implemented to validate not only the application of the recombinant HVT and live attenuated vaccines, but also to establish their replication levels and potential interference with the Rispens Marek Vaccine.

Experiences and practical lessons learned throughout this transition process will be presented. Additional information related to the benefits of administering a live attenuated vaccine to HVT-ILT vaccinated birds on further reduction of clinical signs and viral shedding post-challenge will be presented by Ivan Alvarado et al.

BENEFICIAL EFFECT OF BACILLUS COAGULANS DSM 32016 ON PERFORMANCE AND PRODUCTIVITY OF BROILER BREEDERS

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Probiotics are promising alternative solutions to replace AGPs without losses in animal performance and can contribute to the global efforts on antibiotic reduction. The objective of the study was to investigate the effects of Bacillus coagulans DSM 32016 on the performance of broiler breeder.

In the present trial, 240 broiler breeder hens (Arbor Acres) were randomly allocated to 2 treatment groups (n=12; 10 hens per pen). 30 roosters were also divided into 2 groups (n=3, 5 roosters per pen). 12 roosters from each group were randomly mixed with the hens 6 h daily. The trial was performed from week 22 to week 42 of breeder age. Hens in control group (CON) received a basal diet, whereby the trial group received an identical basal diet supplemented with B. coagulans DSM 32016 with 1×109 CFU/kg diet (BC). Diet composition and daily feed allocations were in accordance with Arbor Acres breeder management recommendations. The laying performance was observed as hen housed egg production (HHEP), calculated as total number of eggs produced per week divided by total number of hens at trial start. The number of settable eggs per hen housed (HHSEP) was calculated equally. Further, egg weights and egg yolk weights were measured weekly (n=48). Data was analyzed with SAS software. Differences between the groups were analyzed through independent sample t-test and multiple comparisons of means produced by LSD. Data was checked for normal distribution through UNIVARIATE procedure and Shapiro-Wilk test. Results are expressed as mean values \pm standard error. Differences at P<0.05 were considered as significant. In the BC supplemented group, the mean cumulative eqq production per hen housed (119.04 \pm 1.19 vs 112.70 \pm 1.94, P \leq 0.01) and the mean cumulative settable eqg production per hen housed (114.06 \pm 0.89 vs 108.93 \pm 1.46, P \leq 0.01) were significantly increased. Further, the mean egg yolk weight was higher in BC than in CON (17.35 ± 0.13 g vs. 16.97 ± 0.15 g, P ≤0.001) throughout the trial period.

In conclusion, the results of the study indicate that the addition of Bacillus coagulans DSM 32016 to the animals' diet improved the production potential of broiler breeder hens. It enhanced the laying performance based on improved total number of produced and settable eggs. Egg quality was supported by an enhanced egg yolk weight.

EXPERIMENTAL CONFIRMATION OF VERTICAL TRANSMITTED ESCHERICHIA COLI IN CHICKENS

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Avian pathogenic Escherichia coli (APEC) infection in chickens is of major concern due to significant economic losses in poultry production and their genetic similarities with isolates involved in human diseases (1). With regards to their potential of vertical transmission published studies were based on in vitro typing of field isolates from parents, eggs and/or progenies, which resulted in conflicting outcomes (2). In such studies, the influence of various other possible routes cannot be neglected thus the true transovarian transmission cannot be ensured. The present study aimed to investigate the possibility of vertical transmission of APEC belonging to 2 different serotypes in layers in a controlled experimental set up.

Three groups of 17-wk-old layer pullets were housed in individual isolators with 16 birds in each. Birds from groups 1 and 2 were inoculated intratracheally with E. coli O1:K1 or E. coli O78:K80, respectively. The remaining group served as negative control. Following infection, gross pathological lesions of colibacillosis in the reproductive tract were reproduced in some birds from the infected groups. Histopathology showed inflammation and edematous changes with infiltration of mononuclear cells and heterophils in the ovary and oviduct of affected birds. Applying immunohistochemistry, E. coli was detected in the ovary and glandular cells/secretome of the oviduct of 3 and 4 birds from groups 1 and 2, respectively. A double immunofluorescence protocol based on the staining of E. coli cells and ovalbumin was newly established, which provided evidence for confirmatory localization of the bacterial cells in the tubular gland of oviductal magnum and within its secretion (ovalbumin) of birds coincidencing with reproductive lesions. In addition, E. coli was re-isolated from unlaid egg yolk in the magnum upon direct plating. To summarize, the findings showed that an egg peritonitis model was successfully developed using intratracheal inoculation of 2 distinct E. coli strains representing different serotypes. Furthermore, localization of E. coli in the ovary, unlaid egg yolk, and egg white formed in the magnum in birds infected via the respiratory route confirms that E. coli can be transmitted vertically from hens to eggs. This finding is of substantial importance not only for the health of newly hatched chicks, but also for food safety.

1 Johnson et al. 2008. Appl. Environ. Microbiol. 74, 43-50.

2 Poulsen et al. 2017. Vet Microbiol. 207, 13-18.

EVALUATION OF IN VITRO METHODS OF ANTHELMINTIC EFFICACY TESTING AGAINST THE CHICKEN ROUND WORM ASCARIDIA GALLI

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Ascaridia galli is an important gastrointestinal nematode infecting chicken. The worm count reduction test, requiring sacrifice of the experimental birds is the only standardised method for evaluating anthelmintic efficacy against this parasite (Yazwinski et al., 2003). Its life cycle, where embryonated eggs are the infective stages and larvae do not hatch outside the chicken host, renders efficacy assays based on natural in vitro egg hatching or larval development unusable. To investigate the potential for in vitro drug sensitivity tests for use in this nematode this study aimed to i) optimise pre-assay sample preparation methods including extracting eggs from chicken excreta using different flotation fluids and comparing the deshelling-centrifugation method and the glass bead method with or without bile, to induce larval hatching in vitro and ii) compare two in vitro efficacy assays, the in-ovo larval development test (LDT) and larval migration inhibition assay (LMIA) using fresh A. galli eggs and artificially hatched larvae, respectively. Four anthelmintics, namely thiabendazole (TBZ), fenbendazole (FBZ), levamisole (LEV) and piperazine (PIP) were tested using a fully susceptible A. galli isolate sourced from a free-range farm. One-way ANOVA and nonlinear logistic regression were used to analyse the data. The results suggested that the LDT and LMIA could successfully be used. The LDT was effective for the ovicidal benzimidazole anthelmintics with TBZ and FBZ having EC50 values for inhibiting egg embryonation of 0.076 and 0.064 μ g/ml, respectively. With the LMIA, the EC50 values for TBZ, FBZ, LEV and PIP were 94.5 nM, 7.91 nM, 437 nM and 65.9 mM, respectively. The saturated sugar solution resulted in a high extraction efficiency (68%) of eggs from excreta while also yielding eggs of the highest morphological normality (98%) and subsequent developmental ability (93%). The larval hatching methods evaluated did not differ in hatching efficiency but the deshellingcentrifugation method seemed to yield larvae with slightly better survival rates. For final standardization of these tests and establishment of EC50 reference values, tests using isolates of A. galli with known levels of resistance to the anthelmintics under test need to be performed.

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ELIMINATION OF PERSISTENT SALMONELLA INFANTIS BY THE IMPLEMENTATION OF PHAGE THERAPY IN THE CLEANING AND DISINFECTION PROCEDURES

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Salmonella is one of the main zoonotic pathogens that causes gastrointestinal diseases worldwide, being poultry products the main source of the infection [1]. Cleaning and disinfection (C&D) are a crucial step to reduce the contamination of new flocks and prevent remaining bacteria in the facilities [2]. However, serovars such as S. Infantis, have been presented high resistance against conventional C&D protocols, with an increase capability to persist on broiler farms environment [1]. This has led to explore alternative methods, such as the use of bacteriophages (BPs) [3], a group of viruses that infect only the bacterial cells. Thus, the aim of this study was to assess the efficacy of BPs as a biocide against S. Infantis in combination with C&D protocols in commercial poultry farms. MATERIAL AND METHODS. BPs were isolated, purified and multiplied [3]. The modification of the C&D protocol included the application of the BPs twice at 24h intervals, between the C&D. A total of 28 broiler houses positive for S. Infantis were sampled. Swab samples from farm facilities were collected after the application of each step. All samples were analyzed (ISO 6579:2017) and serotyped (Kauffman-White-Le Minor technique). A GLM was fitted to the data to determine if there was an association for Salmonella presence at the sampling moment. A P \leq 0.05 was considered to indicate a statistically significant difference, RESULTS, Significant differences were found between the different steps of the C&D modified procedure. In this regard, 78.6% and the 57.1% of the samples were positive to S. Infantis after the cleaning procedure and the first phage application, respectively. A significant reduction after the second application of the BP (25%,), and the total of the bacteria after disinfection was obtained (P≤0.05). elimination CONCLUSIONS. Results of this study show that including two consecutive phage applications in the C&D procedure significantly reduced the contamination of the resistant bacteria of the poultry farms, even eliminating it after disinfection. The poultry sector has an efficient and environmentally sustainable tool capable to remove the Salmonella resistant to the conventional C&D protocols.

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DYNAMICS OF SALMONELLA MICROENCAPSULATED PHAGE TROUGH THE GIT IN THE BROILER CYCLE PRODUCTION SYSTEM.

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Introduction. Bacteriophage therapy is increasingly viewed as a promising tool to control Salmonella in poultry [1]. Nevertheless, challenges from the gastrointestinal tract (GIT), could compromise the efficacy of the orally administered phages [2], especially in the ceca, where Salmonella multiplies. The encapsulation has been proposed to protect the phages against these challenges [2]. In this context, the main objective of this study was to assess the optimal timing and dosage of Salmonella microencapsulated phage with the polymer Eudragit® S100 in the chicken rearing period.

Material and methods. The Salmonella phage FGS011 was microencapsulated using the anionic polymer Eudragit® S100 using the spray drying technique [3]. Sixty 1-day-old Salmonella free chicks were housed to simulate production conditions (CITA, IVIA, Segorbe, Spain). Weekly 10 birds were transferred to another house and randomly separated into 2 independent pens in groups of 5 birds. The treatment group was challenged with a single dose of the microencapsulated phage (108 PFU/g) and after 24 h, animals of each group (n = 5/group) were slaughtered and sampled. Samples were collected from the GIT. A Univariate GLM was used to access and compare the dynamics of the BP. A P<0.05 showed statistically significant difference. SPSS 27.0 software.

Results. The highest counts of the phage were obtained in the crop the 1st and 6th wks of application (6.9, and 8.1 PFU/g respectively) (P<0.05). Samples from the proventriculus and gizzard presented the lowest phage counts. The BP counts in the cecum, statistically significant increased over the production cycle (3.8, 5.3 and 7.8 PFU/g the 1st, 4th, and 6th wks, respectively) (P<0.05). The control group was negative in all samples analyzed.

Conclusions. The S100 was able to reach the cecum regardless of the week of application. In addition, the high concentration of phage in the crop is of special interest since the crop is considered, together with the cecum, the major sites of Salmonella colonization in the chicken [4]. This study demonstrates protective effect of Eudragit S100 against the inactivation by the GIT conditions in the most critical moments of the production cycle.

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EFFECT OF LITTER MOISTURE ON THE DEVELOPMENT OF FOOTPAD DERMATITIS IN BROILER AGE

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Footpad dermatitis (FPD) is a common issue in poultry production with average prevalence rates often hovering around 20%, but sometimes reaching as high as 100% on some poultry operations. FPD is characterized as the loss of integrity in the skin of the footpad of a bird. It starts as a chemical mechanical challenge, often due to wet litter conditions with high pH levels and can later become an infectious challenge. More recently, the disease has become a major point of discussion as an important indicator of animal welfare. This study was conducted to investigate the frequency of occurrence of FPD according to the moisture content of litter and according to age in animal welfare certified hens in Korea.

A total of 20,000 Cobb broilers were placed within a poultry house at a bird density of 18.9 birds/m 2 . A Poultry farm had bedding material 8.9 cm deep. Birds were fed standard broiler diet, including a crumbled starter (0 to 21 d) and a pelleted grower (21 to 30 d). Birds had unrestricted access to feed and water via a hanging feeder and nipple drinker. FPD score was determined for 60 birds randomly selected from the poultry house at 21 and 28 days using a 3-point scale that ranges from 0 to 2, with score of 0 = no lesions, score of 1 = mild lesions, and score of 2 = severe lesions. Litter samples were collected at 21 d and 28 d, excluding the day of water spray and litter turning, from six different sites per house. The litter moisture content was determined by the loss of weight on drying. The decrease in weight was recorded as moisture contents (%). Litter moisture was analyzed using the GLM procedure of SAS. Differences were considered statistically significant when p < 0.05. FPD scores were compared between the groups statistically by ttest. Litter moisture was significantly (p<0.05) higher for 28 d than 21 d. As for FPD, the ratio of no lesions (0) decreased by 6.7% from 90 to 83.3% and the ratio of mild lesion (1) and severe lesions (2) increased significantly as the litter moisture content increased with increasing age.

In conclusion, the results of the present study clearly demonstrate the litter moisture is crucial to the control of FPD. A multifactorial approach to litter management would be essential to achieve a balance with the many other factors involved in poultry management.

Key words: Animal welfare, Broiler, Footpad dermatitis, Litter moisture

SEROLOGICAL RESPONSE PATTERN OF DIFFERENT COMMERCIAL LAYER LINES AFTER A CONVENTIONAL PULLET VACCINATION PROGRAM AND THE IMPACT OF MOULTING ON ANTIBODY LEVELS

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Intensive pullet vaccination contributes significantly to the health status of layer flocks. Standard programs are often applied without knowing possible genotype associated differences in the seroconversion pattern. Furthermore, production times of commercial layer flocks are more and more prolonged these days for reasons including welfare aspects as well as economic pressure. Moulting may be induced to improve health and plumage conditions as well as production and egg quality. But the effect of moulting on the immune status of the hen is not known.

Therefore, the aims of our study were to monitor the seroconversion rates of different layer lines with high and medium production traits after a conventional pullet vaccination program. In addition, moulting was induced in these flocks with an adjusted light and feeding program, and the antibody status before, during and after moulting was determined. Interestingly, line differences in antibody titers were mainly seen during the pullet period, while variations between genotypes were less consistent later on during production. In addition, differences between genotypes were associated with the investigated antigen, and not observed consistently throughout all vaccine targets. The moulting did not have a repeatable effect on the development of antibodies after booster vaccination, although the duration of the moulting process varied between lines despite comparable management conditions.

MOLECULAR EPIDEMIOLOGY OF AVIAN ROTAVIRUSES GROUP A IN POULTRY IN THAILAND

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Avian rotaviruses (ARVs) are among the most important viruses causing gastrointestinal diseases of birds worldwide. ARVs are classified in the family Reoviridae. The strains of ARVs including groups A to H are categorized based on viral structure protein 6. The ARV group A (ARV-A) is primarily found in poultry. In 2018 the intestinal tracts were collected from the chickens with diarrhea. Thin intestinal walls and severe villous atrophy were observed by microscopic examination. The ARV-A RNA extraction and detection were then performed using conventional RT-PCR. The first presence of ARV-A in poultry farms in Thailand were found and investigated further in this study to determine the prevalence of ARV-A in different birds in Thailand. A total of 333 avian fecal samples from different farm types and provinces were collected. The prevalence was calculated along with the 95% confidence intervals using the exact binomial method. The positivity to ARV-A was compared between bird type, age, farm type and region. Statistical analyses were performed using the JASP statistics package. For molecular characterization, the nucleotide sequences of major structural proteins (VP4, VP7 and VP6) and nonstructural protein (NSP4) were obtained and analyzed by constructing phylogenetic trees. An overall prevalence of ARV-A infections was high in Thai poultry. In total, 12.9% of all specimens were positive for ARV-A. All samples were obtained from asymptomatic poultry. The prevalence of ARV-A shedding in broiler breeders was highest among bird species at 38.1%. Birds in the age group from 11 to 20 weeks were more likely to shed ARV-A (31.6%) than other age groups. There was no statistical significance between ARV-A infections and farm type. The shedding rates were significantly increased in farms in the southern region (25.0%). According to VP4 characterization, the genotypes included P [30] and P [31]. The VP7 belonged to G19 and G22. The VP6 and NSP4 genotypes were I11 and E10, respectively. Genetic characterization of the VP4, VP6, VP7 and NSP4 showed that the viruses were most closely related to the strains from chickens in Germany and Korea. The results of this study provide the first epidemiological data and molecular characterization of ARV-A in Thailand. Molecular surveillance of ARVs should be continued to monitor reassortment between mammalian and avian rotaviruses in the future.

MONITORING OF DERMANISSIOSIS IN INDUSTRIAL POULTRY FARMS IN RUSSIA AND TESTING THE BIOSAFE ZOOHYGENIC AGENT OREPOWDER® FOR THE POULTRY RED MITE POPULATION CONTROL.

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INTRODUCTION. The poultry red mite (Dermanyssus gallinae) is an ectoparasite of egg and meat-producing chickens. The parasite is a carrier of a number of diseases of bacterial and viral nature, leads to the development of anemia and stress in poultry. In recent years, there has been a rapid spread of the poultry red mite population on the territory of Russia. The rapid development of resistance of the parasite to chemical means of protection was also noted. The development of biosafe means of controlling the number of poultry red mites is relevant. The aim of the study was to monitor the parasite population and control its abundance by using a biosafe agent made of fine silicon dioxide powder OrePowder((with amorphous silicon dioxide content of 83.3 \pm 2%).

MATERIALS AND METHODS. The degree of infestation of parent stock poultry farms, as well as industrial egg poultry farms was assessed. Monitoring was carried out by the method of traps with daily exposure. The survey was conducted in 18 poultry farms from various regions of Russia. The parasite was detected in 15 poultry farms. A specific OrePowder® treatment program was chosen. The dosage varied depending on the degree of infestation and temperature from 1 to 2.7 g/chicken for a cage rearing system and from 30 to 150 g/m2 for on-floor rearing system. One cycle consisted of three treatments with an interval of 7-10 days. The second cycle was carried out at four poultry farms with an interval of 2 weeks – 3.5 months after the end of the first cycle of treatments.

RESULTS. The number of parasite varied from 7.8 sp./m2 to 146777.8 sp./m2. The application of OrePowder® treatment programs for dermanissiosis control in 15 poultry farms showed the decrease in the number of parasites from 52.3% to 98.8% within 24 hours after treatment (on average 77.3%) and from 88.3% to 100.0% (on average 97.9%) at the end of the first cycle of treatments. One cycle of treatments in the poultry houses with a low level of infestation proved enough to completely eliminate the poultry red mite. Repeated treatment in the case of a high level of infestation led to a stable and long-term retention of the number of Dermanyssus gallinae at a safe level.

CONCLUSION. The poultry red mite is widespread in Russia, migrating from south to north, causing severe outbreaks of dermanissiosis. The use of biosafe OrePowder® is an effective method of Dermanyssus gallinae control.

EVALUATION OF THE IMMUNE RESPONSE GENERATED BY A VACCINE ADMINISTERED THROUGH FOOD FOR THE CONTROL OF NEWCASTLE DISEASE

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Newcastle disease virus (NDV) is one of the most serious respiratory problems causing significant economic losses to poultry producers. The application of live vaccines through food has been postulated as an effective method to control the disease, being ideal to reduce the presence of clinical signs and mortality in backyard birds; however, in Colombia the use of this strategy is poorly understood. The present study determined the viability of two live attenuated NDV viruses (VH and La Sota strains) mixed with cooked rice, oiled raw rice and cooked corn and evaluated indicators of humoral and innate immune responses in birds after oral vaccination The mixtures were inoculated into SPF eggs of 9-11 days of incubation. The viral titer was determined by HA and the Reed & Muench formula. Then RTqPCR was performed to quantify viral load. Subsequently, 160 male chickens were randomly divided into four groups: a negative control, and three vaccinated groups (ocular route, drinking water and corn). The birds were immunized at day 28 of age with the VH strain, and sera were taken at days 1, 15 and 25 of age and at 7, 14 and 21-days post-vaccination for evaluation by HI. Productive parameters such as weight and accumulated conversion were taken. Viral shedding and relative expression of IL-6 and IFN-y were quantified on days 2 and 4 post-vaccination in spleen by RT-qPCR. Finally, lymphoid organ weight indices were calculated and digestive, lymphoid and respiratory tissue samples were taken for histopathological evaluation. The results indicated that the VH strain showed more stability and cooked corn was the candidate as a vehicle. Regarding antibody titers, there were statistically significant differences between administration routes on day 7, but there were no differences at 14- and 21-days post-vaccination. There were no differences in lymphoid indices, but differences in larynx and turbinates histopathological lesion scores were seen on days 2 and 4 post-vaccination. Cloacal viral shedding was evident in vaccinated groups compared to the control being higher in eye and water groups on day 2 and in corn group on day 4 postvaccination. IL-6 expression was evidenced early, but IFN-y expression was evidenced only up to day 4 post-vaccination.

This study showed that the oral route through food activates the immune response and that cooked corn and the VH strain could be a strategy to a NDV control program in backyard poultry in Colombia.

PROTECTION PROVIDED BY VARIABLE VACCINATION COVER AGAINST INFECTIOUS LARYNGOTRACHEITIS CHALLENGE IN MEAT CHICKENS

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Mass vaccination of meat chickens with live infectious laryngotracheitis virus (ILTV) via drinking water can result in variable initial vaccine take with adverse outcomes. Two experiments were conducted to determine the effect of partial ILT vaccination on the level of protection against subsequent virulent ILTV challenge. The experiments had a 4x3 factorial arrangement testing 4 levels of ILTV vaccination (0%, 7 or 10%, 20% or 100% of chicks eye drop inoculated with A20 (Expt. 1) or Serva (Expt. 2) live ILTV vaccine strains and 3 levels of virulent ILTV challenge (no challenge or challenge at 7 and 21- or 25-days post vaccination, dpv). Day old Cobb broiler chicks were placed in 20 isolators (n = 15 - Epxt. 1 or 10 – Expt. 2 per isolator) with the vaccination-challenge treatment combinations replicated in two isolators and the sham challenge treatments not replicated. Natural ILTV challenge was achieved on days 7 and 21 or 25 post vaccination by introduction of two infected chicks per isolator 4 days after eye drop inoculation with virulent ILTV, or normal saline in the case of the sham challenge. Mortality and clinical signs after challenge were recorded daily and birds were weighed weekly to 42 or 46 days of age. Choanal cleft swabs were collected at 4 and 7 dpv and weekly thereafter for gPCR detection of ILTV. The highest clinical sign scores and mortality were recorded in unvaccinated and 7% vaccinated birds and full protection was not achieved by 100% vaccination although clinical signs of lesser severity and fewer mortalities were recorded in fully vaccinated birds. Protection provided by 20% vaccination was comparable with 100% vaccination coverage. The level of protection was not greatly affected by the time of challenge post vaccination (7, 21 or 25 dpv). Vaccination reduced body weight gain in unchallenged birds and challenge in unvaccinated birds also reduced body weight gain. Vaccination at the 20% and 100% coverage level eliminated growth impairment in late ILTV challenged birds. ILTV load in choanal cleft swabs increased by ~ 105 fold in the 0 and 10% vaccinated groups and between 0 and 100-fold in the 20 and 100% vaccinated groups a week after challenge indicating significant reduction in virus shedding by 20% and 100% vaccine coverage. Despite the importance of achieving a uniform vaccine initial vaccine take, partial vaccination of at least 20% provided significant levels of protection against challenge with virulent ILTV.

EVALUATING THE ACCURACY OF FIVE VIRULENT GENES IN PREDICTING THE VIRULENCE OF AN ESCHERICHIA COLI ISOLATES USING EMBRYO LETHALITY ASSAY

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This study aims to first characterize the virulence of E. coli isolates collected from healthy and colibacillosis cases based on 5 primary virulent genes (iroN, ompT, hlvF, iss and jutA) and secondly to establish that virulence by an embryo lethality assay (ELA). A total of 58 E. coli samples were isolated, in which 42 were obtained from healthy layers and 16 were isolated from colibacillosis-infected hens. These isolates were confirmed as E. coli by the presence of ybbw gene. Presence of 5 virulent genes in those isolates were evaluated in a pentaplex PCR. Data were statistically analyzed by Fisher exact test and linear discriminant analysis was used to categorize the virulence of those isolates. For ELA, 29 isolates were selected across 4 classes: avirulent, moderately virulent, virulent from healthy hen and virulent from colibacillosis cases. Each isolate was injected as 102 CFU in 15 embryonated chicken eggs through allantoic cavity at 12th day of incubation. Eggs were broken out at 18th day to assess the embryo mortality caused by each isolate. Embryos that were still alive were euthanized and their weight was recorded as a percentage of their egg weight. Embryo mortality data were analyzed in a binomial model that compared the probability of death of an embryo in relation to the virulence of the isolates it was inoculated with. The hindrance to embryo development caused by different virulence classes was evaluated in an ANOVA test and means were separated by Fisher LSD test. The results showed a significantly higher proportion of isolates from colibacillosis infected hens contained all 5 virulent genes, than the isolates from healthy hens (81.1 vs 47.6%, P=0.01). Eqgs inoculated with virulent isolates had a significantly higher probability of death than eggs with moderately virulent and avirulent isolates (0.16 vs 0.41 vs 0.56, P=0.0002). However, there was no significant difference in probable mortality between virulent isolates collected from healthy or colibacillosis-infected hens (0.69 vs 0.54, P=0.08). Avirulent and virulent isolates retarded the development of live embryos to the same degree, which was significantly higher than the moderately virulent isolates (37.6 and 38.1 vs 41.7, P<0.0001). Thus, we conclude that, the predicted virulence of E. coli isolates based on 5 virulent genes can be established in an ELA. However, ELA cannot differentiate the virulent isolates collected from healthy and colibacillosis infected hens.

POULTRY RED MITE CONTROL IN LAYER HOUSES: RESULTS FROM INTERNATIONAL SURVEYS

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The Poultry Red Mite (PRM), Dermanyssus gallinae, is a major threat to the poultry industry worldwide, causing serious problems to animal health and welfare, and huge economic losses. Treatment of PRM is very challenging. Many chemical acaricides have been unlicensed due to human consumer and safety regulations, and mites have become resistant. Non-chemical treatments emerge, though none seem to be sufficient as stand-alone treatments. A promising course of action is Integrated Pest Management (IPM), focusing on preventive and curative nonchemical treatments, while chemicals are only used as a last resort. In the current study, as part of the Interreg NWE project 'MiteControl', surveys were conducted to gain insight into the current state-of-affairs of PRM control in commercial layer houses, and the farmers' desires for future control strategies. A total of 33 surveys were conducted in France, Belgium and the UK, and included 25 conventional and 9 organic farms. The PRM infestation was visually assessed with Mite Monitoring Score (MMS). Results were analysed using GLMs and (M)ANOVAs. The flock age at which farmers first noticed PRM was very variable among farms, and not correlated with any other factor. The MMS, however, was significantly higher on conventional farms (vs. organic), farms with more hens, and farms where chemicals were used, but was surprisingly uncorrelated with e.g. biosecurity, indoors vs. free-range, and timing of treatment. In conventional farms, chemicals were mostly used (n = 16or 64%): 6 applied multiple chemicals and 5 solely chemicals to control PRM. However, farmers were least satisfied with the effectiveness of chemicals compared to other treatment types. The combination of applied treatments did not differ significantly between countries, nor did the farmers' desires for future treatment features. The latter also did not differ between organic and conventional farms. The ability to use products during production and a quick effect are preferred over cheap costs and not being time-consuming. All farmers were open to use only non-chemical treatments if proven effective. Overall, this study illustrates the variability in PRM infestations in different farms, with still many unknown factors. These results were used to feed the development of IPM strategies for sustainable and efficient control of PRM in poultry farms. The project MiteControl was made possible through financing of the European Regional Development Fund.

IPM FOR THE NON-CHEMICAL CONTROL OF POULTRY RED MITE ON LAYING HEN FARMS: RESULTS FROM A FIELD TRIAL STUDY

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The Poultry Red Mite (PRM) is the most damaging ectoparasite of laying hens in Europe causing economic losses of 31 million euros each year. Conventional treatment primarily consists of spraying synthetic acaricides in the hen house. However, because PRM hide in interstices the effectiveness of such treatment is limited. Moreover, synthetic acaricides have adverse effects on the environment and (human) health, and resistance is rapidly emerging. Sustainable control strategies (e.g. Integrated Pest Management or IPM) are therefore needed to decrease the use of chemicals, improve animal health and welfare, economic profitability and food safety. Non-chemical treatments are emerging though none are sufficient as a stand-alone treatment. Efficiency is increased by combining multiple products. Additionally, thorough cleaning, biosecurity and preventive treatment during the empty period have a clear beneficial effect on PRM control.

The current field trial study was conducted on an experimental farm with multiple housing units: four aviaries with 1920 hens each, four aviaries with 2650 hens each, and four enriched cage units housing 3072 hens each. This allowed for different combinations of at least two non-chemical treatments to be tested in parallel and their compatibility and synergistic effects to be evaluated. The products used were predatory mites, phytoadditives, an autogenous vaccine, and an electrified perch. The trial ran from the age of 61 to 82 weeks and ended in March 2020. PRM populations were intensively monitored but no significant differences between treatment combinations and PRM counts were found. This first field study confirmed that in units with high start infestations, control was more difficult. At 74w, six of the twelve housing units were treated with silica (mean PRM counts > 1000). A significant association (p=0.02) was found indicating the application of silica was effective. Furthermore, production parameters such as laying percentage (%HH) were evaluated in relation to the treatment combinations and PRM monitoring results. No significant differences were found in laying percentage at 61w and 82w of age.

The work carried out within the MiteControl project provides useful information for the development and implementation of non-chemical strategies as well as the general understanding of PRM control. On-going research includes pilot testing three IPM strategies on 11 commercial layer farms between May 2020 and March 2022.

THE EFFECT OF STOCKING DENSITY ON THE MICROBIOLOGICAL PROFILE OF WHITE EGG LAYER EGGS AND CLOACA

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Food safety as a component of welfare associated with stocking density is a major concern for commercial poultry producers due to consumer perception. Therefore, it is important to understand how the potential stress from stocking density can affect the microbiological load on the egg and in the cloaca. Five different density treatments consisting of 6 (208 in2/bird), 9 (139 in2/bird), 12 (104 in2/bird), 15 (83 in2/bird) and 18 birds (69 in2/bird) were examined in colony cage environments. There were three replicates containing 2 cages, for each treatment in colony cages measuring 48 by 26 in. Three birds per replicate were leg banded and swabbed every sampling point. Microbiological tests were performed at 39, 55 and 68 weeks of age. The populations of total aerobic bacteria count, E. coli/coliform, Enterobacteriaceae, and yeasts and molds from the eggshell rinse, egg content, and cloacal swabs were enumerated. The prevalence of Salmonella spp. in these samples were also monitored. The total counts are expressed as log10 CFU/mL. Statistics were performed using Rstudio 4.1. Overall, no microbes were detected in any of the egg content and there were no differences (P>0.05) between treatments for the shell bath. However, the E. coli population on the eqg shell on week 39 was significant higher (0.86 log10 CFU/mL) than weeks 55 and 68 (0.092 and 0.052 log10 CFU/mL, respectively). Furthermore, enterobacteria presence also followed the same trend (P<0.001) where week 39 had a higher presence (1.26 log10 CFU/ml) than weeks 55 and 68 (0.37 and 0.18 log10 CFU/ml, respectively). More molds from the eqg shell (P < 0.001) were detected during week 39 (2.144 log10 CFU/ml) than month 68 (1.16 log10 CFU/ml). For cloacal samples, the 12-bird density had a higher aerobic bacteria count at 4.69 log10 CFU/ml than that in the 6-bird density at 4.19 log10 CFU/ml (P=0.015). Also, the 9-bird density had a higher cloacal presence of molds (0.25 log10 CFU/ml) than the 6, 12 and 15 bird replicates (P=0.015). There were no statistical differences in the presence of Salmonella between treatments for cloacal, shell bath or contents. However, there was a statistical correlation between months for cloacal Salmonella samples with a higher number of positive samples occurring in weeks 55 and 68 (P<0.001).

COLONIZATION OF SALMONELLA ENTERITIDIS IN VACCINATED AND UNVACCINATED LAYING HEN USING ORAL INOCULATION

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The objective of this study was to compare the effect of an oral inoculation of a Nalidixic acid resistant strain of Salmonella Enteritidis (SE) on tissue colonization and fecal shedding of 30-wk-old vaccinated and unvaccinated laying hens. Two experiments (Exp) were conducted as a completely randomized design. Exp 1 utilized 36 hens that had been vaccinated with a live Salmonella vaccine and Exp 2 utilized 36 hens that were unvaccinated. Hens were placed in a layer battery cages (two hens per pen) which included fecal collections pans. In Exp 1, treatments (TRT) included: TRT1 a negative control (NC) that were orally gavaged with 1 mL of tryptic soya broth (TSB); TRT2 were SE challenged with 1 mL of a 1.2×107 CFU of SE; and TRT3 were SE challenged with 1.2×108 CFU of SE at day 0, 7 and 8. Fecal samples were collected at 0, 3, and 7 days post-inoculation (dpi). One bird per pen was euthanized at 7 dpi while the remaining half were euthanized on 14 dpi. Cecal tonsil, ovary, spleen, and liver-gallbladder (LGB) were removed aseptically and processed to determine Nalidixic acid resistant SE concentrations. In Exp 2, TRT's included: TRT1 the NC as in Exp 1; and TRT2 and TRT3 that were SE challenge with 1 mL of a 1.3×108 CFU and 1.3×109 CFU of SE, on day 0 and 1, respectively. All samples and sampling days were the same as that in Exp 1. Data were log transformed and analyzed using PROC GLIMMIX procedure of SAS 9.4. All NC fecal and tissue samples were negative for SE in both Exp. In Exp 1, at 3 dpi, SE was recovered from feces at 1.25 log10 and 1.32 log10 for TRT2 and TRT3 (P= 0.016), respectively. On 14 dpi, 2.13 log10 and 2.68 log10 of SE was detected in the feces (P=<0.01), 3.58 log10 and 3.42 log10 in ceca (P<0.001) and 0.25 log10 and no count in LGB (P=0.036) were observed for TRT2 and TRT3, respectively. In Exp 2, at 3dpi, fecal SE (P < 0.001) of 4.46 log10 was observed in TRT3 compared to 3.73 log10 in TRT2. At 7dpi, SE counts were 1.99 log10 and 2.00 log10 in LGB and 0.81 log10 and 3.34 log10 in ceca (P<0.001) for TRT2 and TRT3, respectively. A higher SE count in the ceca (0.40 log10 vs 1.91 log10) were obtained for TRT2 and TRT3 (P<0.001) even at 14dpi. The results demonstrate that a consecutive 2-day challenge can cause effective SE translocation in the LGB and ceca of either vaccinated or unvaccinated hens. Future research will focus on feeding various dietary interventions to control SE and their effectiveness on SE tissue translocation.

UTILIZING NANOPORE SEQUENCING OF ITS-1, 18S, AND CO1 GENES FOR IDENTIFICATION OF EIMERIA PARASITES AND DIFFERENTIATING VACCINE AND FIELD STRAINS

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Coccidiosis is a costly enteric disease of commercial poultry worldwide caused by single-celled, parasitic protozoa of the Eimeria genus. The parasite reproduces rapidly both sexually and asexually causing substantial damage to intestinal linings, and often mortality. Furthermore, infection with certain species of Eimeria predisposes the chicken gut to development of necrotic enteritis caused by Clostridium. Control of coccidia and necrotic enteritis infections cost the poultry industry billions annually. The most common method for the identification and differentiation of Eimeria of species is microscopy, using morphometric characteristics (size, shape) and region of the gut parasitized. While a simple technique, microscopy can be subjective, and sensitivity is not ideal. As molecular tools have advanced, using PCR to differentiate species has become more common place. Three different genome regions have been used to molecularly differentiate species of coccidia; Internal Transcribed Spacer-1 (ITS1), Ribosomal 18s DNA (18S), and Cytochrome Oxidase C-1 (CO1). Our hypothesis is that, when all three genes are evaluated together for a single sample, inter- and intra-species variation in these regions will allow for distinction of current multispecies samples. The overall goal of our research is to produce profiles from sequence data to promptly differentiate Eimeria species, identify Eimeria strains within a species, and potentially differentiate vaccine and field strains of Eimeria from each other. To test this, DNA was extracted directly from commercially available vaccines in the US. Pan-Eimeria PCR to amplify either the ITS1, 18S or CO1 genes was performed on the mixed species DNA samples. PCR amplicons from each gene were then sequenced using the Nanopore next generation sequencing (NGS) platform and sequences were separated and compiled using Geneiuos software. To date, our results show that using this technique can differentiate species in a multi-species sample, eliminating the need for monoclonal samples or species-specific PCR. We can also see that individual vaccines carry an identifiable sequence combination of these three genome regions, enabling differentiation from each other. Furthermore, there are differences between vaccine and field strains that can be seen when analyzing sequence data. With this data, we hope to build a profile database of vaccine and field strain coccidia, which will be an extremely useful diagnostic tool in the future.

EFFECT OF MOISTURE CONTENT AND HOLDING TEMPERATURE OF POULTRY LITTER ON THE VIABILITY OF MIXED EIMERIA OOCYST, AND SALMONELLA POPULATION

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The effect of poultry litter moisture and temperature on the viability of Eimeria and Salmonella was evaluated. Pine sawdust was used as model litter and the moisture content was adjusted to 20, 30 and 40%. Coccidia vaccine (COCCIVAC®-B52) was used to inoculate sawdust (5 g) at 8.0x104 oocysts/g and equal proportion of marked Salmonella serotypes (nalidixic acid resistant S. Typhimurium, rifampicin resistant S. Infantis and kanamycin resistant S. Reading) was used to inoculate the sawdust. Three independent replications were performed to determine the effect of temperature (43.3, 54.4 and 65.6°C) and moisture on coccidia and Salmonella survival and а mathematical model was developed. To validate the model, Salmonella reduction over time (6, 12, 18, 24 h) at select temperatures and moisture content was determined in poultry litter obtained from Salmonella positive flock. A modified Bigelow-type model was fitted for moisture and temperature effect as D (T, m) = [Dref - zm x(m-20) x 10(Tref - T)/zT]. For Eimeria, lower moisture content and higher holding temperatures (P<0.05) resulted in lower D value. Sawdust (20% moisture) held at 43.3°C resulted in 8.0x104, 4.6x103, 2.9x103 and 4.4x102 oocysts/g count at 0, 24, 48 and 72 h, respectively. Whereas, holding at 65.6°C resulted in complete destruction of oocysts after 48 h. Increasing moisture to 40% and holding at 65.6°C resulted in recovery of oocysts with 8.0x104, 7.9x103, 6.4x103 and 8.1x103 oocysts/g over 0, 24, 48 and 72 h, respectively. For Salmonella, irrespective of moisture levels there was complete destruction after holding for 48 h at 43.3°C or 24 h at 54.4 and 65.6°C. The secondary model D (T, m) = $[56.42-1.212x(m-20) \times 10(43.3-$ T)/2.483], where zm (1.212) suggests that 1% of moisture increase will increase the time by 0.212 h to eliminate 1 log of Salmonella. Also, the zT (2.483) implies that every 2.483°C increase during composting, the time needed to eliminate 1 log of Salmonella is reduced by 1 h. Overall, composting temperatures of $\geq 65.5^{\circ}$ C will assure destruction of Eimeria oocysts (>104 oocysts/g) in the poultry litter $(\leq 30\%$ moisture). Similarly, compositing temperature of 43.4 °C for over 48 h period will eliminate Salmonella (>8 log CFU/g) in poultry litter. Increasing compost temperature or decreasing litter moisture will reduce the time needed for Salmonella reduction.

SUPPLEMENTATION OF PLANT EXTRACT MIXTURE IN DIET SHOWED INCREASED GOBLET CELL NUMBER, REDUCED ILEAL LESIONS AND EIMERIA OOCYST COUNTS IN BROILERS CHALLENGED WITH NECROTIC ENTERITIS

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Plant extracts (PE) are gaining increased attention as potential alternatives to combat enteric diseases in the post-antibiotic era due to their known antimicrobial (AM) activities. This study was conducted to examine the potential of PE, a microencapsulated product composed of eugenol and garlic tincture as an alternative to AM-agent on intestinal health in broilers under necrotic enteritis (NE) challenge. A total of 960 d-old mixed-sex Cobb 500 chicks were randomly distributed to 48-floor pens with 6 treatments replicated 8 times with 20 birds each. Six treatments were applied: UC, unchallenged control; CC, challenged control; PE, challenged group plus PE; AM, challenged group plus AM; FAP, challenged group plus a full dose of AM with PE; HAP, challenged group plus a half dose of AM with PE in starter, grower and finisher phases. Birds in the challenged groups were inoculated with Eimeria spp. on d 9 and Clostridium perfringens on d 14. On d 16, 4 birds (2 males and 2 females) from each pen were randomly chosen and euthanised by cervical dislocation followed by stunning to perform intestinal lesion scoring and to collect duodenal tissue for the histomorphology and caecal, and ileal content to count Eimeria sp. oocysts. All data were analysed using JMP software (v.14). Results showed that the CC group had reduced villus surface area and goblet cell number, increased intestinal lesions, caecal and ileal oocyst counts compared to the UC group (P < 0.05). Birds fed PE had reduced ileal lesion scores in only male birds and increased goblet cell number compared to the CC group (P < 0.05). Birds fed PE had decreased E. maxima and E. acervulina oocyst counts in caecal content than CC group (P < 0.05). Birds fed PE had decreased E. brunetti and total oocyst counts in caecal content, and E. acervulina oocyst counts in ileal content in only female birds compared to the CC group (P < 0.05). These results suggest that, PE supplementation in diet may help to mitigate the effect of NE on intestinal health as indicated by increased goblet cell number, reduced ileal lesions and Eimeria oocyst counts in caecal, and ileal content.

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Ingestion and digestion

Selected short communications

EFFECT OF MICROALGAE ON THE GUT DEVELOPMENT, FUNCTION AND MORPHOLOGY OF BROILERS

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Microalgae can impact broiler gut development and health. Three microalgae were tested for their effects on gut development, function and morphology: Chlorella, Chloromonas and Nannochloropsis. The microalgae biomass was first desalted and defatted before being mixed to a standard basal broiler diet. In total 105 male Ross 308 broilers were reared (day 0 to day 29) for this trial. Seven treatments were included, each with 15 animals: a control group receiving the basal diet, followed by the 3 microalgae added on top at 1 of 2%. Animals were the experimental unit. At the end of the grower phase (day 29), animals were euthanized and sampled, as at this point the development of the gut is completed. Animals were considered as the experimental unit and data was analysed with R, using a linear mixed model. Animals receiving Chlorella reached a significantly higher body weight (1.469 kg and 1.401 kg, for 1 and 2%) with the animals receiving the control and Nannochloropsis (1 and 2%) diets having the lowest body weights (1.165 kg, 1.191 kg and 1.200 kg respectively). A similar result was seen for feed intake and growth, resulting in significantly lower feed conversion ratios (FCR) for the Chlorella groups (1.513 and 1.489, for 1 and 2%) and higher FCR for the Nannochloropsis groups (1.680 and 1.664, for 1 and 2%). Interestingly, animals fed the Chorella, had also significantly heavier livers and pancreas (liver: 38.6 g (1%) and 37.6 g (2%); pancreas: 4.59 g for both 1 and 2%) (weighed as measure for digestibility capacity), while the control animals and the Nannochloropsis fed animals had the lightest livers and pancreas (liver: 28.5 g (control), 29.9 g (1%) and 29.8 g (2%); pancreas: 3.62 g (control), 3.88 g (1%) and 3.81 g (2%)). The heavier livers and pancreas could suggest a better digestive capacity of the animals, resulting in higher body weights. Curiously, this supposedly higher digestive capacity of Chlorella fed animals was not followed up by a longer gut. It was animals fed Chloromonas (1%) that had the longest gut (162 cm), followed by the animals fed Nannochloropsis at 2% (160 cm) with the control group having the shortest gut (145 cm). One could speculate that Nannochloropsis biomass is more challenging to digest, hence the need for an increased absorption capacity through gut elongation. Histological data is being analysed and will contribute to the understanding of microalgae impact on gut development, function and morphology.

IS SELECTION FOR IMPROVED PERFORMANCE LIMITING THE GASTROINTESTINAL CAPACITY OF MODERN BROILERS?

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Genetic selection for improved carcass yield may limit the capacity of the gastrointestinal tract (GIT) of modern broilers to deal with bulky feeds. Interest has grown around the use of alternative feed ingredients in broiler diets, which are often bulky materials. Reductions in the capacity of the GIT will lead to a limitation in feed intake and consequently performance on such diets. We investigated the capacity of male Ross 308 broilers to deal with increasing levels of dietary bulk and aimed to identify a feed bulk dimension related to limited feed intake (FI). Two bulky ingredients were selected: oat hulls (O), and sugar beet pulp (S), due to the differences in their physicochemical properties. 480 day-old broilers were allocated in 48 pens and offered a common starter until d8 and 1 of 7 feeds from d8-d36 of age; a control feed (C), which was diluted to three levels (15%, 30% or 45%) with either O or S. FI was measured daily and two birds per pen were dissected for organ measurements and empty carcass weight (ECW) at d36. Cumulative FI (CFI) was calculated over the experiment and scaled relative to ECW at d36. It was hypothesised that bulky feeds would increase the visceral weight of the birds, therefore ECW was used as a scalar for CFI (q/ kg CW). Results were analysed with GLM using the nlme package in R.

Broilers were able to accommodate a greater increase in CFI on the O rather than S feeds (P < 0.001). CFI (g) of the birds offered C, O15, O30, O45, S15, S30 and S45 were 2683, 3678, 3977, 3795, 3521, 3041, 2808 (\pm 178) g. ECW was reduced by the inclusion of S, whereas the inclusion of O was limiting only at the 45% level (P < 0.001): 2486, 2610, 2498, 2211, 2262, 1876, 1301 (\pm 35.1) for birds offered C, O15, O30, O45, S15, S30, and S45 feeds respectively. Scaled CFI were 1084, 1444, 1593, 1814, 1702, 1816, 1956 (\pm 64) g/ kg ECW, respectively. Scaled CFI was increased to a greater extent in the S and O45 birds than the O15, O30 and C birds (P < 0.001). The reductions in performance of the S and O45 birds were consistent with the limitations in FI, as they are unable to increase FI to the extent that they meet the nutrient requirements for optimum growth. Nonetheless, our data show that modern broilers can substantially increase FI in response to bulky feeds, almost double their FI on feeds high in S. However, we were unable to identify the property of bulky feeds which was responsible for limiting FI and consequently performance.

THE USE OF SINGLE OR MIXED-SEX BROILERS IN RESEARCH: HOW TO REDUCE EXPERIMENTAL VARIATION IN THE POST FEATHER SEXING ERA

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Currently researchers in poultry science are finding it difficult to source sexed dayold broiler chicks for use in animal trials as feather sexing is no longer an available option due to the change in genetics of the common commercial broiler breeds. It is well known that male and female broilers differ in terms of their growth performance with males having a higher body weight gain (BWG), feed intake (FI) and lower feed conversion ratio (FCR) compared to female broilers (Da Costa, et al. 2017; Goo, et al. 2019). These differences make it important in the way research is conducted with the presence of both sexes as it has an effect on between-pen variation and experimental power. Alternate sexing methods that can be used to sex birds prior to placement include vent sexing or DNA sexing using feathers through high resolution melt curve analysis (England, et al. 2021). In this case researchers can make use of equal numbers of male and female birds in each pen/cage and it then needs to be determined if this approach will result in the between-pen variation the use same as of single sex birds. The growth response of birds reared as single or equally mixed-sex given a reduced (2% lower than breed guidelines) or standard (formulated to meet breed quidelines) crude protein diet was recorded. This study was designed as a 2×3 factorial arrangement of treatments consisting of 672 Cobb 500 broilers which were vent sexed upon arrival and assigned to 48 floor pens with 6 treatments, 8 replicates, and 14 birds per pen. The factors were rearing method (male singlesex, female single-sex or equally mixed-sex) and dietary crude protein level (standard or reduced). Both the birds and feed were weighed on arrival and on d 10, 24, and 35, and mortality was recorded daily. Body weight gain (BWG), feed intake (FI), and mortality adjusted feed conversion ratio (FCR) were calculated for all phases. The data was analysed by SPSS statistics package version 22 (IBM Corporation, Armonk, NY, United States). Mean values of the treatments were compared within the confidence interval adjusted by Tukey test. All significant differences were determined at P < 0.05. The mixed-sex pens fed the reduced crude protein diet had the lowest CV for BWG and FI whilst the single-sex male pens fed the reduced crude protein diet had the lowest CV for FCR, meaning the use of equal numbers of male and female birds per pen will result in a similar between-pen variation as the use of single sex birds.

SEX AS A COVARIATE INCREASES THE STATISTICAL TEST POWER WHEN MIXED-SEX BROILERS ARE USED IN NUTRITIONAL EXPERIMENTS

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Currently researchers in poultry science are finding it difficult to source sexed dayold broiler chicks for use in animal trials as feather sexing is no longer an available option due to the change in genetics of the common commercial broiler breeds. This means that researchers often need to make use of as-hatched birds in their experiments which are then unevenly distributed according to sex into pens/cages. It is well known that male and female broilers differ in terms of their growth performance compared to female broilers (Da Costa, et al. 2017; Goo, et al. 2019). These differences make it important in the way research is conducted with the presence of both sexes as it has an effect on between-pen variation and experimental power. It has not become a common practice amongst researchers who make use of unequal numbers of male and female birds in their pens to make use of sex as a covariate, and this can result in less powerful outcomes. This makes it important to highlight the benefits of analysis of covariance (ANCOVA) to reduce bias and improve observed power when the sex effect is significant for the parameters of interest in research. Performance data was used from 5 separate trials that made use of mixed-sex broilers. From this data the male % in each pen was then determined and used as the covariate in the statistical analysis. The data from the experiments was analysed using ANOVA and ANCOVA, with male % for each pen included as a covariate, as a completely randomized design using the General Linear Model procedure of SPSS statistics version 22 (IBM Corporation, Armonk, NY, United States). Results from this study show that in order for ANCOVA to be performed a set of assumptions first need to be met. One of these assumptions state that there should be a significant correlation between the covariate and dependent variable. In most cases this assumption was not met during the early feed phases and therefore ANCOVA is not needed for the data. In addition to this, it was discovered that if there is already a high level of significance and observed power when ANOVA is performed then ANCOVA analysis will not add value for test power improvement. Aside from these exceptions and when all other assumptions are met it was found that performing ANCOVA analysis using sex % as a covariate reduces MSE, increases the F-statistic and improves model significance, adjusted R2 values and observed power compared to ANOVA.

EFFECTS ON BROILER CARCASS PIGMENTATION ASSOCIATED WITH A DIETARY THREE-STRAIN PROBIOTIC AND VARYING LEVELS OF INGREDIENT PIGMENT INCLUSION

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Broiler skin pigmentation levels are influenced by many aspects of the production phase particularly dietary ingredients and disease. The objective of this study was to evaluate the effect of the dietary inclusion of a three-strain Bacillus probiotic on hot and cold carcass pigmentation levels of 43 days old broilers. This variable was measured in association with differing levels of natural and synthetic pigment inclusion levels in the grower and finisher feeds in a controlled study. A total of 2100 Ross 308 male chicks were randomly distributed in 42 floor-pens and allocated to 7 dietary treatments: T1 (basal diet), T2 (T1 + probiotic + pigment 40g/50g), T3 (T1 + pigment 50g/60g), T4 (T3 + probiotic), T5 (T1 + pigment 60q/70q), T6 (T5 + probiotic), T7 (T1 + pigment 100q/110q). Pigmentation of hot carcasses were evaluated immediately post-scald. Pigmentation of cold carcasses were evaluated immediately after the post-harvest chilling process. A Konica Minolta CR-400 Chroma Meter was used for pigmentation assessment. A mixed statistical analysis was used for data assessment. A significant ($P \le 0.05$) increase in both hot and cold carcass pigmentation yellowness was observed with the inclusion of the probiotic and/or the pigment products. A significant increase in hot carcass pigmentation yellowness levels was observed with the pigment 60q/70q +probiotic (T6) treatment compared to other treatments. Numerical differences were observed between treatments that included the probiotic and/or the pigment products. The results of this trial indicate that the inclusion of a probiotic in addition to pigmentation products may positively affect broiler skin pigment levels. These results also indicate that industry and consumer-preferred skin pigmentation levels may be achieved with lower and sustainable levels of natural and synthetic pigments when combined with a probiotic in feed rations.

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Ingestion and digestion

Posters

MUCOSAL AND MICROBIAL B-GLYCOSIDASE ACTIVITY IN THE GASTRO-INTESTINAL TRACT OF POULTRY

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IIn recent years, the use of phytogenic solutions in livestock production has increased. These phytogenics are generally secondary plant metabolites, often present as glycosides. Glycosidation can alter the bioavailability and the fate of the agylcon in the gastrointestinal tract. Additionally, hydrolysis by glycosidases might be necessary for absorption of the aglycon. Glycosidase activity has been described for the gastrointestinal tract of pigs [1]. In poultry, the presence of glycosidase producing microbiota also indicates such enzyme activity [2]. However, the effect of type, breed and age of poultry on glycosidase activity in the gut has not yet been examined. Samples were collected on d7, d21 and d35 of age from Ross308 and Cobb500, and on d7, d35 and d70 of age from Sasso broilers. With regard to Ross308, ad libitum and a meal-fed group was included. Samples from laying hens were collected at 26, 50 and 66 weeks of age. All animals received standard commercial diets. Digesta samples were taken from 5 intestinal locations and a mucosa sample was taken from mid-jejunum. Three pooled samples of 3-6 birds each were analysed for β -glucosidase activity by a colorimetric method using pnitrophenyl glucosides as a substrate [1]. Data from broilers and layers were analysed separately by ANOVA and Tukey correction was used for post-hoc comparison.

β-Glucosidase activity increased with age in the small intestinal digesta and mucosa in Ross308 and Sasso (p < 0.05). There was no age dependency in the other sampling sites. In the crop, β-glucosidase activity at all ages was highest in meal-fed Ross308 (p < 0.05) and lowest for Sasso (p < 0.05), but markedly higher than in small intestine. Caeca of all birds and ages showed an enzyme activity that was 5 – 10 times higher than in the small intestine. There was no age difference in β-glucosidase activity of layers and activity along the gut was similar to broilers but on a slightly higher level. In conclusion, poultry intestines show β-glucosidase activity. In contrast to results found in swine [1], activity is highest in caeca and crop and there is only negligible activity in the mucosa. This suggests that β-glucosidase activity in poultry is originating from the microbiota.

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THE USE OF A MONO-COMPONENT XYLANASE IN LAYERS: EFFECT ON NUTRIENT DIGESTIBILITY AND GUT MICROBIOME

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Exogenous carbohydrases are commonly added to monogastric feed to degrade non-starch polysaccharides. The objective of the current study was to investigate the effect of supplementation of a new xylanase on laying hen nutrient digestibility and caecal microbiome. A total of 96 ISA-Brown layers were placed in 48 cages, with 2 laying hens per cage. There were 3 treatments, with 16 replicates each. The experiment was carried out over 28 days, from the age of 22 weeks. Xylanase was added to the diets at concentrations of 0, 30,000 U/kg (10 g/t XygestTM HT, Kemin Europa NV) and 45,000 U/kg (15 g/t XygestTM HT). The diets were based on wheat, wheat bran and soybean meal. Overall, the egg yolk color of the 30,000 U/kg group seemed to be more intense compared to the control treatment (P=0.05). Also, average daily feed intake tended to be lower in the 30,000 U/kg treatment group compared to the control group. Crude protein digestibility was numerically improved with 5 % in birds fed 45,000 U/kg, compared to the control. Furthermore, dry matter and gross energy digestibility significantly improved when supplementing the diet with 30,000 and 45,000 U/kg. Compared with the nonxylanase fed birds, the AME values were significantly increased with 3 and 4 % in the 30,000 U/kg and 45,000 U/kg treatments, respectively (P < 0.05). Importantly, 16S rRNA genes were sequenced to characterize diverse microbial communities of the cecal content. The gene expression analysis revealed that xylanase supplementation significantly increased, 1.95 times, the relative abundance of Lachnospiraceae (P<0.05). Furthermore, the proportion of Bifidobacteriaceae (2.14-fold), Lactobacillaceae (1.48-fold) and Ruminococcae (1.92-fold) numerically increased in the 45,000 U/kg group compared to the control. In conclusion, supplementation of the new xylanase to a laying hen diet noticeably improved laying hen digestibility and positively modulated the laying hen microbiome. It is generally accepted that a healthy balance between the number of beneficial and pathogenic bacteria in the intestinal tract is vital for an optimal growth of the host.
SUPPLEMENTATION WITH CAPSICUM-BASED ADDITIVE IN STARTER FEEDS IMPROVES BROILER PERFORMANCE BY INCREASING NUTRIENT DIGESTIBILITY.

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Previous studies have shown that the phytogenic additive based on capsaicin LUCTAROM® Convert (LOM) improves broiler chicken performance. Recent studies show that in addition to antimicrobial, antioxidant and anti-inflammatory effects, phytogenic additives can also improve animal performance by enhancing digestive enzyme secretions. The aim of the present study was to determine the effects of LOM in starter diets on broiler performance, nutrient apparent ileal digestibility (AID), and pancreatic enzyme activities to test the hypotheses that this additive improves animal performance by favoring digestive processes. For this purpose, 480 male broiler chickens (Cobb 500), with an initial average live weight of 42.6 \pm 0.06 g, were randomly assigned to 2 experimental treatments, a control diet (C) without additives and a diet supplemented with 250 ppm of LOM, with a total of 12 experimental units/treatment (20 chickens/pen). Animals were fed ad libitum their respective diets for three weeks and then samples of ileal content and pancreas were taken for digestibility and enzymatic analysis respectively. In the period from 0 to 7 days of age, a higher average daily gain (ADG) was observed in chickens fed with LOM (P < 0.05), which resulted in a higher body weight (BW) (173 vs 177 g for C and LOM respectively; P < 0.05). Despite BW was numerically higher in the following periods measured, and at 20 days of age it was 973 vs 982 q for C and LOM respectively, it did not reach statistical significance. The AID of Dry Matter (DM), Gross Energy (GE) and Crude Protein (CP) at 21 d of age was significantly higher (P < 0.05) in broilers fed LOM. Finally, no significant differences were observed in pancreatic trypsin and lipase activities, but amylase activity tended to be higher in birds fed LOM (174 vs. 254 U/mL for C and LOM respectively; P = 0.06). It is concluded that LOM positive effects on weight gain might be attributable, at least partly, to an improvement in nutrient digestibility probably by enhancing pancreatic amylase activity.

OPTIMIZING FEED COST AND IMPROVING GROWTH PERFORMANCE WITH A BIO-EMULSIFIER IN BROILER CHICKENS UNDER REAL FARMING CONDITIONS

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The physiological capacity to digest and absorb fats is poorly developed in young broilers. Optimizing the utilization of dietary fat has never been more critical for cost-effective production in the poultry industry. We hypothesized that the use of a bio-emulsifier can reduce feed cost via reduced-fat inclusion and maintain or even improve performance, providing thus added cost benefits. Here, a corn-soybased diet was formulated with two ME levels (standard and low) and the growth performance, abdominal fat, footpad lesions (FPL), and cost-benefit with(out) a bio-emulsifier were evaluated. In a 5-week study period, the day-old chicks were distributed in 27 pens, housed inside a commercial production facility, and allotted into 3 treatments (n=9 pens/treatment; 30 birds/pen). T1: Positive Control (PC) with standard ME value; T2: Negative Control (NC), low ME (PC minus 75 kcal/kg of feed), T3: NC + bio-emulsifier (Maxilys® 500 ppm). Maxilys® (Innovad) is a natural soy-lysolecithin bio-emulsifier. ANOVA and Tukey post-hoc tests were used to determine statistical differences (P<0.05). Regarding performance, no differences were found between (NC+Maxilys®) and PC for all the parameters measured. No significant differences in BW were found between the three treatments, although the values were numerically higher in NC+Maxilys® and PC compared to the NC on days 14, 28, and 35 (P = 0.09, P = 0.07, P = 0.10, respectively). Maxilys® significantly improved the FCR of broilers on all days (P<0.05) compared with the NC. FI was not affected by any treatment. On day 35, a significantly lower percentage of abdominal fat was observed in chicks fed with Maxilys® (2.10 %) compared to the NC (2.14 %) and PC (2.22 %) (P=0.045), accompanied by the lowest numerical prevalence of FPL (1.556) compared to the NC (1.778) and PC (2.444) (P = 0.14). The EPEF over the 35-d cycle was significantly increased in the PC (477) and NC+Maxilys® (475) treatments compared to the NC group (452) (P<0.001). The reformulated diets with the bioemulsifier reduced feed costs significantly and increased the corresponding ROI nearly by a factor of 3 (1: 2.66) compared to the NC. In conclusion, supplementation of a bio-emulsifier in an energy-reduced diet showed a positive effect on growth performance, fat deposition, and FPL. Importantly, it was concluded that the bio-emulsifier can be added to feed formulation to decrease the current high costs of added dietary fat and to maximize growth performance.

EVALUATION OF ENCAPSULATED CALCIUM BUTYRATE WITH OR WITHOUT MONOBUTYRIN ON PERFORMANCE OF BROILER CHICKENS

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A 35-day trial was conducted to evaluate the efficacy of encapsulated calcium butyrate (Ca-B) with and without monobutyrin (MB) in iso-butyric levels on performance parameters in broiler chickens. Butyric derivatives in general supplemented to broiler feed positively contribute to the performance of broilers, as they have a positive effect on gut integrity and anti-oxidative stress and inhibit microbial intestinal growth. However, the effects are less well known when combining both Ca-B and MB. A total of 90 one-day old broiler chicks (Ross 308, sexed males) were allocated randomly based on their initial body weight (BW) to 3 dietary treatments. Birds received a grain-soybean meal-based pelleted diet according to the GfE nutrient requirements of broilers in 3 phases (starter d1-7; grower d7-21 and finisher d21-35). Broilers were housed pairwise in 90 cages with 30 broilers per treatment until day 21, afterwards number was reduced to 15 birds per treatment. Treatments were (1) negative control, (2) single Ca-B (supplementing 240/170/170 butyric acid (BA) mg/kg in starter, grower, and finisher period) and (3) combination of Ca-B (120/85/85 BA mg/kg) with MB (120/85/85 BA mg/kg), iso-butyric in total (50/50) to treatment 2. Body weight (BW) and feed intake (FI) were recorded weekly. Average daily gain (ADG) and feed conversion ratio (FCR) were calculated for the starter/grower (d1-21), finisher (d21-35), and total period (d1-35). Data was analysed using the UNIANOVA procedure in SPSS and the post-hoc test Tukey HSD to identify differences between treatments. Birds fed the diet supplemented with the combination of Ca-B and MB (T3) tended to have an improved BW at d28 (1.94 vs 1.84 kg; P = 0.07) and d35 (2.74 vs 2.62 kg; P = 0.08) as well as a trend in higher ADG in both finisher (112 vs 105 g/day; P = 0.08) and overall period (77 vs 74 g/day; P =0.08) when compared to the negative control (T1). In addition, feed intake during the finisher, but not total phase tended to be increased in birds of T3 compared to T1 (176 vs 167 g/day; P = 0.07). When only considering T1 and T3 in the statistical analysis, the differences mentioned before were found significantly different (P<0.05). In general, FCR was not different between treatments. In conclusion, a combination of both encapsulated calcium butyrate and monobutyrin contribute performance mav to improved growth of broilers.

Key Words: encapsulated calcium butyrate, monobutyrin, growth, feed conversion, broiler

EFFECT OF BROILER BREED AND GENDER ON DIETARY PROTEIN AND ENERGY DIGESTIBILITY AT THREE DIFFERENT GROWING PERIODS

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The aim of the study was to compare the evolution of dietary protein and energy digestibility in males and females of two different broiler breeds (A and B) at three different periods (1-14, 15-29 and 30-42 days of age). A total of 384 animals grouped per gender and breed (8 replicates) were used; housing five, four and three animals for the first, second and third period, respectively. Animals were fed ad libitum with a common starter (2875 kcal AMEn/kg, 8% EE and 22% CP), grower (2925 kcal AMEn/kg, 7.5% EE and 21% CP) and finisher diet (3000 kcal AMEn/kg, 8% EE and 20 % CP). Titanium dioxide was added as indigestible marker for faecal and ileal digestibility determination. Faeces and ileal content samples were collected and pooled per experimental unit. They were analysed for dry matter (DM), nitrogen (n), energy and titanium dioxide. The uric acid content present in the faeces was also analysed to correct the crude protein excretion. The apparent faecal digestibility of the DM (AFDDM), nitrogen (AFDn), energy (AFDE), apparent metabolizable energy (AME) and apparent metabolizable energy corrected by nitrogen (AMEn) were calculated. Also, the apparent ileal digestibility of the DM (AIDDM), nitrogen (AIDn) and energy (AIDE), and the apparent ileal digestible energy content (AIDE kcal/kg) were estimated. The effect of breed, gender and their interaction were analysed using a mixed model. All the coefficients varied across the time with minimum values at 14 days and maximum values at 29 days to later, slightly decline at 42 days regardless breed and gender (P < 0.001). Breed and gender did not have any effect in the digestibility coefficients at 14 days of age. However, at 29 d of age, breed B had a higher AFDDM, AFDn and AFDE (73.8 vs. 73.3%, 83.7 vs. 82.3 %, 77.5 vs. 76.8 %; P < 0.05) comparing with breed A. Consequently, AME and AMEn (kcal/Kg) were greater at this age in breed B (3518 vs. 3490 and 3325 vs. 3283, respectively). At 42 days, this comparison remained constant progressing as a statistical trend. Breed B had a higher AFDDM (71.2 vs. 70.8 %; P = 0.09), AFDE (75.6 vs. 75.1%; P = 0.076), and, therefore, a bigger AME (3416 vs. 3392 kcal/kg; P = 0.076) and AMEn (3228 vs. 3197; P = 0.089) than breed A. Moreover, females reached a higher nitrogen ileal digestibility than males (77.5 vs. 75.4%; P = 0.045). We can conclude that faecal and ileal digestibility fluctuate over the time and there are significant differences between breeds and genders.

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Metabolism

Selected short communications

ENCAPSULATED CRYSTALLINE LYSINE AND DL-METHIONINE HAVE HIGHER EFFICIENCY THAN THE CRYSTALLINE FORM IN CHICKENS

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Crystalline amino acids (AA) supplementation is necessary in AA deficient or lowprotein diet. The different absorption rate of crystalline AA and protein-bound AA may take an effect on AA utilization. In this study, the effect of encapsulated lysine-HCl (Lys) and DL-methionine (Met) on the performance of broiler and hen was investigated. In Exp. 1, a total of 432 one-day-old male AA broilers were subjected to three dietary treatments for 42 days: fed with a basal diet supplemented with crystalline Lys and Met (control), and the basal diet supplemented with encapsulated Lys and Met at the levels of 80% and 60% of control diets (80CLM, 60CLM), respectively. The growth performance, intestinal development, and transcription of AA transporters were determined. In Exp. 2, a total of 135 forty-seven-week-old Hy-Line Brown hens were subjected to three dietary treatments for eight weeks: basal diet supplemented with crystalline Lys and Met (control) and the basal diet added with encapsulated Lys and Met at the levels of 60% (60CLM) or 80% of control (80CLM), respectively. In Exp. 3 and 4, 24 broiler and laying hens were respectively subjected to the same treatments as in Exp. 1 and 2 and the plasma levels of free AAs were measured 0, 2, 4, and 6 h after feeding. In broilers, 80CLM treatment had no significant influence on production performance and plasma free AAs content (P>0.05). 80CLM group moderately enhanced gut morphology development and increased AAs absorption capacity. However, broilers fed the 60CLM diet had lower performance and breast muscle weight (P<0.05), but increased villi height and BOAT mRNA expression level (P<0.05). At h 4 after feeding, the 60CLM broilers exhibited higher level of Ala, Cys, and total dispensable AA than control (P<0.05). In laying hens, AA treatment had no significant influence on the laying performance of hens. At the 2-h time point, the 80CLM hens exhibited higher concentrations of Lys (P<0.05). In contrast, plasma Met concentration was not influenced (P>0.05), while Cys was reduced in the 60CLM hens at all the time points. The result suggests that crystalline Lys and DL-Met can be effectively saved approximately for 20% by using the encapsulated form. The improved postabsorptive AA balance contributes to the reduced crystalline AA supplementation.

Reference

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EFFECTS OF VARYING LEVELS OF DL-METHIONINE ON AMINO ACID METABOLISM

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Essential amino acids play an important role in protein synthesis and cellular metabolism. Specifically, the total sulfur amino acids, methionine (Met) and cysteine (Cys), and their metabolites are involved in crucial functions such as tissue building and repair, as well as the reduction of oxidative stress. Considering Met is the first limiting amino acid in poultry, understanding the metabolism of this amino acid is important. One thousand Cobb 500 chicks were assigned to five different treatments fed grower diets containing varying levels of standardized ileal digestible (SID) methionine + cysteine ranging from deficient (0.36 Met %, 0.66 % TSAA) to excess (0.45% Met, 0.984% TSAA) based on Evonik recommendations (AMINOChick® 2.0). At 21d, birds were selected for either control, intravenous (IV) or intragastric (IG) infusion of [1-13C]Met, followed by sampling at intervals ranging from 5-420 min post-infusion. Blood was collected and 13CO2 enrichment was determined by isotope ratio mass spectrometry (IRMS). Large intestine, small intestine, pectoralis major, pancreas, kidney, and liver tissues were harvested at each of the time periods, along with plasma, in order to determine the amino acid enrichment over time (10 reps/timepoint). Samples were analyzed using a gas chromatography-mass spectrometer (GC-MS) and high-pressure liauid chromatography (HPLC). Results were analyzed using JMP Pro 16. Performance results showed that decreasing intakes of TSAA results in poorer feed conversions. Additionally, a deficiency of TSAA resulted in a greater loss of Met label as 13CO2 (p<0.05). Comparison of 13CO2 showed differences in IG and IV infusions (p<0.05), suggesting differences in parenteral and enteral uptake of nutrients. Understanding the TSAA pathways is increasingly important as the poultry industry moves towards complete antibiotic free production practices, considering methionine and cysteine are the precursors to glutathione, which plays several roles in immunity and antioxidant capacity. Additionally, determination of requirements has the potential to lead to reductions in nutrient loss, thus reducing nitrogen waste. Ultimately, the loss of the Met labels to 13CO2 proves worthy for understanding TSAA utilization and metabolism, considering greater 13CO2 production from [13C]Met may signify increased Cys synthesis.

EFFECT OF TWO MICROENCAPSULATED BLENDS OF ORGANIC ACIDS AND ESSENTIAL OILS ON BROILER PERFORMANCE UNDER A SAMONELLA TYPHIMURIUM CHALLENGE FROM 0 TO 21 D OF AGE

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In the last decades, much emphasis has been placed to the search for alternatives to the use of antibiotics in poultry production. One of those alternatives is the use of organic acids (OA), among which is found butyric acid. Even though there is a significant amount of studies about the use of butyric acid in poultry production, there are still many unknows regarding the synergies with other OA or essential oils (EO). Therefore, an experiment was performed to evaluate the effect of two different microencapsulated blends of OA and EO on growth performance and gut health of broilers Ross 208 from 0 to 21 d of age under a Salmonella typhimurium challenge. The two encapsulated blends of OA and EO were supplied by TECNOVIT and consisted a) ACITEC [calcium butyrate (CB) and fumaric acid + EO] and BUTYTEC (CB + EO). A total of 255 one-day-old, male Ross 708 broiler were randomly allocated according to a completely randomized block design and distributed into 3 treatments: 1) negative control (NC) based on standard commercial diet; 2) NC supplemented with 2 kg/tn of ACITEC and 3) NC supplemented with 0,75 kg/tn of BUTYTEC. Chicks were challenged by an oral inoculation with a S. typhimurium ATCC 14028 suspension on day 0. Data was analyzed using the MIXED procedure of SAS. The experimental unit was the pen and each treatment were replicate 5 times (17 birds/pen). Body weight and feed intake were measured at d 3, 7, 14 and 21 and feed conversion ratio was calculated. The use of ACITEC-MC increased (P<0,05) body weight and average daily gain at day 3 and day 21 and improved feed conversion ratio (P<0.05) from 0 to 3 d of chicks compared with NC. However, supplementation of ACITEC did not affect feed intake in any of the period studied. The supplementation of BUTYTEC did not affect any of the growth parameters studied. Salmonella concentration in cecum (log10/g) a was significantly (P<0,05) lower in the BUTYTEC group, meanwhile there was no significant effect on the ACITEC group (P>0,05). On d 21, ACITEC group showed significantly lower Alpha-1-acid glycoprotein (ng/ml) (P<0,05) than the NC, but no significant effect on serum amyloid A (ng/ml) (P>0,05). The use of BUTYTEC did not show any significant effect on this parameter (P>0,05). In conclusion, supplementing a targeted-released ACITEC and BUTYTEC blend exhibits some benefits on performance and Salmonella concentration in cecum, which may suggest an improved capacity of resist to Salmonella infection on broilers.

CAECAL METABOLOMIC FINGERPRINT AND PERFORMANCE OF BROILERS FED PLANT SENSORY MOLECULES AS ALTERNATIVE GROWTH PROMOTERS

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The massive use of antibiotics as growth promoter in broilers led to antibiotics resistance, hence the ban of this use. Sensory feed additives (SFA) are a nutritional alternative to antibiotic growth promoters (AGP) due to their functional properties. This study evaluated two SFA [capsaicin (CAP) and a blend of essential oils (mostly carvacrol) and oleoresins of spices (CAR)] as AGP and their impact on caecal metabolites. Ten-days old ROSS 308 broilers (n=432) were randomly placed in 36 cages (n=12/cage) and assigned to 3 treatments: 1) CON (control without SFA, 2) CAP (capsaicin) and 3) CAR (blend of essential oil and spice) that were given during growing (10-21 d) and finishing (21-34 d) phases. Animals and feed were weighed at d10, 21, 28 and 34 to assess body weight (BW), average daily gain (ADG), feed intake (ADFI), and feed conversion (FCR). One broiler per cage was euthanized for sample collections and organ weighing. Liquid chromatography coupled to mass spectrometry was used to perform untargeted metabolites profile of caeca content (n=10/treatment). Animal performance data was analysed using MIXED procedures of SAS and statistical multivariate was used for metabolomic data analysis through workflow4metabolomics. At d34, greater BW was observed when animals received CAP and CAR compared with CON (3.7%, P<0.001). The CAP diet increased ADFI as compared with CON (2.2%, P<0.001) and decreased FCR (3.6%, P<0.001). In comparison to CON, CAP decreased gizzard weight (8.9% BW; P=0.02). Lower liver weight (6.6% BW; P=0.03) was also observed with CAR compared with CON. Caecal metabolome analyse (variable importance in projections; VIP > 1.8) showed an increase of indole-3-acetic acid and a decrease of taurocholic acid, lysine, asparagine, arginine and serotonin in birds receiving CAP as compared with CON. These metabolites indicate implications of CAP on primary bile acids and amino acids, more specifically tryptophan metabolism. Broilers fed CAR have increased, capsaicin, saccharopine, pyrimidine, and jasmone, while was decreased arginine and valine levels as compared with CON. Metabolite modulation induced by CAR implies potential greater structural components (nucleotides, vitamins) and amino acids metabolism. Overall, these results showed that SFA supplementation induces an improvement in broiler performances and changes in caecal and liver metabolism. In further steps we will study the plasma metabolome profile associated with each SFA.

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Posters

SULFATION IS THE PATHWAY OF METABOLISATION OF DEOXYNIVALENOL TO DEOXYNIVALENOL-SULPHATE IN CHICKENS

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Recent experiments observed that deoxynivalenol (DON) is extensively metabolized to DON-sulphate (DON-S) in chickens. The aim of the study was to evaluate the most relevant biomarker of exposure of DON in broiler chickens plasma after intravenous and oral administration. Twenty- four 35 day -old chickens Ross 308 were used for the trial. Birds received intravenously and orally DON mycotoxin. A dose of 0.75 and 2.25 mg /kg of BW were administrated intravenously and orally. The administrated dose of 0.75 mg/kg BW is in coincidence with 5 mg DON/ kg of poultry feed (maximum level recommended by the European Commission. Blood was collected at different time points at (0) preadministration and at 15, 35, 60, 75 and 120 minutes post administration. The centrifugation of samples was (10 min at 2851*g) and plasma was stored at <= -20° until further analysis. Extract plasma was prepared adding acetonitrile to precipitate proteins, pursued by evaporation of the supernatant. LC-MS/MS revealed that DON-S was the major metabolite of DON in broiler chickens after intravenous and oral administration. The maximal concentration of DON in plasma was 36.5 and 101.5 ng/ml after IV administration of 0.75 and 2.25 mg of DON /kg BW, respectively. After, oral administration of 0.75 and 2.25 mg/kg BW of DON to broiler chickens DON concentration in plasma was below the LOO of 5 ng/mL. Only DON-S was found. Since no commercial standard of DON-S is available, the results were evaluated using the absolute chromatographic peak areas. The maximum areas of DON-S were 2,178,419 and 7,053,108 for the intravenous administration at low and high level, respectively and were 122,623 and 713, 515 for the oral bolus administration at low and high level, respectively. The maximum peaks of areas of this metabolite were achieved in plasma after 15 min post administration. The mean concentration of DON under the curve (AUCO-2h) for IV application at low level was 28.48 and at high level was 76.81 counts. The mean area of DON-S under the curve (AUC0-2h) for IV application at low level was 952, 087 counts and at high level was 2, 824, 109. The mean area of DON-S under the curve (AUC0-2h) for oral application at low level was 52,518 counts and at high level was 343, 970. This study confirms results recently reported, that DON-S is the most suitable biomarker for DON exposure in broiler chickens.

EFFECT OF BUTTIAUXELLA PHYTASE DOSE ON PERFORMANCE OF LAYING HENS FED NUTRITIONALLY REDUCED CORN/SOYBEAN MEAL DIETS

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The objective of this study was to ascertain the optimal inclusion level of a Buttiauxella phytase (PhyB) in diets of laying hens (during 24-60 weeks of age). A total of 240 Lohmann LSL Lite hens (20 replicates/treatment, 3 hens/cage) were distributed into four treatment groups at 24 weeks of age. Distribution was a randomized block design to achieve uniform bodyweight across the treatments at the beginning of the feeding trial. Diets were corn/soybean meal based. A pullet/grower adaptation diet supplemented with PhyB at 300 FTU/kg was fed from 12-23 weeks of age. Two diets were formulated based on common commercial practices: phase 1 (24-50 weeks of age) and phase 2 (51-60 weeks of age). The positive control (PC) diet was formulated to meet nutritional requirements based on breeder recommendations. The PC diet was reformulated to be reduced in AvP (0.16%), Ca (0.17%), ME (58 kcal/kg) and CP (0.35%) vs breeder recommendations and was supplemented with PhyB at 300 FTU/kg. A negative control diet (NC1) was formulated to be reduced in AvP (0.19%), Ca (0.20%), ME (63.5 kcal/kg) and CP (0.65%) vs breeder recommendations. NC1 was supplemented with PhyB at 600 FTU/kg. A second negative control, NC2, was formulated to be reduced in AvP (0.20%), Ca (0.21%), ME (64 kcal/kg) and CP (0.92%) and was supplemented with PhyB at 900 FTU/kg. Egg production and feed intake were measured daily and weekly respectively, and all eggs were collected from each replicate cage to determine egg weight at weekly intervals. Data were analysed across age and treatment by analysis of variance. Means were compared using Tukey's test. The NC1 diet reduced (P<0.05) hen-day egg production, egg weight, egg mass and FCR vs PC. Supplementation of PhyB at 600 FTU/kg to NC1, and 900 FTU/kg to NC2, maintained hen-day egg production, egg weight, egg mass, FCR vs PC. In conclusion, Buttiauxella phytase can replace up to 0.20% AvP, 0.21% Ca, 64 ME kcal/kg and 0.92% CP in laying hen diets, whilst maintaining performance.

Keywords: layers, matrix, optimal dosing, phytase

DIETARY CANNABIDIOL AFFECTS BREAST MEAT VOLATILE COMPOUNDS IN CHICKENS SUBJECTED TO DIFFERENT INFECTION MODELS

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Chickens are intensely selected for increased weight gain and feed efficiency. This selection has resulted in compromised health status in most key areas. The use of bioactive compounds in poultry feed is among the investigated potential alternatives to the antibiotic-based approach. Such additives might improve number of aspects of avian wellbeing and provide final products that are improved in quality for consumers. One phytogenic substance that exhibits potential to beneficially modulate gut health and functionality of final product is cannabidiol (CBD) from hemp, which has recently gained considerable attention. However, to date, no study has demonstrated the use of Cannabis-derived CBD to alter the stress response in chickens or examined its effects on meat volatile compounds (VOCs). Here, we subjected chickens to dysbiosis via C. perfringens infection or E. coli endotoxin (LPS) treatment and investigated the potential link between meat VOCs and cecal bacterial activity and the ameliorative effect of CBD. Cecal bacterial production of short-chain fatty acids (SCFAs) and meat VOCs were mostly closely correlated. The present study demonstrated the existence of close correlations between gut bacterial activity and chicken meat VOCs that were based on the production of SCFAs. Under the optimal conditions (no challenge), increased production of the main SCFAs, including acetic and propionic, was associated with increased formation of meat spoilage markers. CBD supplementation did not contribute to meat quality deterioration; in contrast, it reduced the formation of spoilage VOCs, including alcohols, trimethylamine and pentanoic acid, in challenged birds. CBD supplementation decreased the production of putrefactive fatty acids, which resulted in decreased production of spoilage VOCs in breast meat. Moreover, our findings indicated that the association between VOCs and SCFAs differed depending on the applied challenge (C. perfringens vs. LPS), and that CBD more effectively altered the effect of C. perfringens than LPS challenge to meat VOCs. The findings in this study provide a basis for understanding the influence of gut bacterial activity on meat VOCs and the role of pathogenic stimuli in this regard. The results also provide new insights into the interactions among bioactive agent application, gut microbiota activity and meat properties in chickens.

This work was supported by the National Science Centre in Poland, Grant No. 2018/29/B/NZ9/01351.

THE MODULATION OF MYO-INOSITOL (INS) TRANSPORTER PROTEINS IN THE JEJUNUM AND KIDNEY OF BROILER CHICKENS SUPPLEMENTED WITH DIETARY PHYTASE, INS AND GLUCOSE.

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Supra inclusion rates of phytase have led to improvements in feed efficiency and understanding of phytate destruction in the gastrointestinal tract (GIT) of broiler chickens. These improvements are often associated with myo-inositol (Ins). Based on previous research, it is understood that any free Ins in the GIT is rapidly absorbed and circulated to various tissues. However, it is not fully understood if there are any changes in the expression of the Ins transporter genes (SMIT1, SMIT2 and HMIT) or glucose transporter genes (SLC5A11) when diets are supplemented with phytase, glucose or Ins. A total of 100 Ross 308 male chickens of 7 days of age were allocated to five treatment groups with 10 replicates (2 birds per pen) and fed until 21 days of age. The birds were fed a pelleted diet either supplemented with Ins at 4.5 g/kg or 13.5 g/kg, glucose at 4.5 g/kg, phytase (Quantum Blue P40, ABVista) at 4,500 FTU/kg or un-supplemented control. Levels of mRNA for Ins transporter genes were measured by RT-qPCR and transformed using log10. Data was analysed by one-way ANOVA with a post hoc protected Fisher Least Significant Difference test to separate means. Treatment diets did not significantly affect the relative mRNA expression of HMIT, SMIT2 and SMIT1 in the kidney or HMIT and SMIT2 in the jejunum (P>0.05). The expression of SLC5A11 was affected by treatment diets (P<0.001), treatments 4.5 g/kg Ins and 4.5 g/kg glucose had lower relative expression compared to the other treatment groups in the kidney. Within the kidney overall expression was highest for SMIT1 followed by HMIT, SMIT2 and SLC5A11. The expression of SMIT1 in the jejunum of control birds was significantly higher than the other treatment diets (P<0.006). Furthermore, the gene SLC5A11 was expressed significantly more than the other diets by 4500 FTU/kg phytase diet (P<0.003). In jejunum tissue the overall expression was highest for SMIT1 followed by SMIT2, HMIT and SLC5A11. Overall, this study indicated that Ins is actively transported into the jejunum and kidney via SMIT1, SMIT2 and HMIT. Expression of these transporters are not regulated by dietary availability of Ins.

EFFECT OF MYCOTOXINS ON CHICKEN AND RABBIT EMBRYONIC DEVELOPMENT AND CHICKEN PGCS

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Nowadays, a very actual topic is the investigation of the effect of mycotoxins on food safety, animal breeding and animal health. Many researchers examine the impact of these mycotoxins on the animal's development because these toxins in high concentration cause severe problems in meat-, milk-, egg production, and animal health. Our group investigates the effect of 2 different mycotoxins (T-2, Zearalenone) on chicken embryonic development. We used different concentrations of toxins in our experiments. In case of chicken, the toxins were injected into blood vessels of 3 days old embryos. On the 10th day of incubation, we collected different organs from the embryos (gonads, muscle tissue, liver, egg white and yolk). We injected T-2 toxin in 1, 5, 8, and 10 $ng/\mu L$ concentration into chicken embryos and Zearalenone in 1, 5 and 10 ng/µL. Finally, we mixed the two toxins (T-2 and Zearalenone) in different concentrations (2.5-20; 5-10 and 5-20 $nq/\mu L$) and injected back into chicken embryos. Altogether 140 chicken embryos were injected with different toxin solutions. We investigated the embryo survival rate, the male-female ratio and the accumulation of the toxins in different tissues of isolated embryos. We found significantly lower survival rate in case of T-2 toxin. When 25 control embryos were injected 20 (80%) were living to the 10th day of embryonic development. When the lower concentration of T-2 toxin was injected $(1 \text{ ng/}\mu\text{L})$ the survival rate was 61.5 %. Finally, in case of the most concentrated T2 toxin (8ng/µL) we injected 19 embryos and 10 embryos survived (52.6%). After zearalenone injection we did not get significant decrease in the survival rate. We injected 18 controls (16 survived, 87.5%), 21 embryos from 5 ng/µL concentration (17 survived, 82.2%) and 21 embryos from 10 ng/µL concentration (17 survived, 77.2%). We did not find any difference between the two genders. In case of T2, the highest accumulation rate was found in the yolk and the mesonephros from the examined tissues. We established chicken PGC lines from the embryos of white Hungarian chicken breeds. We investigated the effect of different toxins on these PGC lines' proliferation rate. We added toxins to the culture medium in different concentrations (T-2: 0.5-1-2.5-5 ng/µL; ZEA: 1-5-10-20 ng/µL). We placed 2500 cells/well into 96 well plate. We calculated the proliferation rate on the 3rd day. We found that the proliferation rate of PGCs declined from 1.27 to 1.09.

Mineral nutrition

Selected short communications

DETERMINATION OF CA AND P DIGESTIBILITY OF INDIVIDUAL FEED INGREDIENTS AS AFFECTED BY LIMESTONE

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For Industry to progress towards formulating broiler diets on a digestible Ca basis, there is a need to generate robust values of digestible Ca for ingredients and factors affecting this digestibility. The current study had 3 main objectives: 1) determine for 7 ingredients the standardized ileal digestibility (SID) of Ca and P in broilers 2) determine the impact of phytate source on SID Ca of limestone (LS) and 3) determine the impact of phytate source on SID P of the ingredient when LS is present. Two experiments (Exp) were done to accomplish this. In Exp 1 a 4 x 2 factorial design was used with 4 ingredients (corn, wheat, sorghum, and full fat soy (FFS) and 2 inclusions of LS (0 and to achieve 0.65% Ca in final diet). In Exp 2, a 3x2 factorial design was used with 3 ingredients (soybean meal (SBM), rapeseed meal (RSM), and sunflower meal (SFM)) and 2 inclusions of LS (0 and to achieve 0.65% Ca in final diet). Each ingredient was formulated into a basal diet such that phytate P (PP) from the ingredient contributed 0.144% (corn, wheat, FFS and sorghum) or 0.23% PP (SBM, RSM, SFM). Experimental diets were fed for 36 hrs, before distal ileal digesta collection for 8 replicates per treatment of 6 All data were analyzed using a Proc mixed model (SAS 9.1, SAS birds/rep. Institute, 2012) with significance accepted at P < 0.05. In Exp. 1 SID Ca of wheat (72.9%) and FFS (69.9%) were higher (P<0.05) than for sorghum (54.5%) and corn (46.3%). In Exp. 2 SID Ca of SFM (61.0%) was higher (P<0.01) than for RSM (42.7%) and SBM (46.8%). SID Ca from added LS was affected by ingredient being lowest for diets containing wheat versus corn and sorghum in Exp. 1 and lower for SBM vs. RSM and SFM in Exp2. There was a 2-way interaction (P<0.01) of LS addition and ingredient on SID P in both Exp. The result of this study provides SID Ca and SID P from the selected ingredients and suggests that phytate from different ingredients reacts differently with Ca from limestone and should be considered when developing SID coefficients of Ca and P for use in commercial broiler feed formulation.

AVAILABLE PHOSPHORUS REQUIREMENT FOR BROILERS: INTEGRATED ASSESSMENT OF PERFORMANCE RESULTS USING META-ANALYSIS

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Phosphorus is an essential nutrient that plays an important role in many biological functions. Precisely knowing the requirements is essential to improve the productivity on broiler production. This study was developed to reassess the available phosphorus (aP) requirements of broilers by carrying out a meta-analysis of previously published performance responses. Studies available in electronic databases (PubMed, Web of Science, and Scopus) were searched using systematic review methods. Then, publications were selected for the meta-analysis if presenting performance responses of broilers fed at least three dietary aP levels. Data from 76 studies (64,503 animals from 1 to 53 days old) published from 1997 to 2019 were extracted to an electronic spreadsheet. Due to the high variability among studies, the aP concentration and the performance responses were standardized. First, the aP intake was relativized to the recommendations of Brazilian Tables for Poultry and Pigs (i.e., values were considered 100% if in complete agreement with the recommendations of Rostagno et al., 2017, 4th edition, Viçosa, Brazil). After, the average daily weight gain (ADG) and feed conversion ratio (FCR) were also expressed as relative values to the best response in the study (i.e., best performance in each study was considered 100%). Linear broken-line (LP), quadratic broken-line (QP), and exponential asymptotic (EXP) models were used to estimate the relative aP intake that maximized performance responses (NLMIXED procedure with random effect of study, SAS 9.3). When using LP model, the relative aP intake required to maximize ADG was 71% of the recommendations from Brazilian Tables, indicating an overestimation. The values obtained with QP and EXP models were 85 and 104% of Brazilian Tables recommendations, respectively. Overestimation was also found for FCR, with best performance estimated at 57 (LP) and 94% (QP) of Brazilian Tables recommendations. In this last case, EXP model failed to converge. The LP models showed the lowest Akaike Information Criterion for both responses. Interaction of the response of broilers to increasing aP intake was found (P<0.05) for growing phase (ADG and FCR) and for the use of animal origin ingredients (ADG), while was not significant for phytase, feed form or calcium level. Reassessing the aP recommendations is essential for the implementation of precision feeding strategies and the meta-analysis is a suitable tool in this very important task.

EFFECTS OF GRADED LEVELS OF DIETARY MICROBIAL 6-PHYTASE ON PERFORMANCE, INTESTINAL HISTOMORPHOLOGY, CECAL MICROBIAL POPULATION, AND SHORT-CHAIN FATTY ACID COMPOSITION OF LOHMANN WHITE-CLASSICS

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This study was conducted to determine the effects of graded levels of phytase on performance, egg quality, and gut health of white laying hens. Treatments consisted of a negative control (NC) diet containing 0.14 avP (available phosphorus), positive control (PC) diet containing 0.35 avP provided via dicalcium phosphate (DCP), and DCP in PC diet was replaced with three graded levels of phytase derived from Komagataella phaffii ((OptiPhos Plus, Huvepharma NV) at 500, 750 and 1000 FTU/kg which provided 0.176, 0.188 and 0.200 % of avP, respectively). Diets analyzed phytase levels were < 13, 55, 605, 755, and 975FTU/kg for NC, PC, PC-500, PC-750, and PC-1000 respectively. One hundred and fourty Lohmann white classic laying hens were raised in 3-floor battery cages from 30 to 56 weeks of age. Data for all response variables were analysed in a completely randomized block design consisting of 5 treatments and 7 replicate blocks with 4 birds in each. Egg production, body weight, feed intake, feed conversion ratio (FCR), and eggshell quality were determined. At the end of the experiment, 2 birds per replicate were manually slaughtered and subjected to jejunal and cecal sampling for further analysis. Egg production, feed intake, FCR, and jejunal morphometry were negatively affected in NC birds (P<0.001). Neither of the dietary treatments affected cracked eggs, dirty eggs, eggshell breaking strength, and eggshell thickness. Considering the whole period, birds fed on a diet supplemented with graded levels of phytase shared the same egg production and feed intake levels with PC birds (P<0.001). FCR was significantly lower by 4.9, 1.6, and 7.4 % in PC-500, PC-750, and PC-1000 birds respectively compared to PC (P < 0.001). Dietary supplementation of phytase significantly increased villus surface area by 15, 36, and 40 % in PC-500, PC-750, and PC-1000 birds respectively compared to PC (P < 0.001). In addition, a significant increase in Lactobacillus count was observed in line with increasing the level of phytase (P<0.002). Dietary treatments had no effect on the cecal coliform and total aerobe bacterial population. Furthermore, phytase supplementation significantly increased the concentrations of total cecal short-chain fatty acid (SCFA) (P<0.001). In conclusion, along with improving performance parameters, the inclusion of phytase in laying hens' diet can ameliorate intestinal morphology, influence cecal microflora compositoin, and increase cecal concentration of SCFA.

META-ANALYSIS: GROWTH PERFORMANCE AND CARCASS YIELDS OF BROILER CHICKENS ARE IMPROVED BY HYDROXYCHLORIDE ZN AT EUROPEAN COMMERCIAL AND LOWER DIETARY LEVELS

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There is an increasing trend in Europe to reduce trace mineral excretion by animals into the environment. Feeding a more efficient mineral source may allow reducing dietary Zn levels without hampering performance. The objective of this study was to compare the effect of commercial Zn level (80 mg/kg) and lower Zn level (20 mg/kg) when fed as hydroxychloride form (HTM) or sulfate form (STM) on growth performance and carcass yields in broiler chickens. Nine studies were combined into one meta-analysis. The selected studies: 1) included a comparison between HTM and STM fed at 80 mg/kg and/or 20 mg/kg Zn, 2) were performed in Europe in experimental conditions mimicking commercial practice by using a wheat-based diet, high stocking density, and/or environmental challenges. Raw data was compiled and analyzed using SAS. Overall growth performance was analyzed using PROC MIXED and carcass yields were analyzed using PROC GLIMMIX following a beta distribution. All models were corrected for within study and between study variation. Data was analyzed as a 2 (Zn sources) x 2 (Zn levels) ANOVA, followed by pairwise comparisons between treatments. Growth performance and carcass yields were significantly improved in the HTM group compared to the STM group regardless of Zn levels. This was demonstrated by a higher final body weight (BW) (+32.7 g, P=0.0026), average daily gain (ADG) (+1.0 g/bird/day, P=0.0021) and average daily feed intake (ADFI) (+1.2 g/bird/day, P=0.0110), while feed conversion ratio was not significantly different. Furthermore, the breast meat yield (BMY) was significantly improved (+0.4%, P=0.0064), while carcass yield tended to be higher (+0.2%, P=0.0803). No significant main effect of Zn level or source*level interaction were observed. Pairwise comparisons showed that at 80 mg/kg HTM had a higher BW (+24.9 g, P=0.0374) and ADG (+0.8 g/bird/day, P=0.0294) compared to STM at the same Zn level. Reducing Zn to 20 mg/kg fed as HTM resulted in a tendency towards a higher BW (+30.3 g, P=0.0694), ADG (+0.9 g/bird/day, P=0.0674) and ADFI (+1.4 g/bird/day, P=0.0531), while BMY was significantly (0.4%, P=0.0169) improved compared to 80 mg/kg Zn fed as STM. In conclusion, feeding HTM results in an improved growth performance and carcass yield compared to feeding STM. Feeding 20 mg/kg Zn from HTM resulted in a similar or even higher response as feeding 80 mg/kg Zn from STM, with possibly great benefits for the environment.

26th World's Poultry Congress, abstracts selected in 2022

Mineral nutrition

Posters

EFFECTS OF DIETARY CALCIUM LEVEL AND MICROBIAL PHYTASE ON THE BIOAVAILABILITY OF ZINC DEPENDING ON ZINC SOURCE USED IN GROWING BROILERS DIET

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The bioavailability of zinc (Zn) is related to its solubility in the gut, which is determined by source's physicochemical properties and dietary factors, e.g., calcium (Ca) and phytase. It was hypothesized that Zn source modulates phytase efficiency and Ca level interferes with Zn bioavailability depending on the source. A starter diet set at 40 mg native Zn/kg was given for one week to 144 one-day broiler chickens which then were assigned to 8 conventional diets containing 27 mg of native Zn supplemented with 23 mg of Zn as oxide (ZnO) or sulfate (ZnSO4)/kg, 6 or 10 g Ca/kg and microbial phytase (0 or 750 FTU/kg). On 21 d of age, after 6 hours of fasting, 5 chicks per diet were re-fed 30 min and the others 150 min before sampling. Data was analyzed using the GLM procedure. Body weight gain (BWG) was improved in high Ca group supplemented with phytase (Ca \times phytase, P=0.049). Zn oxide resulted in higher BWG (P=0.007), lower FCR (P=0.0003) and heavier (P=0.03), denser (P=0.04) and longer (P=0.003) tibia compared to ZnSO4. Phytase improved (P<0.0001) bone Zn content and deposition, and Zn content of tibia especially in birds given ZnSO4 compared to ZnO (Zn source×phytase, P=0.04). High Ca level improved (P \leq 0.01) tibia traits and Zn deposition compared to low Ca. Increasing the re-feeding length and Ca level, respectively, decreased and raised the pH of proventriculus plus gizzard content (P+G) (P<0.0001), but not in jejunum. Plasma Zn tended (P=0.08) to be higher in birds fed diets supplemented with ZnO and phytase. Re-feeding for 150 min decreased (P \leq 0.009) and phytase increased (P \leq 0.004) plasma Zn. The $Ca \times re-feeding$ length or $Ca \times Zn$ source were significant (P<0.05) with higher soluble Zn (%) in P+G for low Ca group re-fed for 30 min or fed ZnO. Longer refeeding decreased (P<0.0001) total and insoluble Zn (μ g/g DM) in P+G. Low Ca level decreased (P<0.0001) insoluble Zn in P+G and phytase increased (P<0.05) soluble Zn (%) and decreased (P<0.05) insoluble Zn (%) in P+G and jejunum contents. A tendency for higher soluble and lower insoluble Zn in jejunum with ZnO in the low Ca group supplemented with phytase was observed (P=0.082). In conclusion, the speed of dissolution can determine the fate of Zn in the gastrointestinal tract and its deposition in the body tissues. Phytase efficiency in releasing Zn depends on the zinc source and the response criterion. Dietary calcium level affects digestive physiology of zinc depending on the zinc source.

THE EFFECT OF VARIOUS LEVELS OF A DACITIC (RHYOLITIC) TUFF BRECCIA ON GROWTH PERFORMANCE OF BROILERS UNDER HEAT STRESS

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Heat stress (HS) negatively impacts animal production worldwide, being an economic and welfare issue. In poultry, HS was shown to reduce feed intake (FI) and increase mineral excretion, suggesting a marginal mineral deficiency. AZOMITE® (AZOMITE Mineral Products, Inc), a dacitic tuff breccia (DTB), contains over 70 trace minerals, including rare earth elements. The aim was to investigate the effect of DTB on growth performance of birds under chronic cyclic HS. Cobb 500 male one-d-old chicks (N=1,280, 39.7 g \pm 0.19 SD) were randomly assigned to a complete block design, with 4 dietary treatments (0, 0.125, 0.25, and 0.5%) DTB), 16 replicates of 20 birds. Birds were raised in floor pens (0.09 m2/bird, wood shaving) from 0-42 d with ad libitum water and feed. From 7-42 d, all birds were exposed to chronic cyclic HS ($32^{\circ}C/8$ h/ d). The treatments were obtained by adding 0, 0.125, 0.25, or 0.5% of DTB to the basal diet (corn-soybean meal-wheat based, CON) in the place of corn. Diets were divided into starter (0-14 d), grower (14-28 d), and finisher (28-42 d). At the end of each phase, average daily gain (ADG), average daily FI (ADFI), and gain efficiency (GE) were calculated. Data were analyzed by Mixed procedure, SAS (SAS Institute, Cary, NC; v9.4) with linear and quadratic contrasts for dose-response. Significance was considered at P<0.05 and trend at P<0.10. From 0-14 and 14-28 d, ADG and ADFI were similar among aroups (P>0.36). GE was higher in 0.125% than 0.25% DTB (P=0.04, 0-14 d), and the highest in 0.125% DTB (linear effect, P=0.02, 14-28 d). From 28-42 d, ADFI was similar among groups (P=0.17), and ADG was higher in 0.25% than 0.5% DTB (P=0.06). GE was higher in 0.25% than 0.5% DTB (P=0.04; 0.508 vs. 0.483, respectively). Quadratic effect was found for ADG (P=0.01) and trends were found for GE (P=0.05) and ADFI (P=0.06), with maximum values at 0.25% DTB (65.9 g ADG and 0.508 GE) and 0.125% DTB (131.4 g ADFI). These values were 5.1% (ADG), 3.9% (ADFI), and 2.6% (GE) higher than CON. From 0-42 d, ADG and ADFI were similar among groups (P>0.44). However, ADG and ADFI were 2.2% and 1.9% numerically greater in 0.25% DTB than CON (47 vs. 46 g CON, ADG; 80.9 vs. 79.4 g CON, ADFI). Moreover, birds fed 0 and 0.25% DTB had higher GE than 0.5% DTB (P=0.02, 0.579 and 0.582 vs. 0.568, respectively), with a linear effect (P=0.01) showing maximum value at 0.25% DTB (0.582). The inclusion of DTB at 0.25% showed potential in improving growth performance in HS birds.

AVAILABLE PHOSPHORUS REQUIREMENT FOR BROILERS: INTEGRATED ASSESSMENT OF BONE CHARACTERISTICS USING META-ANALYSIS

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Precise estimations of available phosphorus (aP) requirements are essential in modern broiler production. Although several studies have been conducted to study requirements, there is no definitive consensus regarding its ideal aP recommendation. This meta-analysis was conducted using results published in the literature to evaluate the requirements for aP to maximize tibia characteristics in broilers. Studies were search in three different electronic databases (PubMed, Web of Science, and Scopus). All publications were critically evaluated. For inclusion in the database, the articles must compare at least three dietary levels of aP. After applying the selection criteria, data from 47 scientific publications (35,592 animals) published from 2000 to 2019 were extracted to an electronic spreadsheet. Standardization procedures were adopted to deal with the high variability among studies. Thus, raw values of tibias ash, phosphorus (P), and shear force were expressed as relative to the greatest response in the study (i.e., greatest value for each variable in each study was considered 100%). Relative aP intake was also evaluated according to its relation to the recommendations of Brazilian Tables for Poultry and Pigs (i.e., values were considered 100% if in complete agreement with the recommendations of Rostagno et al., 2017, 4th edition, Vicosa, Brazil). Linear broken-line (LP), guadratic broken-line (QP), and exponential asymptotic (EXP) models were used to estimate the relative aP intake that maximized bone characteristics (NLMIXED procedure with random effect of study, SAS 9.3). Relative aP intake required to maximize tibia ash, P, and shear force were, respectively, 89, 89, and 88% of the recommendation proposed by the Brazilian Tables when using LP models. The QP and EXP models estimated higher relative aP intake to maximize tibia ash (QP: 111%; EXP: 113%), P content (QP: 137%), and shear force (QP: 104%; EXP: 116%). However, the LP models showed the best (lowest) Akaike Information Criterion for all studied responses. Interaction of the response of broilers to increasing aP intake was found (P<0.05) for growing phase for all studied responses, and also for phytase and feed form when evaluating tibia ash. No interaction was found for the use of animal origin ingredients or calcium level. Integrating available results to reassess the aP recommendations is an important task toward feeding programs that maximize the efficiency of P utilization in broilers.

EFFECT OF CA LEVELS ON PERFORMANCE, BONE ASH AND ILEAL DIGESTIBILITY OF CA AND P IN BROILERS FED PHYTASE AND ENDO-B1,4-XYLANASE

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A broiler trial was set up (33 floor pens, 12 male ROSS 308 broilers per pen) to investigate the effect of lowering Ca on performance, Ca and P digestibility and bone ash when fed a thermostable 6-phytase (OptiPhos® Plus). Birds were fed a starter (d1-8), grower (d9-21) and finisher feed (d22-35), to which 1000 FTU/kg phytase, and 1500 EPU/kg of an endo-1,4 β -xylanase (Hostazym® X) was added. Levels of available P (aP) in starter, grower and finisher were 0.43%, 0.37 % and 0.22 %, using a contribution of 0.176 % aP from the phytase. Feeds were pelleted at \pm 80°C (starter was crumbled); to the finisher TiO2 was added at 0.4 %. Three treatments were implemented: normal Ca level (0.85, 0.7 and 0.6 %), reduced Ca level (0.7, 0.6 and 0.5 %), and low Ca (6.5, 5.0 and 4.0 %) in starter, grower and finisher respectively by reducing the inclusion of limestone (average particle size 0.39 mm). Technical performance was measured after every feeding phase. At day 21, 3 birds per pens were killed and the right tibia was removed, pooled to one sample, and analysed for ash on fat free dry matter. At day 35, 9 birds of each pen were killed to collect ileal material for the determination of Ca, P and Ti, to calculate Ca and P digestibility. Reducing the Ca levels led to an increased technical performance, with the low Ca level giving a higher end weight vs the normal Ca level (2770 g vs 2679 g, P< 0.05) while the reduced Ca level results in an end weight of 2767 g. Feed conversion over the whole trial period was not impacted (all 1.40). The Ca digestibility was significantly higher at the lowest Ca inclusion level vs the normal and reduced Ca level (64.1 vs 50.2 and 53.9 % respectively; P< 0.05). The P digestibility was highest at the lowest Ca inclusion level (60.6%) and significantly different from the normal Ca level (54.5%) and the reduced a level (51.3%). Tibia ash was significantly lower for the low Ca level (46.8 %) versus the normal Ca level (48.4%) while the reduce Ca level was in between (47.6 %). It could be concluded that lowering the Ca levels optimised technical performance but tended to reduce bone ash. Therefore, and considering the aP level of the feed, an appropriate Ca level needs to be chosen to fulfil the Ca requirement for optimal bone growth, but not to impact performance.

P-FOWL - THE ACTIVE CORE MICROBIOTA OF TWO HIGH-YIELDING LAYING HEN STRAINS FED WITH DIFFERENT LEVELS OF CALCIUM AND PHOSPHORUS

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The nutrient availability and supplementation of dietary phosphorus (P) and calcium (Ca) in the feed of avians, especially in laying hens, plays a vital role in phytase degradation and mineral utilization during the laying phase. The required concentration of P and Ca has its peak during the laying phase and the direct interaction between Ca and P concentration shrinks the availability of both supplements in the feed. Ca supplementation modifies the microbiota of the ileum and caeca, however, the extension of its effect together with P supplementation at different levels is not yet understood across the gastrointestinal tract (GIT) of laying hens. Our goal was to characterize the entire GIT of two high-yielding strains of laying hens (Lohmann Brown Classic, LB; Lohmann LSL-Classic, LSL) under different P and Ca supplementation levels. Eighty animals of two different strains (LB and LSL) were fed with four experimental diets (P+Ca+; P+Ca-; P-Ca+; P-Ca-). A total of 678 samples were taken from the digesta and mucosa of five GIT sections (crop, gizzard, duodenum, ileum, caeca). RNA was extracted to target the active bacterial community with a commercial kit and subjected to target amplicon sequencing followed by bioinformatic analysis with Mothur (1). Statistical significances were tested with PERMANOVA routine. The core microbiome was identified with the phyloseq & microbiome library in R. Statistical significances were observed for strain, GIT section, Ca, and the interaction of section x strain, P x Ca, Ca x strain and P x Ca x strain (p < 0.05). Lactobacillus and uncl. Lactobacillaceae were more abundant in the LSL while uncl. Lachnospiraceae and Ligilactobacillus were prevalent in the LB strain. A core microbiota of five species was detected in more than 97% of all samples. They were represented by an uncultured Lactobacillus (average relative abundance (avra) 12.1%), Lactobacillus helveticus (avra 10.8%), Megamonas funiformis (avra 6.8%), Ligilactobacillus salivarius (avra 4.5%), and an uncultured Fusicatenibacter (avra 1.1%). Our findings indicated that Ca and P levels of supplementation have a minor effect on the microbiota compared to the strong effect of the bird's genetic background. Moreover, a core active microbiota across the GIT of two high-yielding laying hens was revealed for the first time.

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MEASUREMENT OF ELEMENTAL SELENIUM (SE0) IN SE-YEAST

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Selenium is an essential trace mineral for animal nutrition largely related to its key roles in the antioxidant defense, immunity and inflammatory modulation. Selenium is included into livestock premixes either in inorganic forms (sodium selenite, selenate) known to have low bio-efficacy, or organic forms such as inactivated veasts - SY and pure chemically synthetized forms (selenomethionine, hydroxyselenomethionine) known to have a higher bio-efficacy. It has been known for a long time that SY largely vary in their selenomethionine (SeMet) content from supplier to supplier as well as from batch-to-batch of production (Fagan et al., 2015) which explain Se bio-efficacy differences in poultry (Simon et al., 2013; De Marco et al. 2021). Whereas the other Se forms present in SY can be characterized but rarely quantified. Here, using recent analytical methods (Vacchina et al. 2021), the Se composition of 13 fresh commercial samples of SY products from different suppliers and from batches within the same supplier (CNCM I-3060, CNCM I-3399, NCYC R397, NCYC R645, NCYC R646) has been investigated for total Se, SeMet and elemental Se (Se0), this last inorganic form being only recently identified in SY. Results in terms of total Se confirmed adequation with expected Se concentration of all 13 SY samples. Results of proportion of this Se present as SeMet and Se0 was as followed: SY1: 60% and 9%, SY2: 69% and 6%, SY3: 68% and 8%, SY4: 72% and 4%, SY5: 61% and 13%, SY6: 19% and 52%, SY7: 33% and 30%, SY8: 72% and 9%, SY9: 60% and 9%, SY10: 54% and 11%, SY11: 41% and 8%, SY12: 52% and 18%, SY13: 65% and 7%. These results first confirmed the high variability in the proportion of SeMet in SY products. They also indicated that the inorganic form of selenium, Se0, could represent between 4% up to 52% of SY products, with proportions that were observed to be negatively related to the SeMet one. It can be hypothesized that selenite used during the fermentation process of those SY may be reduced to Se0 in some situations. Such advances in the analytical characterization confirm that measuring Se0 and SeMet contents is important to guarantee the effect of the organic Se supplemented by SY.

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EXOGENOUS PHYTASE AND A MULTICARBOHYDRASE CONTAINING A-GALACTOSIDASE INFLUENCES BROILER PERFORMANCE, PROCESSING YIELD, AND NUTRIENT DIGESTIBILITY FROM 1 TO 42 DAYS OF AGE

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This study evaluated the effects of phytase and a multicarbohydrase containing agalactosidase activity (CAG) on broiler performance, processing yield, and nutrient digestibility from 1 to 42 d of age. A total of 2,250 d-old Ross 708 x YPM male broilers were distributed randomly in 90 floor pens and assigned to 9 treatments with 10 replicates per treatment. A positive control sufficient in phosphorus (PC-P) and a PC for energy (PC-E) with 100 kcal/kg more ME were formulated. A negative control (NC) was formulated to contain 0.20% less available phosphorus and calcium than the PC-P and 100 kcal/kg less ME than the PC-E at each feeding phase. Six additional treatments contained 500 or 1,500 FTU/kg of phytase combined with 0, 0.1, or 0.2 g/kg of CAG in the NC diet. Feed intake and BW were determined at 14, 28, and 42 d of age, and FCR corrected for mortality. Ileal digesta was collected from 5 birds per pen on d 42 to analyze nutrient digestibility. On d 43, 10 birds per pen were processed to determine processing yields. Data were analyzed using the GLM procedure of JMP, and means separated using Tukey's HSD with statistical significance considered at $P \leq 0.05$. During the entire study, broilers fed the PC-E had 7.2% heavier BW and 5-point lower FCR compared to broilers fed the NC (P < 0.05). Combining 0.2 a/kg of CAG with either 500 or 1,500 FTU/kg of phytase resulted in similar FCR to broilers fed the PC-E, and a 1.6- and 3.0-point improvement when compared to the non-CAG supplemented groups, respectively. Fillet, tender, and wing weight increased 8.4, 8.4, and 3.7%, respectively, when broilers were fed a diet with 500 FTU/kg of phytase and 0.2 q/kq of CAG compared to broilers fed the NC (P < 0.05). Broilers fed a diet with 1,500 FTU/kg of phytase and 0.2 g/kg of CAG had 3.4 and 2.7% higher fillet yields compared to the NC and PC-E, respectively (P < 0.001). Crude protein digestibility (82.53 vs 77.21%; P = 0.002) and apparent ileal digestible energy (3,585 vs 3,254)kcal/kg; P < 0.001) improved when broilers were fed both enzymes at their highest inclusion rate compared to the NC. Regardless of CAG inclusion, phosphorus digestibility improved an average of 46.7 and 59.1% when broilers were fed a diet with 500 or 1,500 FTU/kg of phytase, respectively, compared to the NC (P <0.001). Overall, supplementation of phytase and CAG improved broiler performance, processing yields, and nutrient digestibility in phosphorus and energy deficient diets.

DOSE RESPONSE OF A PHYTASE ON (PHYTATE) PHOSPHORUS, PROTEIN AND ASH DIGESTIBILITY AND ON BONE ASH IN BROILERS

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The objective of this trial was to investigate the effect of different doses of a phytase (OptiPhos) on the ileal digestibility and tibia quality of male broilers (Ross308). Starter diet was the same for all animals. From day 9 on, broilers were divided into eight treatment groups (pelleted grower): a positive control group (PC; corn-soybean meal-based diet) and a negative control group (NC, -1.5 g/kg available phosphorus (aP) and calcium), and six different doses of the phytase (added to the NC): 250, 500, 750, 1000, 1250, and 1500 FTU/kg (T3 to T8). On day 17, broilers were transferred to digestibility units (six replicates of four or five broilers per treatment) and on day 27 ileal digesta and right tibia were collected and pooled per digestibility unit; No significant differences were found in crude protein and crude ash digestibility. Adding the phytase (except for T5), significantly increased the total P digestibility (72.5-74.8%) compared to the PC (55.8%). Concerning the phytate P digestibility, all groups had a significantly higher digestibility (range 57.6-81.1%) compared to the PC (41.1%). Compared to the NC (57.6%) only the two highest doses (T7 and T8; 74.3% and 81.1%, respectively) were significantly higher. The tibia crude ash concentration was significantly higher for all treatments (range 39.5-45.4%) compared to the NC (36.3%), where the addition of the two highest phytase doses (T7 and T8; 43.6%) and 43.9%, respectively) no longer significantly differed from the PC (45.4%). Based on these results, adding the phytase at 1250 and 1500 FTU/kg could compensate for the decrease in available phosphorus.

"EFFECT OF NANOPARTICLE TRACE MINERALS ON REPLACEMENT OF INORGANIC TRACE MINERALS IN DIET ON GROWTH PERFORMANCE, IMMUNE STATUS AND ECONOMICS OF BROILERS"

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The supplementation of mineral in Nano form increases bioavailability and efficiency of utilization. In perspective to this study was performed to evaluate the effect of nanoparticles supplementation in diet on growth, immune status and economics of broiler chicken. Three hundred straight run day-old chicks were randomly distributed into three treatment groups. Each treatment had four replicates with twenty-five chicks in each. The three treatments were, control group (T1), basal diet replacing inorganic trace minerals + Nanoparticles @ 0.500 kg/ton of feed (T2), Basal diet replacing inorganic trace minerals + Nanoparticles @ 1 kg/ton of (T3). Birds from group T3 receiving diet with trace mineral nanoparticles @ 1kg per ton of feed recorded the highest live body weights at the end of sixth week. However, cumulative feed consumption was significantly (P<0.05) reduced in treatment group T3 as compared to control group. Thus, treatment groups T2 and T3 recorded not significantly different but numerically better cumulative feed conversion ratio than control group T1. EPEF indices in treatment groups T2 and T3 showed superior performances than control group. The ND titers at the end of 3rd week in treatment group T3 was significantly (P<0.05) reduced as compared to control group. However, at 6th week, IBD titer was significantly reduced in treatment groups T2 and T3 compared to T1. The net profit rupees per kg live weight in treatment groups T2 and T3 were increased by 7.73 and 7.00 percent, respectively, over control group. Hence, study concluded that supplementation of trace mineral Nanoparticles at 0.5 and 1kg per ton of feed by replacing inorganic trace mineral in broiler diets to be useful to improve the overall performance as well as immunity and due to better growth resulted into increase in profit margin of broiler production.

PRODUCTIVE TRAITS IN BROILER CHICKENS ARE IMPACTED BY BOTH THE SOURCE AND THE LEVEL OF DIETARY TRACE MINERALS

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Traditionally, trace minerals have been supplemented in broiler chickens' diet in the form of inorganic salts, such as sulphates, to meet requirements. As inorganic (INO) trace mineral sources are considered inexpensive, diets fed to broiler chickens are formulated with trace minerals in excess of NRC requirements. However, the ionic bonds in INO trace minerals are very weak, allowing the metal ion to disassociate as it encounters water, binding up diet antagonists such as other minerals, vitamins and other nutritional components. To mitigate these negative effects new technologies e.g. organic minerals or hydroxychloride minerals, have been introduced to the poultry industry. Such minerals either on their own or in combination have higher bioavailability and therefore can be incorporated at lower inclusion levels to support maximum performance. The current study was designed to determine the efficacy of different sources and levels of Zn, Mn, Cu, and Se on productive performance of broiler chickens. There were 4 dietary treatments as: 1. positive control or INO diet with 80 ppm of Zn and Mn, 15 ppm of Cu and 0.25 ppm of Se added as INO (sulphate and selenite); 2. hydroxychloride + organic (proteinate) treatment (Hyd/PRT) with Zn, Mn, Cu and Se matching the levels of INO diet, but added as Hyd (87.5 %) + PRT (12.5 %)%); 3. organic (ORG) blend treatment (chelated to amino acids) which provided 20 ppm Zn and Mn, 2.5 ppm Cu, and 0.15 ppm Se; 4. Hyd/PRT treatment matching the trace mineral levels of T3. Each treatment was replicated 9 times with 25 male ross 308 chicks per replicate. On day 35 birds in Hyd/PRT (T2) recorded the highest BW (P < 0.001) which was higher by 2.5 % than the T3 and T4 treatments. Birds in INO treatment exhibited the highest FCR over the entire production period (1-35 d - P < 0.001). Feeding the Hyd/PRT diets (T2) improved FCR by of 3.2 points compared to INO diet (FCR 1-35 d; P < 0.01). Birds in both T3 and T4 had the lowest BW gain, feed intake and consequently FCR (1-35 d; P < 0.001), but FCR was not statistically different to that of Hyd/PRT (T2) when corrected for BW. These results indicate that replacing inorganic Zn, Mn, Cu and Se, with a blend of Hyd and PRT when supplemented at 80 ppm for Zn and Mn, 15 ppm for Cu, and 0.25 ppm for Se in broiler diets, improves BW gain and FCR. However, decreasing supplemental Zn and Mn levels to 20 ppm and Cu to 2.5 ppm either from an organic source or Hyd/PRT blend compromises FI and BW gain.

VALORISING LIMESTONE AS A DIETARY CALCIUM SOURCE ON QUAIL PRODUCTION PERFORMANCE IN THE SUDANO-GUINEAN ZONE OF CAMEROON

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Shellfish powder is one of the main calcium sources in poultry farming (Parker et al., 2020; NRC, 1994; INRA, 1989). It is relatively expensive and frequently unavailable in the northern part of Cameroon. In the aim of finding alternative solutions to this problem and valorizing a local product in poultry feed, a study has been conducted in the city of Ngaoundere to evaluate the impact of the substitution of shell powder by a local limestone powder on quail production performance. To this end, 240-day-old quail were divided into 15 batches of 16 birds. Each of the five experimental diets (T0, T1, T2, T3, and T4) was formulated on the basis of the level of substitution (0, 25, 50, 75, and 100% respectively) of shell powder by limestone powder in a basic diet was randomly distributed to 3 batches in a completely randomized design with 5 treatments repeated 3 times each. At 49 days of age, 3 males and 3 females per treatment were sacrificed. Data were collected on biochemical parameters, growth performance, carcass, and reproductive characteristics. The main results show that feed consumption increased significantly with the substitution of shellfish while live weight, weight gain, and feed conversion ratio were comparable. However, for these different parameters, a slight increase in values was noted compared to the batch without limestone powder. Carcass characteristics were generally comparable. Testicular weights were significantly higher with the 25% (6.92±0.34g) and 50% (5.89±0.60) substitution levels compared to the 0% substitution batch whose testicular weight was 3.98±0.54g. The 50% limestone substitution induced a high egg-laying rate (24.91%) compared to the control (20.04%) although the highest egg weight was induced by the control (12.05±0.97q). Association of limestone in the diet induced an increase in Haugh Unit and proportion of edible material but a reduction in shell proportion and thickness. The biochemical parameters were generally comparable between the substitution levels. Based on these results, it was concluded that limestone powder could be used not only to substitute shellfish powder, but also to improve the production performance of the quail, and the rates of 25 and 50% seem to be more suitable for growth and early breeding respectively.

EFFECT OF INCREASING A NOVEL CONSENSUS BACTERIAL 6-PHYTASE VARIANT (PHYG) DOSE LEVEL ON INSULIN LIKE GROWTH FACTOR (IGF-1), MYO-INOSITOL CONCENTRATION IN THE SERUM AND EXPRESSION OF NUTRIENT CO-TRANSPORTER GENES IN THE SMALL INTESTINE IN BROILER CHICKENS

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This study evaluated the effects of increasing dose level of a novel consensus bacterial 6-phytase variant (PhyG) on insulin like growth factor (IGF-1), myoinositol (MI) concentration in the serum and expression of nutrient co-transporter genes in the small intestine in broilers. Five treatments were tested including: a positive control (PC) diet adequate in Ca and available P, a negative control (NC) diet deficient in Ca (-0.21%) and available P (-0.21%), the NC supplemented with PhyG at 500, 1000 and 4000 FTU/kg respectively. Ross 308 male broilers were randomized allocated to 50 cages, with 10 cages (2 birds/cage) per treatment. Birds had ad libitum accesses to water and corn and soybean meal-based starter (1-14 d) and grower (15-28 d) feed (pelleted at 82oC for 40 seconds). Body weight (BW), feed intake and feed conversion ratio (FCR) were measured per phase. Blood samples were collected at 28 d for analysis of insulin like growth factor (IGF-1) and myo-inositol in the serum. Tibia was collected at 28 d. The composite small intestinal tissues collected at 28 d were analysed for expression of the nutrient cotransporter solute-carrier-family-2-member-5 (SLC2A5) gene by the $2-\Delta\Delta$ Ct method, using a polymerase chain reaction and expressed relative to the PC group. Data were analysed with cages as the experimental units in a GLM. Curve fitting was performed to evaluate the linear or exponential responses of increasing phytase dose. Increasing phytase dose from 0 (NC) to 4000 FTU/kg exponentially (P<0.05) improved 28 d BW (from 1499 to 1635 g), overall 1-28d FCR (from 1.33 to 1.26), tibia ash (from 43.4 to 46.3%), IGF-1 (from 11.8 to 13.0 ng/mL) and MI (from 195 to 290 nmol/mL) in the serum and expression of nutrient transporter gene (SLC2A5) in the small intestine. The increase in plasma myo-inositol may indicate a more complete phytate degradation by PhyG (potentially together with the function of intestinal alkaline phosphatase to break down the lower esters such as IP2 and IP1). On comparison to PC, the NC group reduced (P<0.05) BW, ADFI and increased FCR, PhyG supplementation at >500 FTU/kg improved BW and FCR. vs. PC. At 4000 FTU/kg, expression of nutrient transporter gene increased (P<0.05) compared to the PC group. It can be concluded that increasing PhyG dose to 4000 FTU/kg improved performance which is related to complete phytate degradation that reduced anti-nutritional effect of phytate and increased nutrient absorption.

EFFECT OF REPLACING MCP BY CALCIUM HUMOPHOSPHATE ON THE GROWTH PERFORMANCE OF BROILER CHICKENS

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Phosphorus (P) and calcium (Ca) are essential nutrients, involved in physiologic and metabolic pathways in poultry. One of the mostly used inorganic feed phosphate sources available on European market for broilers is monocalcium phosphate (MCP; P=22.7%; Ca=15-17%). Calcium humophosphate (CHP; P=21.5%; Ca=15%), is a newly patented phosphate molecule that is obtained by chemical reaction of a calcium source, humic substances and purified phosphoric acid. A broiler experiment was conducted to evaluate the effect of replacing MCP by CHP on the growth performance. A total of 528 male Ross 308 broilers were randomly distributed along 24 floor pens. Pens were allocated to three different dietary treatments (8 pen-replicates/treatment, 22 birds/pen) in a randomized block design. The experiment had three feeding phases: starter (D0-11), grower (D11-27), and finisher (D27-35). Treatment 1 (T1) contained MCP (at 0.918%; 0.458%; 0.308% for each phase respectively), whereas treatments 2 (T2) and 3 (T3) contained CHP fed at different regimes. In T2, CHP incorporation was set in all phases at 85% of MCP incorporation. In T3, CHP incorporation was set at 100% of MCP incorporation in starter and 85% for grower and finisher. All experimental diets were wheat-corn-SBM based and supplemented with 500 FTU/kg phytase and NSPases. In each treatment, total P was 9.0; 7.0 and 6.3 g/kg for starter, grower and finisher phases respectively. The analysed response parameters were production performance, mortality, litter score, and footpad dermatitis. Statistical analysis was carried out with GenStat (20th edition). Treatment means were compared by least significant difference, using Fisher's post-hoc test. After outlier removal, production performance was analysed with ANOVA. Results showed no differences (P>0.05) neither on the growth performance, among dietary treatments during all feed phases, nor other parameters. Based on these results, MCP can be replaced by CHP, as T2 and T3 maintained growth performance while decreasing phosphate incorporation compared to Control treatment. This could be explained by a better feed efficiency (to be tested on suboptimal P level), which may be improved by chelation properties of CHP specific molecular complex, on antinutritional factors (deserving further research). To conclude, Calcium humophosphate incorporation allows to optimize feed formula and respond to market evolution regarding sustainability challenge in broiler production.

THE EFFECT OF DIETARY CALCIUM AND PHOSPHOROUS LEVELS DURING PULLET PHASE ON THE EGG PRODUCTION PERFORMANCE AND EGGSHELL QUALITY IN TWO STRAINS OF BROWN LAYERS

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Feeding high levels of calcium (Ca) and phosphorous (P) to avoid issues related to bone development could have detrimental effects on nutrient absorption and unnecessarily increase feed costs. A total of 288 day-old brown pullets of strain A and B were distributed into 3 dietary treatments with eight replications in a randomized complete block design to test the impact of different rearing Ca/P levels on production performance and eggshell guality. The standard diet (ST) contained analyzed Ca of 9.00, 7.60, 6.70 %, and available P of 4.5, 4.2, 3.8 %, the Low diet contained analyzed Ca of 6.75, 5.93, 5.29 %, and available P of 4.1, 3.8, 3.4 %; and Very Low (Vlow) diet contained analyzed Ca of 5.85, 5.17, 5.03 %, and available P of 4.1, 3.8, 3.4 %, respectively for 0-3, 4-10, and 11- 16 WOA. At the end of each feeding phase, two birds/cage were euthanized to collect the tibia and other tissue analyses. After completing the rearing phase, birds were fed the same diet and allocated in individual cages to track down the effect during the laying phase. Tibia breaking strength was higher in ST than Vlow at 2 and 10 WOA (P < 0.05), while there were no differences among the treatments in tibia strength at 16, 25, and 49 WOA (P > 0.05). Egg production rate (EP), egg weight (EW), egg mass (EM) and feed conversion ratio (FCR) were not affected by the diets during the onset of lay to 25 WOA (P > 0.05). During 25-50 WOA, the effect of the diets was dependent on the strain in which A layers in the ST group had higher EM than Low and Vlow (P = 0.0437). B birds in the ST group had higher EM and lower FCR than B birds in Low and Vlow during 50-75 WOA, while A did not show any response (P > 0.05). The eggshell breaking strength was higher when the birds were fed a Vlow diet during their pullet phase than ST during 50-75 WOA (P <0.001); however, there was no effect of dietary treatments during 25-50 WOA on eggshell breaking strength (P > 0.05). The main effect of the strain showed A birds had stronger eggshells, better FCR, higher EW with lower EP, and more incidence of shellless eggs than B birds. In conclusion, the impact of pullet feeding strategy is dependent on the breeds, and as the hen ages, there is more chance of emerging any carryover effect. Feeding pullets with Vlow diets improved the eggshell quality after 50 WOA, while the effect of this feeding strategy appeared to be negative on EW and EM after 50 WOA; thus, it has to be further studied.

COMPARATIVE EFFICACY OF FIVE COMMERCIAL PHYTASES ON GROWTH PERFORMANCE, PHYTATE DEGRADATION, NUTRIENT DIGESTIBILITY, AND BONE QUALITY IN BROILERS

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The efficacy of 5 commercial phytases on growth performance, nutrient digestibility, and bone quality in broilers were evaluated. Cobb 500 as-hatch chicks (31 birds \times 13 floor pen replicates per diet) were assigned to five diets. A basal diet based on corn, wheat, SBM, rapeseed meal, and sunflower was formulated to have 2,866, 2,971, 3028, and 3057 kcal/kg of ME; 1.18, 1.07, 0.97, and 0.90% of dig Lys; 0.70, 0.63, 0.54, and 0.53% of Ca; and 0.17, 0.16, 0.15, and 0.14% dig P (inorganic P-free) at each of starter (0-10d), grower (11-21d), finisher 1 (22-35d) and finisher 2 (36-42d), respectively. The five tested diets were the basal diets supplemented with either 1) a novel consensus bacterial 6-phytase variant expressed in Trichoderma reesei (PhyG), 2) a Citrobacter braakii phytase expressed in Aspergillus oryzae (PhyC), 3) a hybrid phytase expressed in Aspergillus niger E. (PhyH), 4) an E. coli phytase expressed in Trichoderma reesei (PhyE1), or 5) another E. coli phytase expressed in Pichia pastoris (PhyE2) at 1,000 FTU/kg in starter and grower diets, 750 FTU/kg in finisher 1 diets, and 500 FTU/kg in finisher 2 diets. Diets were pelleted and fed ad lib. Data were analyzed using JMP 15.1 and treatment means were separated by Tukey HSD test. The d 42 BW was higher (P<0.05) for PhyG (2,837 g) vs PhyC (2,696 g) and PhyE2 (2,675 g), while PhyE1 (2,744 g) and PhyH (2,741 g) were intermediate. The d1-42 FCR was lowered (P<0.05) for PhyG (1.636) vs all other phytases (PhyE1 (1.678), PhyH, (1.684), PhyE2 (1.711) and PhyC (1.711)), while PhyE1 reduced 1-42d FCR vs PhyC and PhyE2. Digestibility of sum of myo-inositol hexa-, penta-, tetra-, triphosphates-P was greater (P<0.05) for PhyG (70.9%) vs PhyE2 (48.3%) and intermediate in PhyE1 (60.7%), PhyH (61%), and PhyC (57.8%) (P<0.001). The d21 P digestibility and d42 tibia breaking strength were higher (P<0.05) for PhyG (71.5% and 51.3 kgF) than all other phytases (PhyE1 (67% and 47.7 kgF), PhyH (67.1% and 47.1 kgF), PhyC (65.7% and 44.4 kgF), and PhyE2 (64% and 44.8 kgF)). Feed cost, USD / BWG, kg was lowered (P<0.05) for PhyG (0.411) vs PhyC (0.429) and PhyE3 (0.425), while PhyE1 (0.418) and PhyE2 (0.42) were intermediate. In consideration to the d42 BW and d1-42 FCR objectives for Cobb 500 (2,952 g and 1.61, respectively), PhyG proved efficient in alleviating the nutrient and energy reduction and restore broilers growth performance, vs other evaluated commercial phytases.
EFFECT OF METHIONINE SOURCE (DL-MET OR HMTBA) AND TRACE MINERAL SOURCE ON GROWTH PERFORMANCE AND GUT HEALTH IN BROILER CHICKENS

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HMTBA (2-hydroxy 4 methylthio butanoic acid) has been shown to bring benefits in gut health and heat stress, while the use of mineral methionine hydroxy analog chelate (MMHAC; MINTREX®) has proven to decrease mineral excretion and improve food pad quality and gut health. Therefore, a pen study was conducted to evaluate the replacement of DL-Met, inorganic trace minerals (ITM) and calcium butyrate for HMTBA and reduced levels of MMHAC on grow performance under a mild gut challenge. A total of 26,240 as hatched Ross 308 broilers were assigned to one of the 4 treatments with 8 replicates per treatment, 820 birds per replicate, in a Randomized Complete Block Design. The experiment consisted in a 2*2 factorial arrangement with main factors as methionine sources (DL-Met vs HMTBA), and ITM (Zn, Cu, and Mn), plus a source of 500g/MT calcium butyrate (ITMCB) vs same trace minerals as MMHAC. Levels of ITM include a reduction from starter (0-10d), Zn: Cu: Mn, (90:10:120) to finisher phase (28-38d) (70:10:90), while the MMHAC contained the same levels (50:10:50) across the 3 phases. Birds received a wheat-soybean meal basal diet throughout the 38 days of grow out, following CVB recommendations. Body weight, feed intake, feed conversion ratio, and mortality were determined on d 10, 28, and 38. Litter quality and food pad scores were measured at 23 and 35d. To create a mild gut challenge, the diets did not contain xylanase. Data were subject to ANOVA and means were separated by Fisher's post hoc LSD test with significance at P < 0.05. No significant differences were observed in broiler performance, nor litter quality, at 10, 28 and 38d of age. However, an interaction trend (P=0.092) showed that when DL-Met was used, the ITMCB treatment produced numerically greater BW than the MMHAC treatment. This trend might be explained by overall higher feed intake (P=0.032). Food pad dermatitis scores on day 38 tended (P=0.072) to be lower in birds fed ITMCB compared with the MMHAC group. HMTBA improved FCR during the starter phase and tended to reduce food pad lesions on 35d compared to DL-Met. Moreover, the MMHAC tended to improve BW uniformity on day 10, although the effect disappeared during the grower and finisher phase. Results from this study suggest that the use of DL-Met, with ITMCB can be replaced by HMTBA in combination with low inclusion of MMHAC -Zn, Cu and Mn, and the economic response might be the decision driver.

CUPPER REQUIREMENTS OF BROILERS SUPPLEMENTED WITH OR WITHOUT PHYTASE

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Phytic acid is strongly negatively charged at physiological pH values indicating a tremendous potential for complexing positively charged molecules, such as copper (Cu). The objective of the present study was to reassess Cu requirements for broilers fed diets supplemented or not with phytase at a concentration known to break most of phosphate groups out of phytate (2,500 FTU per kg). A total of 560 d-old Cobb male broilers were randomly distributed in battery cages (0.9 \times 0.4 m²) and assigned to 10 treatments with 8 replicates. A basal corn-soybean mealbased control diet was formulated (8.3 ppm analyzed Cu). Additional treatments contained 3, 6, 9, and 12 ppm of added Cu from sulfate pentahydrate (CuSO45H2O) with or without phytase supplementation. Performance was evaluated at 14, 21, and 28 d. On d 28, locomotor disorders were evaluated, and all birds were euthanized to kidney, liver, Pectoralis major muscles, gastrocnemius tendons collection. Samples of feed and ileal digesta were analyzed to determine the ileal digestible energy (IDE). Data were analyzed using the SAS MIXED proc and means were separated using Tukey test with statistical significance considered $P \leq 0.05$. Broiler responses were adjusted to the quadratic (QP) and linear (L) polynomial models. The analyzed Cu levels in the experimental diets with phytase were 8.3, 11.4, 13.8, 16.6 and 19.9 ppm Cu with corresponding 2752, 3200, 2831, 2446, 2947; and 7.7, 11.1, 14.6, 16.5 and 19.0 of analyzed Cu levels when fed no phytase. No effects of increased Cu levels were observed for factorial analysis of cumulative growth performance of broilers from 8 to 28 d (P > 0.05). However, phytase supplementation presented higher growth performance during all the experimental periods (P < 0.05). Treatments with phytase inclusion presented higher IDE (P < 0.05). Dietary Cu did not influence (P > 0.05) hematocrit, hemoglobin, locomotor disorders, Cu breast content as well as in tendons and kidney. Liver Cu increased linearly (P < 0.05, $R^2=0.352$) as the dietary level of Cu increased. Data from the present study suggest that dietary supplementation of Cu for broilers from 1 to 28 d of age is needed in average lower amounts than what is commercially used. However, phytase supplementation is essential to achieve maximum growth rate.

SUPPLEMENTATION OF A NOVEL CONSENSUS BACTERIAL 6-PHYTASE VARIANT TO AN ALL-PLANT-BASED DIET WITHOUT ADDED INORGANIC PHOSPHORUS MAINTAINED TIBIA ASH AND PERFORMANCE IN BROILERS RAISED UNDER A COMMERCIAL PRODUCTION SETTING

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The effect of a novel consensus bacterial 6-phytase variant (PhyG), administered alone or in combination with xylanase, on growth performance, tibia ash content and bone strength was evaluated from 1 to 37 d of age in broilers fed all-plantbased diets containing no added inorganic phosphate (Pi) and raised under a commercial production setting. A total of 26,240 mixed-sex Ross 308 one-day-old broilers were assigned to pens (820 birds/pen, 8 pens/treatment, stocking density at 37 d of age ~37.7 kg/m2) in a randomized complete block design with four treatments, comprising 1) a nutritionally adequate diet containing Pi from monocalcium phosphate (PC1); 2) PC1 reduced in metabolizable energy (ME) (-75 kcal/kg) and supplemented with 2,000 XU/kg xylanase (PC2); 3) and 4) PC1 and PC2, respectively, reformulated without added Pi, reduced in calcium (-0.15 percentage units, % unit), and supplemented with PhyG at 3,000 FTU/kg in starter [d 1 to 10, 0.33% phytate-P (PP), retainable P -0.23% unit vs. PC], 2,000 FTU/kg in grower (d 10 – 22, 0.31% PP, ret P -0.16% unit vs. PC), and 1,000 FTU/kg in finisher (d 22-37, 0.26% PP, ret P -0.13% unit vs. PC) phase (IPF1 and IPF2). Data were analyzed by two-way ANOVA. No interaction was observed between phytase and xylanase for any measured parameter. During all growth phases and cumulatively, phytase added to the Pi-free diets, with or without xylanase, maintained final body weight (BW) and feed conversion ratio (FCR) relative to PC diets, except during 22 to 37 d and 1-37 d of age when FCR was improved (-5.7 and -3.4 points, respectively vs. PC, P < 0.05). Feed and water intake were reduced in IPF treatments during grower and finisher phases (-2.7 to -4.5% vs. PC; P < 0.05) but water-to-feed intake ratio was unaffected. Phytase in Pi-free diets, with or without xylanase, maintained tibia ash and breaking strength at 21 and 36 d of age, except for breaking strength which was higher with phytase at d21. Compared to the PC, phytase supplementation in diet completely free of Pi reduced the total 1 to 37 d feed costs per kg BW gain. The data demonstrated that Pi can be totally replaced from diet by using PhyG during all growth phases under a commercial production setting. If implemented in practice, Pi-replacement by phytase has potential to improve the sustainability of commercial poultry production.

SUPPLEMENTATION OF SULFUR AND FOLIC ACID IMPROVES PERFORMANCE OF BROILER CHICKEN FED SUB-OPTIMAL CONCENTRATIONS OF DIETARY METHIONINE

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Supplementation of sulfur (S) and folic acid (FA) spares the dietary requirement of methionine (Met) for methyl donor and other metabolic functions of in chicken. An experiment was conducted to determine the effects of feeding diets supplemented graded concentrations of FA and inorganic S on performance, slaughter and anti-oxidant variables, immune responses and serum protein fractions in broiler chicken. FA was supplemented at 0, 2, 4, 6, 8 mg/kg alone or in combination with S (2 g/kg) in basal diet (BD) containing no supplemental Met and FA. Standard diet having the recommended concentration of Met was used as the control diet. Each diet was offered to 10 pens of 20 male chicks each and fed ad libitum from day 1 to 42. The data were analysed by nesting of the two-way structure within the grouping of control versus treatments. The broilers fed low Met BD had lower weight gain, high FCR, lesser ready to cook yield, higher lipid peroxidation (LP) and reduced concentrations of total protein and albumin in serum. The body weight gain (BWG) improved with FA at d 21 and a trend of improvement at d 42 was observed. Similarly, the BWG improved with S supplementation at d 42. Breast meat weight showed a trend of improvement with S supplementation compared to those fed FA alone. The FA and S interaction indicated significant reduction in LP with S supplementation at all the concentrations of FA. The serum protein fractions increased with increased FA content in the diet and the concentrations further increased with S supplementation. Based on the results, it is concluded that the combination of FA (2-8 mg/kg) and S (2 g/kg) improved the performance and breast meat weight in broiler chicken fed diets without supplemental Met. The improvement was probably mediated through the increase in protein metabolism and reduction in lipid peroxidation compared to those fed the low Met basal diet.

EFFECT OF A SYNERGISTIC BLEND OF ORGANIC ACIDS IN COMBINATION WITH HYDROXY COPPER CHLORIDE ON PERFORMANCE AND HEALTH OF BROILERS

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The increased pressure to phase out the use of antibiotic growth promoters (AGP) has stimulated interest in using alternatives. Several additives when combined exert a synergistic and stronger effect on the performance and health of animals. This study evaluated the effects of a synergistic blend of short and medium-chain fatty acids including butyrates and a phenolic compound (PFY) in combination with hydroxy copper chloride (HyC) on growth, footpad health, and the economics of broilers. Cobb broiler chicks (n=840) were assigned to one of five dietary treatments with 12 replicates of 14 birds each. The treatments were (1) NC, an AGP-free diet with 15 ppm CuSO4, (2) AGP, as NC with 50 ppm zinc bacitracin, (3) HyC, an AGP-free diet with 200 ppm HyC, (4) PFY, as NC with PFY, and (5) PFY+HyC, an AGP-free diet with PFY and HyC. Birds were reared in floor pens under high stocking density with 14 birds/m2 and a house temperature of 32°C from d1 to 31 then at 28°C till d42. Data were analyzed using the GLM procedure in SAS. In the starter period, birds supplemented with AGP and HyC had the highest feed intake compared with those given other treatments (P<0.05). During the succeeding phases, however, no significant effect was observed (P>0.05). The BW gain was higher for AGP and HyC during the starter period and for PFY+HyC during the last phase. AGP and HyC treatments had the lowest FCR during the starter period, whereas PFY+HyC had the lowest from growing to finishing periods (P<0.05). Overall, the feed additives either alone or in combination showed a similar effect as AGP on growth performance, livability, and broiler index (P>0.05). Compared with NC, ADG increased with PFY+HyC (+4.6%), while the FCR was reduced with HyC (-2.5%), PFY (-2.0%), and PFY+HyC (-4.2%)supplementation (P < 0.05). The feed cost per kilogram liveweight for treatments with additives was lower (USD0.01-0.02) compared with the NC. The birds supplemented with PFY or HyC as a single product had healthy footpads compared with those supplemented with AGP (P<0.05). This study demonstrated that PFY+HyC supplementation in broilers reared under stressful environmental conditions improved growth performance and cost-benefit. The application of PFY or HyC alone maintained growth performance similar to AGP and can potentially enhance the FCR of broilers compared with NC. In addition, the use of either product promoted good footpad integrity.

INFLUENCE OF DIETARY OMEGA-3 FATTY ACIDS ON BONE QUALITY IN EGG- AND MEAT-TYPE POULTRY: A META-ANALYSIS

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Poultry egg and meat production continue to be optimized for productivity and efficiency through genetic selection. However, modern birds are still prone to leg weakness, osteoporosis and, subsequently, fractures – despite genetic selection programs. Dietary omega-3 polyunsaturated fatty acid (n-3 FA) enrichment has been proposed to benefit bone development, quality, and strength; however, there are no conclusive and quantitative results across studies. Therefore, a metaanalysis approach was used to evaluate published studies to determine the effects of dietary n-3 PUFA enrichment on bone quality in laying and meat type birds. Publications were retrieved from multiple sources, and ten studies were selected for inclusion in the dataset. A model to predict bone ash content (BAC) was developed in Proc MIXED of SAS, treating the study as a random effect. The dietary concentration of n-3 PUFA, n-3 PUFA: n-6 PUFA ratio, calcium (Ca), phosphorus (P), feeding duration (day) and were used as independent variables to predict tibial bone ash content (BAC). The final model included n-6: n-3 ratio and calcium concentration in the diet. The model was selected based on corrected Akaike information criteria and evaluated using the root mean square prediction error (0.999) and its components and concordance correlation coefficient (0.99). The model for laying type birds was as follows: BAC (%) = $44.01 (\pm 5.229) - [0.03]$ $(\pm 0.49) \times N6:N3] - [3.26 (\pm 1.558) \times Ca (\%)]$. In laying type birds, BAC was reduced (P = 0.001) by n - 3 PUFA but was increased by Ca (P = 0.014). The model for meat type was as follows: BAC (%) = $44.01(\pm 5.229) - [0.25(\pm 0.060)]$ x N6:N3] – $[0.07 (\pm 2.780) \times Ca (\%)]$. In contrast, in broiler chickens BAC was increased by n-3 PUFA (P = 0.001) and decreased (P = 0.014) by Ca. The influence of n-3 PUFA: n-6 PUFA ratio on tibia BAC in laying type birds was not statistically significant from zero (P=0.505), whereas, in meat type birds, the influence of PUFA ratio was significantly different from zero (P<0.05). These results may indicate a low biological significance in laying type birds but not in meat type birds.

EFFECT OF HYDROXY-SELENOMETHIONINE ON BROILER BREEDERS PERFORMANCE

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Selenium (Se) is an essential micronutrient for birds, then the deficiency of this nutrient can affect broiler breeder's production and reproduction. The quality of chicks depends on the composition of the egg. Selenium as an antioxidant in animal metabolism could improve the embryos development and hatchability. The aim of this study was to evaluate the egg production and hatchability of eggs of broiler breeders, fed with organic or inorganic Se, in the second production cycle. A total of 600 broiler breeders (Cobb 500) in the second production cycle were fed diets supplemented by: 0 ppm of Se or 0.3 ppm hydroxy-selenomethionine or 0.3 ppm sodium selenite with 10 repetitions of 20 birds. The trial lasted 12 weeks, divided into three cycles of 28 days each. Egg production was counted daily. At week 12, 1050 eggs were collected for 8 consecutive days and stored in a climate room (19°C and 60% RH). The eggs were distributed in a hatching machine (CASP) in 10 replicates of 35 eggs according to dietary treatments. During 18 days of incubation, it was controlled the temperature of 99.5° F in the dry bulb and 85° F in the wet bulb, a ventilation of 1.1 m^3 / s, and the eggs were turned every hour. At 18 days, the eggs were transferred to the hatcher and kept at temperature of 98.9° F in the dry bulb and 86.5° F in the wet bulb, and ventilation of 0.5m³ / s. At 21 d, hatched chicks and fertility were measured. Hatchability was calculated considering the number of hatched chicks by the number of fertile eggs. Differences were tested by means of one-way ANOVA, followed by Duncan post hoc test (p < 0.05). Significant difference (p < 0.05) in egg production was observed only in the third cycle. The egg production of birds fed 0.3 ppm hydroxyselenomethionine was significantly higher (57.91%) than those without selenium supplementation (52.86%). However, the diet with 0.3 ppm sodium selenite (54.70%), no significant difference was observed. No difference was observed between source for fertility. The hatchability of eggs from broiler breeders supplemented with hydroxy-selenomethionine (91.32%) was significantly higher than sodium selenite and no supplemented (87.06 and 85.20%, respectively). In conclusion, hydroxy-selenomethionine showed to positively influence egg production and in particular hatchability in eggs of broiler breeders.

THE COMPARATIVE EFFICACY OF TWO NEW PHYTASES ON BONE ASH AND PERFORMANCE IN 14-DAY OLD BROILERS.

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Phytases are supplemented to poultry diets to hydrolyse phytate, improve digestibility of P and thereby decrease P excretion and feed cost. It has been shown that the in vivo efficacy can differ between 6-phytases, leading to differences in the slope and asymptote in the response in digestible P with increasing phytase dose. The objective of this study was to compare the efficacy of a novel consensus bacterial 6-phytase variant expressed in Trichoderma reesei (PhyG), against an E. coli phytase expressed in Komatagaella phaffii (PhyE) on 14-day old broiler bone ash and performance (body weight (BW), body weight gain (BWG), and mortality adjusted feed conversion (FCRm). A total of 1440, 4-day old Ross 308 male broilers were randomly assigned to one of 14 treatments (16 birds/cage, 6 replicates for treatments 2-14 and 12 replicates for the negative control (NC)). A mash cornsoy based NC diet (3.4 g/kg P and 7.0g/kg Ca) was supplemented with each phytase product based on analysed phytase units (FTU) at the five dose levels (500, 1000, 1800, 2500 and 3500 FTU/kg) to form a dose response which was compared against three positive control (PC) diets that contained incremental amounts of monodicalcium phosphate. PC1: (2.70 g/kg rP (retainable P), 4.88 g/kg P & 5.98 g/kg Ca), PC2: (3.00 g/kg rP, 5.24g/kg P & 6.82g/kg Ca) and PC3: (3.1 g/kg rP, 5.6 g/kg P & 7.2 g/kg Ca). BW, BWG and FCRm were determined from 4-14 days. On day 14, eight broilers/cage were sampled, the right tibia removed, pooled by cage, and defatted tibia bone ash weight and content were determined. Bone ash, BW, BWG and FCRm were plotted against analysed phytase dose using Genstat 18th edition to compare the slope and asymptote parameter estimates in the nonlinear equation: $y=a+b*c^x$. The asymptote of the response to PhyG vs. PhyE phytase was significantly greater for bone ash (47.14% vs. 45.51%); BW (390.9 vs 372.7 g); BWG (218.1 vs 201.3 g) and FCRm (1.38 vs 1.48). Similarly, PhyG phytase exhibited a steeper gradient for all three performance parameters measured. Based on the exponential models obtained PhyG exhibited equivalency values to that of the PC3 diet at predicted values of 2050 FTU/kg for BW and 600 FTU/kg for FCRm, whereas PhyE was unable to match BW and FCR of the PC irrespective of dose. The results from this study suggest large differences in the in-vivo efficacy of newly developed phytases based on differences in the gradient and asymptote of the response in bone ash, BW, and FCRm.

26th World's Poultry Congress, abstracts selected in 2022

Molecular genetics

Selected short communications

RNA-SEQ DATA FOR RELIABLE SNP DETECTION AND GENOTYPE CALLING IN LIVESTOCK SPECIES: INTEREST FOR CODING VARIANT CHARACTERIZATION AND CIS-REGULATION ANALYSIS BY ALLELE-SPECIFIC EXPRESSION.

F. Jehl (1), F. Dégalez (2), M. Bernard (3), F. Lecerf (2), L. Lagoutte (4), O.
Bouchez (5), B. Abasht (6), M. Tixier-Boichard (7), B. Bed'hom (7), T. Burlot (8),
D. Gourichon (9), T. Zerjal (7), F. Pitel (10), C. Klopp (11), S. Lagarrigue*(12)

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In addition to their common usages for studying gene expression, RNA-seg data that have accumulated over the last 10 years are a yet-unexploited resource of SNPs in numerous individuals from different populations. SNP detection by RNA-seq is particularly interesting for livestock species since whole genome sequencing is expensive and tools for whole exome sequencing are unavailable. These SNPs detected in expressed regions can be used to characterize variants affecting protein functions, and to study cis-regulated genes by analyzing allele-specific expression (ASE). However, gene expression can be highly variable, and filters for SNP detection using the popular GATK tool are not yet standardized, making SNP detection and genotype calling by RNA-seq a challenging endeavor. We compared SNP calling results on two independent chicken populations (n = 15 & n = 8) for which both RNA-seq and DNA-seq data was available for the same samples. The RNA-seq SNP detection was performed with GATK RNA-seg specific suggested filters. We showed that, in expressed regions, 91% of SNPs detected by RNA-seg were also detected by DNAseq (at 20X coverage); we characterized the remaining 9% SNPs. We then studied the impact of two factors (genotype (GT) call-rate and read number supporting the GT) on the concordance of genotypes detected by DNA-seq versus RNA-seq and proposed thresholds for them that led to a 95% concordance. Applying these filters to 744 RNA-seq samples collected on 10 chicken populations, we found 9.9M SNPs in total, with on average 560,000 SNPs per population with a GT call rate \geq 50% and 340,000 with a MAF \geq 10%. We then showed that, such RNA-seq data can be used for i) detecting SNPs with a severe predicted impact on proteins, despite their rarity in each population (more than 17,000 SNPs), and ii) studying, on a large scale, cis-regulations of gene expression by using the ASE approach: in particular we showed that on average 80% of protein-coding and 70% of long noncoding genes with an expression level \geq 1TPM could be analyzed for ASE. Finally, we illustrated the possibility to perform population genetic analysis using such SNPs detected exclusively in expressed regions, as an alternative set to the standard low- or high-density SNP genotyping arrays. This work shows that RNA-seq data can be used with good confidence to detect SNPs and associated GT within various populations and used them for different types of analysis.

EFFICIENT AND ACCURATE IDENTIFICATION OF CRISPR-INDUCED MUTATIONS BY AMPLICON SEQUENCING

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CRISPR gene editing technology is accelerating animal genetics and breeding. The identification of desired mutations is one of the most important processes to assess the effect of gene editing. Several methods were developed to identify desired mutations, among which T7E1 digestion and Sanger sequencing are widely used. However, due to their self-existing defects, they may no longer meet the evolving requirements of CRISPR biology. Nowadays, the development of next-generation sequencing technology makes it possible for developing a gene mutation identification method with high efficiency and low cost. Thus, a method was developed to efficiently and accurately identify CRISPR-induced mutations based on amplicon sequencing. Experimental design. Our method consisted of the following steps: (1) amplified targeted areas of genome DNA using pair-end barcode primers; (2) constructed a PCR-free amplicon library; (3) sequenced amplicons on NovaSeq 6000; (4) split the amplicon sequence data according to barcodes using AdapterRemoval; (5) identified CRISPR-induced mutations, analyzed gene editing efficiency and visualized results using CRISPResso2. In addition, a shell script was written to standardize and automate the analysis. All the advantages of this method were demonstrated by: (1) performed on tyrosinase knockout DF-1 cells to compare with traditional ones; (2) performed on tyrosinase knockout chimeric chicken tissue samples to test under actual conditions. Results and discussion. This method was more accurate and sensitive than traditional methods. It was able to identify all types of mutations including deletions, insertions and substitutions. Meanwhile, this method was cost efficient. The method typically cost less than \$1.50 / sample and had a processing time of less than 30 sec / sample on a general office computer. In the actual condition test, this method was able to identify CRISPR-induced mutations. The gene editing efficiency was 4.55±1.9712% for chimera chicken and 0.03±0.0087% for control, with a well-presented mutation pattern. The false positives ware relatively low, and the resolution was very high. Conclusions. A CRISPR-induced mutation identification method based on amplicon sequencing was developed. This method is superior to traditional methods, more accurate, more sensitive, more economical, and easy to use.

SELECTIVE SWEEP MAPPING IN THE R+ R- CHICKEN LINES DIVERGENTLY SELECTED FOR RFI (RESIDUAL FEED INTAKE).

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Intense artificial selection of populations leads to the acceleration of evolutionary processes and often results in extreme phenotypes with associated changes across the genome. Selective-sweep mapping makes it possible to identify the genomic regions where allele-frequencies have moved after selection. In this project we performed a selective-sweep mapping in two experimental chicken lines, developed from a common Rhode Island Red base population, by long term divergent selection (40 years) for high (R+) and low (R-) residual food intake, an indicator of feed efficiency. High-density genotyping was performed using the 580K Affymetrix® Axiom® HD array on 96 animals per line sampled at three different generations: the 18th, the 25th and the 40th generation of selection. Such an interval was shown by simulation to be the optimal solution to infer the presence of selection allowing to characterize the dynamic of selection. From the 580 961 SNPs genotyped 326 004 were informative. A filtering was applied to keep only the SNPs with data missing <10 and a MAF> 0.05, leading to 255 103 SNPs suitable for analysis. We looked for genomic signatures of positive selection using the HapFLK method, which focuses on the differences of haplotype frequencies between populations, and retained those for which the differentiation was too large to result from a neutral evolution model. In total 5 regions were detected, at a FDR of 15%, on GGA1, GGA2, GGA6, GGA7 and GGA11 and their size varied between 60kb and 3Mb. When we looked at the haplotype frequency among generations for these 5 regions, we identify relatively similar selective scenarios suggesting, a divergent before the first generation (18th) analyzed in this study, and little evolution in the following generations (25th and 40th). The five selected regions contained in total 78 long non-coding genes and 77 protein coding genes. These included candidate genes involved in important functions known to differ between lines, as the modulation of innate and adaptive immune responses (WWP1, RIPK2), the mitochondrial activity (ATP6V0D2, TFAM, CISD1) and physiological processes such as respiration, acid-base balance, bone resorption, calcification metabolism (CA2, CA13) and fatty acid uptake, transport, and metabolism (FABP4). Among all genes identified, the Fatty Acids Binding Proteins 4 (FABP4) gene is particularly interesting because it is mainly expressed in adipocytes and macrophages and plays an important role in the development of insulin resistance and non-alcoholic fatty liver disease, conditions that are observed in the R- line. This study was supported by the CRB-Anim (SelMaR project) and the ANR (Chickstress project).

A TRANSCRIPTOMIC ANALYSIS UNRAVEL CHICKEN GENES POTENTIALLY INVOLVED IN RESISTANCE TO HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS INFECTION

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Avian influenza viruses (AIVs) represent a severe challenge to the global economy, avian health, and human health, as highlighted in the latest European H5N8 highly pathogenic AIV (HPAIV) outbreaks. Gallinaceous birds are susceptible to HPAIVs, but the disease outcome, caused by the same viral strain, depends mainly on the host genetic background. The objective of this study was to perform a transcriptomic analysis in HPAIV resistant and susceptible chickens to underscore the host genetic mechanisms involved in the disease outcome. Chickens (Gallus gallus) were challenged with clade 2.3.4.4b H5N8 HPAIV, euthanized at day 3 post-challenge, and classified as resistant or susceptible depending on the following criteria: chickens that presented i) clinical signs, and ii) histopathological lesions consistent with HPAIV infection, and iii) presence of AIV nucleoprotein (NP) antigen in tissues were classified as susceptible, while chickens lacking all these criteria were classified as resistant. From the total challenged chickens (n=15), two of them were identified as outliers and discarded after a principal component analysis. Lung and spleen samples from resistant (n=3), susceptible (n=4), and non-infected control chickens (n=6)were used to characterize transcriptomic signatures by RNA-Seq. Differentially expressed genes (DEGs) in samples from infected chickens compared to control chickens were obtained with a Wald test and the p-value was adjusted with a Benjamini and Hochberg method (BH-adjusted p values), DEGs with a BH-adjusted p value < 0.05 were considered significant. We identified 5,873 and 5,422 DEGs in lung and spleen, respectively, from susceptible chickens compared to non-infected controls. As expected, gene ontology (GO) analysis showed an enrichment in GO terms related to Type-I interferon and inflammatory responses. In contrast, only 29 DEGS in lung and 20 DEGs in spleen were identified in resistant chickens compared to non-infected controls. Interestingly, six DEGs were downregulated in resistant birds and upregulated in susceptible birds. Some of these genes belong to the NF-kappa B (NF- κ B) and/or mitogen-activated protein kinase (MAPK) signaling pathways. Among these six genes, a serine protease-encoding gene was of particular interest, being the most significantly downregulated gene in resistant chickens. Expression levels of this protease were further validated by qPCR in a higher number of experimentally infected chickens. Altogether, our results suggest that some immunerelated pathways and genes could play a role in the disease outcome of HPAIV in chickens. An early and optimal regulation of the NF-κB pathway and the MAPK cascades could confer some resistance to HPAIV in chickens. In particular, early inactivation of important genes could prevent an exaggerated immune response and viral replication.

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Molecular genetics

Posters

AN EAV-HP INSERTION IN THE PROMOTER REGION OF SLCO1B3 HAS PLEIOTROPIC EFFECTS ON CHICKEN LIVER METABOLISM BASED ON THE TRANSCRIPTOME AND PROTEOME ANALYSIS

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Solute carrier organic anion transporter 1B3 (SLCO1B3) is an important liver primarily highly expressed gene, its encoded protein (OATP1B3) involved in the transport of multi-specific endogenous and exogenous substances. We previously reported that an EAV-HP inserted mutation (IM+) in the 5' flanking region of SLCO1B3 was the causative mutation of chicken blue eggs, and a further research showed that IM+ significantly reduced the expression of SLCO1B3 in liver. Herein, we confirmed a cholate response element (IR-1) played an important role in activating SLCO1B3 and in vitro experiments showed that the activation of IR-1 can be significantly reduced by the EAV-HP IM+. We performed transcriptome and proteomic analysis using the same set of IM+ and IM- liver tissues from Yimeng hens (a Chinese indigenous breed) to study the effect of SLCO1B3 and OATP1B3 expression reduction on chicken liver function. The results showed that common differential expression pathways were screened out from both transcriptome and proteome, in which fatty acid metabolism and the metabolism of xenobiotics by cytochrome P450 were significantly enriched in the KEGG analysis. The lipid-related metabolism was weakened in IM+ group, which was validated by serum biochemical assay. We unexpectedly found that EAV-HP fragment was highly expressed in the liver of the IM+ chickens. We cloned the EAV-HP full-length transcript and obtained the complete open reading frame. It is worth noting that there was some immune related differential expressed genes, such as NFKBIZ, NFKBIA and IL1R1L, which were higher expressed in the IM+ group, which may due to the high expression of EAV-HP. Our study showed that EAV-HP IM+ reduced the expression of SLCO1B3 in liver, resulting in the decrease of fatty metabolism and exogenous substance transport capacity. The mutation itself also expressed in the liver and may be involved in the immune process. The mechanism needs further study.

GENOME-WIDE SNP ANALYSIS OF 23 ITALIAN LOCAL CHICKEN BREEDS

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Local breeds are an important biodiversity source, especially for genetic features; indeed, those populations show unique alleles or allelic combination coding for specific traits. The local biodiversity of Italian chicken breeds was investigated through Affymetrix 600 K SNP Array. A total of 582 animals (half males and females, unrelated) from 23 local breeds and 4 commercial stocks were considered. This study aimed to assess a genome-wide comparative analysis of 23 Italian local chicken breeds, such as: Ancona Bianca di Saluzzo, Bionda Piemontese, Cornuta di Caltanissetta, Livorno Bianca, Livorno Nera, Mericanel della Brianza, Modenese, Mugellese, Ermellinata di Rovigo, Millefiori di Lonigo, Padovana Argentata, Polverara Bianca, Padovana Camosciata, Padovana Dorata, Polverara Nera, Pepoi, Robusta Lionata, Robusta Maculata, Romagnola, Siciliana, Valdarnese, and Valplatani. Regarding the four commercial stocks: Broiler Ross 708, Eureka, Hy-lyne white eggs, and ISA Brown. The analysis allowed to evaluate i) the level of genetic diversity; ii) the genetic distance among the studied breeds; iii) the runs of homozygosity. In general, the levels of genetic diversity were lower in the local breeds compared to commercial stocks. The level of genomic inbreeding, based on runs of homozygosity, was markedly different among the breeds and ranged from 0.121 (Valdarnese) to 0.607 (Siciliana). In all breeds, short runs of homozygosity (ROH) (<4 Mb in length) were more frequent than long segments. The analysis of genetic differentiation model-based clustering and neighbour networks showed that most breeds formed distinct clusters and separate populations, which indicated the gene flow events, especially among breeds that originated from the same geographical area. Investigation of ROH islands identified four regions as hotspots of autozygosity among the breeds, highlighting the presence of several candidate genes involved in morphological traits, as body weight and feed conversion ratio. This is the first exhaustive genome-wide study of Italian local chickens, that show a conserved authentic genetic pattern. The results have significant importance for designing and improving conservation strategies. Finally, the conservation of local breeds may play a role in the local economy and biodiversity as a genetic reservoir.

MOLECULAR CHARACTERIZATION OF POLYMORPHISMS OF PROLACTIN GENE AND ITS ASSOCIATION WITH PRODUCTION TRAITS IN NATIVE INDIAN BREED OF CHICKEN

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Prolactin (PRL) is a polypeptide hormone that plays a crucial role in reproductive events of chicken and is directly associated with the onset of broodiness and eqg production. The avian PRL gene is highly conserved, located on chromosome number 2 and most sequence polymorphisms occur in the exon 5', 3' flanking region, and the coding region. In poultry, traditional selection approaches based on phenotypic performance have successfully improved production levels and the response has reached a plateau. Besides, selection cannot be performed at an early age in traditional selection methods. Also, it has a high generation interval with slow genetic gain. In view of these drawbacks, newer molecular technologies like marker-assisted selection (MAS) have been efficiently employed in poultry breeding over the past few years. Furthermore, studies on PRL gene polymorphism are comparatively less in indigenous breeds of chicken. Therefore, the present study was undertaken to identify single nucleotide polymorphisms (SNPs) in the PRL gene and its association with egg production traits in native chicken of Kerala (India). A total of 200 female birds (Tellicherry breed of chicken) were randomly selected from All India Co-ordinated Research Project on Poultry breeding farm, Mannuthy, Kerala, India, and genomic DNA was isolated from the venous blood. Polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis was done to identify the SNPs (C-2402T and C-2161G) at the promoter region of the PRL gene. In addition, sequence analysis was performed on the RFLP products using Sanger's di de-oxy chain termination method. Based on the RFLP patterns birds were designated into three genotypes as CC, CT, and TT and CC, CG, and GG for the SNP site C-2402T and C- 2161G, respectively. Allele and genotypic frequency were calculated, and the association between the polymorphisms of PRL gene and production traits viz. broodiness, age at sexual maturity, egg weight at 28, 32, and 40 weeks, and egg number up to 40 weeks of age were determined by one-way ANOVA using the SPSS software (Version 21.0). Association analysis of our study showed that SNP C-2402T in PRL gene was significantly associated (p < 0.05) with age at sexual maturity, egg weight and, egg number of native chickens. This result suggests that SNP C-2402T of the PRL gene could be used as a potential molecular marker in poultry selection and breeding programme for increasing egg production.

RELATIVE EXPRESSION OF GROWTH AND STRESS RELATED CANDIDATE GENES OF CROSSES PRODUCED UNDER PARTIAL DIALLEL CROSS DESIGN INVOLVING THREE GENETIC GROUPS OF CHICKEN

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A partial diallel cross design was applied to three chicken breeds viz. Coloured Synthetic Male Line (CSML), Native chicken (Desi) and Dahlem Red (CARI-Red) to produce crosses suitable for rural poultry production, b. Three purebreds viz. CSML x CSML, D x D and CR x CR; three crossbreds viz. CSML x D, CSML x CR and D x CR and a triple cross [D x (CSML x CR)] was produced. Relative m-RNA expression of growth and stress related candidate genes viz. IGF-1. IGF-1R, cGH, GHR and HSP-70 was determined for the crosses from liver tissue at 8 and 12 weeks of age using real time g-PCR technique with specific primers, 28s rRNA served as internal control gene and CR x CR was identified as control group as having lowest body weight among all. Expression of IGF-1 was non-significant at all ages. At 8 weeks, CSML x D followed by CSML x CSML, D x CR showed IGF-1 upregulation over control (CR x CR), but, D x D and CSML x CR were downregulated. Reverse trend was noticed at 12 weeks with upregulation only in CSML x CR. IGF-IR showed significant (P<0.05) upregulation in D x D and CSML x CSML at 8 weeks, while crossbreds showed downregulation. Contrarily, significant (P<0.05) upregulation of IGF-IR in D x CR (9.53 folds), triple cross (5.22 folds) and CSML x D (3.74 folds) was noticed at 12 weeks. cGH expression was significantly upregulated (P < 0.05) in D x D at 8 weeks, while downregulated in others. Significant upregulation (P<0.01) was evident in all groups except CSML x CSML at 12 weeks with highest in D x CR. GHR expression significantly lowered (P<0.01) in all groups than control at 8 weeks, while at 12 weeks, all crossbreds except CSML x D showed significant (P<0.01) GHR upregulation. GHR expression was downregulated in CSML x CSML, Desi x D and CSML x D. Expression of HSP-70 gene was significantly upregulated (P<0.05) in D x D, whereas downregulated in others at 8 weeks. At 12 weeks, HSP-70 expression was significantly higher (P<0.001) in crossbreds with CSML x CR, D x CR and triple cross, whereas downregulated in others. It is concluded that the wider genetic differences in parent breeds had considerable effect on gene expression pattern. Yet, their expression is positively correlated with body weight at 8 weeks, especially for CSML purebred and CSML x D. Expression of IGF-1 and IGF-IR showed inverse relationship. Upregulation of HSP-70 gene in Desi and its crosses indicates its resilience to heat stress.

Key words: Candidate genes, Chicken, growth, Partial diallel

HAPLOTYPE-RESOLVED GENOME ASSEMBLY AND POPULATION GENOMIC ANALYSIS OF ALECTORIS CHUKAR

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Introduction

The Alectoris chukar (chukar) in the order Galliformes and the family Phasianidae, is native to Asia, Western Europe, and the Middle East's stony semi-desert areas and has been introduced through the world as the game bird. In China, chukar was mainly bred for the supply of high-quality meat and egg. Currently, there are few genetic resources for chukar, which has limited investigations on its domestication and adaptive evolution. In this study, we assembled and annotated the haploid genome of the domestic chukar by using third and second-generation high-throughput genome sequencing and transcriptome sequencing technology. Six chukar subspecies (pubescens, potanini, pallida, falki, pallescens, and domestic) sampled from 12 distinct locales representing the majority of chukar's geographical area in China, were collected and resequenced to investigate the population structure, population history, and signals of selection. We acquired two contiguous, precise haplotype-resolved chukar genomes made up of 38 pairs of relatively complete diploid chromosomes. Population structure, history, and signals of selection of six chukar subspecies revealed that adaptive evolution occurs in these chukar populations under artificial or natural selection. For example, select genes, such as DGAT2, AOC3, and BRCA1, may improve the growth and reproductive qualities of domestic chukar. Several genes, including EXD2, BLM, and BRCA2, were shown to be selected and involved in DNA repair processes, which may aid pallescens chukar adaptation to the high plateau's intense UV radiation. Remarkably, we also provided evidence to support the hypothesis that chukar originated in the center of Asia. This study attempts to address scientific questions about the origin of chukar, genetic improvement of domestic chukar, and adaptive evolution of chukar populations in varied environments by assembling a high-quality reference genome, providing an important reference for future breeding and conservation of chukar.

SEQUENCING OF MC4R AND POU1F1 GENES: GENETIC VARIABILITY IN TWO SLOW-GROWING ITALIAN CHICKEN BREEDS AND RELATIONSHIP WITH GROWTH PERFORMANCE

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The interest in local poultry breeds is linked to environmental changes and energy use reduction. Improved performance could make autochthonous chickens more commercially attractive and productive efficient. Study of the genetic polymorphisms and their association with the productive traits are necessary for genetic improvement plans. In North-west Italy there are two slow-growing chickens breeds: the Bionda Piemontese (BP) and Bianca di Saluzzo (BS), traditionally considered as dual-porpoise breeds. In avian species, few studies have been conducted on the gene paired box 7 (PAX7), which is considered a marker of precursor cells during myogenesis. Two further genes have been identified as candidate genes for body weight trait: melanocortin 4 receptor (MC4R) and pituitary-specific positive transcription factor 1 (POU1F1). The aim of this research was to deepen the genetic knowledge of the candidate genes POU1F1 and MC4R in the two considered chicken breeds. By PCR amplification and NSG sequencing (Miseg Illumina), it was possible to identify the DNA polymorphisms (SNP) and to evaluate the associations with the birds' body weight (BW) at slaughter at the age of 180 day. The samples were selected from a database of 201 birds and for each breed (BP or BS), the following categories were defined: low weight (BW< M-1SD) female (LWF) and male (LWM) and high weight (BW>M+1SD) female (HWF) and male (HWM). 73 samples were analyzed:12 BP-LWF (1261-1473g), 12 BP-HWF (1961-2149g), 9 BP-LWM (1929-1940g), 7BP-HWM (2510-2994q), 9BS-LWF (1340-1499q), 8BS-HWF (1997-2023q), 7BS-LWM (1767-2058g), 10BS-HWM (2546-2712g). The sequences were aligned with the reference sequence of the Gallus gallus genome. It was possible to identify for POU1F1 218 SNP in BP breed, of which 2 in the exonic region, and 265 SNP in BS breed, of which 5 in the exonic region. For the MC4R gene, 5 exonic SNPs have been identified in both breeds. For the gene MC4R, in BP females, two SNPs (rs313708918 and rs318235419) and one for BS females (rs318235419) were significantly associated to the live weight. For the gene POU1F1 were associated 3 SNPs in males BS (rs740768161, rs737328551 and rs314443057) (P<0.05, chisquared test).

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New traits for poultry breeding

Selected short communications

GENETIC PARAMETERS FOR THE OVIPOSITION TIME USING HIGH-THROUGHPUT PHENOTYPING FROM INDIVIDUAL ELECTRONIC NESTS IN LAYING HENS

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Oviposition time is a predictor of the laying rhythm. Drift in oviposition time (i.e. more than 24h between 2 oviposition times) can lead the hen to make a pause day. The recent availability of an individual electronic nest enables highthroughput phenotyping of laying behavior like the record of Daily Oviposition Time (DOT). In this study, the genetic background of DOT along the production time was investigated through the estimation of heritability coefficients for 10 successive periods. For the genetic evaluation purpose, the use of the mean or the variance of DOT per hen, instead of the repeated data, to estimate the breeding values of hens was then considered. Data were continuously recorded on laying hens raised in a barn thanks to individual electronic nests, which use radiofrequency identification of hens. The oviposition time was recorded on 1,180 Rhode Island Red hens (RIR) and 932 White Leghorn hens (WL) between 24 and 64 weeks of age. Heritability coefficients of DOT were estimated for 10 periods of 28-days between 24 and 64 weeks of age, using REML methodology applied to an animal model. Heritability coefficients of DOT, of the mean of DOT per hen (MOT) and the Logarithm of the DOT Variance per hen (LVOT) were estimated for the whole period (24 to 64 weeks of age) and the Estimated Breeding Values (EBVs) of hens for DOT, MOT and LVOT were compared. Earliest recorded DOT was 2h05 and 3h07 after turning on the light for RIR and WL respectively. According to the period of 28-days, low to moderate heritability was estimated for DOT with values ranging from 0.28 \pm 0.06 and 0.42 \pm 0.07 for RIR, and 0.13 \pm 0.03 and 0.24 \pm 0.05 for WL. For the whole period, moderate to high heritability was estimated for MOT $(0.73 \pm 0.08 \text{ for RIR and } 0.56 \pm 0.08 \text{ for WL})$, and LVOT $(0.42 \pm 0.07 \text{ for RIR and } 0.07 \text{ for$ 0.45 ± 0.08 for WL). The Pearson correlations between the EBVs of hens for DOT and MOT were 0.99 in both lines. The Pearson correlations between the EBVs of hens for DOT and LVOT were 0.87 and 0.65 for RIR and WL respectively. These results indicate that DOT captures genetic information closer to MOT than LVOT. The rankings of candidates are the same for MOT and DOT. However, the calculation time of EBVs is faster for MOT than for DOT, suggesting that MOT is more interesting for the genetic evaluations. The existence of genetic background for the oviposition time is promising to identify candidate genes implicated in the expression of the laying rhythm.

CHANGES IN OVIPOSITION TIME OF BROWN HENS DURING THE CYCLE IN AN AVIARY SYSTEM

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The oviposition time of hens is known to be affected by genotype and age. In cagefree systems, egg collection should be adjusted to the oviposition pattern more strictly compared to cage systems to optimize egg quality. Thus, the present study evaluated changes in oviposition time during the production cycle in 1,800 Lohmann Brown-Classic hens kept in an aviary system made of two tiers, equipped with collective nests, perches, nipple drinkers and automatic feeding, and a third level with perches and feeders. The aviary was divided in 8 pens (225 hens per pen) which were joined by 45 weeks of age by removing the nets between pens to have a unique system. Egg production at different time intervals (from light turning off to 7:30, 7:30-9:30, 9:30-11:30, 11:30-13:30, 13:30-15:30, 15:30-light turning-off) was measured every two weeks from 26 to 30 weeks and once per month from 37 to 58 weeks of age. Data were analysed using a mixed model with week as the main effect and pen as a random effect by the PROC MIXED of SAS (Statistical Analysis System) and means were compared by the Bonferroni t-test. Most of eggs were laid within 11:30 (average of all weeks: 95.29%). As for the first interval, the rate of laid eggs significantly decreased with age from 79.75% of the daily total (average of data collected during weeks 26-30) to 50.85% (data of weeks 37-58) (P<0.001). On the other hand, the rate of eggs laid in the second time interval increased from 14.28% (average of weeks 26-30) to 32.35% (average of weeks 37-58), and from 3.71% to 10.64% in the third time interval. Changes in the rate of eggs laid in the intervals 11:30-13:30 and 13:30-15:30 showed a rather consistent trend between the weeks 26-30 and the weeks 37-58 with lower values in the former period compared to the latter, whereas changes among weeks referred to the last interval (after 15:30) were most erratic. As regards the eggs laid in the nest (as rate of the total eggs laid in each time interval), a significant effect of the age was recorded only for data referred to the first and second time intervals, i.e. within 7:30 and 7:30-9:30. In details, eggs laid in the nest increased from 77.26% (average of weeks 26-37) to 85.92% (average of weeks 41-58) at the first interval and from 70.21% to 84.35% at the second interval. In conclusion, the oviposition pattern was affected by age with earlier deposition in younger hens and improved use of nests in elder ones.

GENETIC VARIABILITY OF CHICK QUALITY WITHIN TWO CHICKEN LINES DIFFERING FOR GLYCOGEN RESERVES

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The quality of the chick is one of the main concerns of the broiler industry, because of its determining effect on the bird's robustness and ability to cope with challenging starting conditions. Chick guality is a complex trait, including multiple components related to the metabolic status, physical conditions and behavior of the chick, or ability to survive and grow well during the first days of life. Up to now, the role of genetics in the determinism of chick quality has been poorly explored. We have taken advantage of two divergently selected lines on meat pH ultimate (pHu), a proxy for muscle energy reserves, to evaluate the phenotypic correlated responses to selection on chick quality and to estimate the genetic parameters of several indicators or biomarkers. Quality of 321 pedigree chicks of the 15th generation was assessed at hatching (D0), the day after hatching after a challenging start (D1) and at 7 days of age (D7). It was based on a visual scoring adapted from the Tona's grid (Tona et al., 2003) and on some plasmatic measurements (at D7 only) related to the metabolic or antioxidant status of the chick (concentrations of glucose, triglycerides, uric acid, and Total Antioxidant Status: TAS). The selection for an increased ultimate pH (pHu+ line), which impairs muscle glycogen content as soon as hatching (Métayer-Coustard et al., 2021), was associated with a decrease in the quality score of the chick at the three ages. Despite a higher body weight at hatching, birds of the pHu+ line showed a lower relative growth during the first week of rearing and a lower weight at slaughter than in the pHu-line. Within the pHu lines, the quality score at hatching showed a rather low heritability (h2=0.14) but a high positive genetic correlation with the weight gain between D1 and D7. Interestingly, uric acid and TAS showed positive genetic correlations with the relative weight loss between D0 and D1 (rg of 0.70 and 0.49 respectively), which may suggest the establishment of a biological response against oxidative stress, partly controlled by genetics. To conclude, this study evidenced that genetic selection for increased muscle glycogen reserves was favoring the robustness of the chick. It suggested that several indicators or biomarkers could constitute valuable traits for a better chick quality, but they deserve further studies at larger scale and in other genetic lines to define the best selection strategies.

BIOELECTRICAL IMPEDANCE ANALYSIS (BIA) TO PREDICT IN VIVO BODY COMPOSITION IN BROILER CHICKENS FROM 0 TO 42 DAYS

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The aim of this study was to estimate in vivo body composition using BIA in broiler chickens from 0 to 42 days of age. A total of 528 birds were used grouped per gender and breed (A and B) equally distributed. Animals were fed ad libitum with a common starter (0-14d; 2875 kcal AMEn/kg, 8% EE and 22% CP), grower (15-29d; 2925 kcal AMEn/kg, 7.5% EE and 21% CP) and finisher diet (30-42d; 3000 kcal AMEn/kg, 8% EE and 20% CP). BIA was measured at different ages (0, 7, 14, 20, 29, 35 and 42 days) with a body composition analyser. Then, animals were euthanized, gutted and frozen. Afterwards, they were thawed and ground. Samples were collected which were frozen, freeze-dried and milled for further chemical analysis of dry matter, fat, protein, ash and energy. Water (%), protein and ashes, expressed as % DM, decreased with the age (77.5 to 67.5, 69.1 to 52.2 and 8.12 to 7.29, respectively; P < 0.001). On the contrary, fat (% DM) and energy (kJ/100 g DM) rose with the age (20.7 to 36.4 and 2268 to 2574, respectively; P < 0.0001). Multiple statistical differences were observed between breeds and genders as well as an interaction trend between gender and breed for the protein (P = 0.089) and fat (P = 0.083) content, expressed as % DM. Resistance (Ω) and reactance (Ω) were significantly (P < 0.05) correlated with the different variables. A multiple linear regression analysis was carried out to select the most suitable equation model to predict body composition. The prediction accuracy of the equations was measured calculating the relative mean prediction error (RMPE; %) through cross validation and using independent data. The coefficient of determination (R2) to estimate water (%), protein (% DM), fat (% DM), ash (% DM) and energy (kJ/100 g DM) content were 0.909, 0.825, 0.795, 0.493 and 0.838, and the relatives mean prediction errors (RMPE) were 1.26, 3.46, 7.73, 8.85 and 1.86 %, respectively. The coefficient of determination (R2) of the equations obtained to estimate water (g), protein (g), fat (g), ash (g) and energy (MJ) content were 0.998, 0.996, 0.970, 0.986 and 0.991, and relatives mean prediction errors (RMPE) were 2.70, 5.90, 11.9, 11.1 and 6.64 %, respectively. A t-test analysis was run, observing no differences in any of the parameters under study. Based on these results, we can assert that BIA can be used as a valid and accurate non-invasive technique to predict in vivo body composition in broiler chickens from 0 to 42 days of age.

26th World's Poultry Congress, abstracts selected in 2022

New traits for poultry breeding

GENOME-WIDE ASSOCIATION STUDY FOR TIBIA STRENGTH IN DAWEISHAN MINI CHICKEN × WHITE PLYMOUTH ROCK F2 RESOURCE POPULATION

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INTRODUCTION

Skeletal problems in poultry represent both an economic and an animal welfare concern. Due to genetic, nutritional and other factors, the occurrence of bone diseases such as osteoporosis and fractures cause huge economic losses. Yunnan Daweishan Mini Chicken, which have excellent bone properties, are used to study the genetic mechanism of bone properties of poultry. In current study, GWAS was carried out for tibia bone strength in 800 F2 chickens derived from reciprocal crosses between Daweishan mini and While Plymouth rock.

MATERIALS AND METHODS

Five tibia strength indicators were measured and calculated after 800 F2 chickens slaughtered at the age of 12 weeks, including modulus of elasticity (MOE, N/mm2), yield strain (YS, N/mm2), energy absorbability (EA, J), maximum load (ML, N), maximum stress (MS, N/mm2). According to the manufacturer's protocol, genomic DNA was extracted from blood of experimental population using phenol-chloroform method. The construction of DNA library and the performance of paired-end sequencing using an Illumina HiSeq2000 platform (Illumina, USA) were processed at Beijing Novogene Co., Ltd (Beijing, China). After quality control and SNP calling by GATK software, all 5 tibia strength indicators were applied to GWAS analysis with mixed linear model (MLM) in GEMMA program. The heritability and genetic correlation were estimated using a genomic relationship matrix (GRM) and restricted maximum likelihood (REML) method implemented by GCTA software.

RESULTS

After quality control, a total of 91 SNPs were detected for 5 tibia strength indicators with genome wide significance ($-\log 10(P) > 7.44$). Twenty-two candidate genes were found to be associated with tibia strength. The heritability of MOE, YS, EA, ML, MS were 0.34 (0.07), 0.36 (0.07), 0.22 (0.07), 0.31 (0.08), 0.38 (0.07) respectively.

CONCLUSION

A total of 91 SNPs were detected to be significant associated with 5 different tibia strength indicators. Besides, 22 candidate genes were found to be candidate genes for tibia strength in chicken. GO enrichment analysis revealed that voltage-gated potassium channel complex (GO: 0008076) was with the lowest P value. This study would be helpful to illustrate the genetic mechanism of tibia strength in chicken, and further would provide a reference for chicken breeding.

KEY WORDS: chicken, tibia strength, genome-wide association study

BIOELECTRICAL IMPEDANCE ANALYSIS (BIA) TO PREDICT IN VIVO CARCASS COMPOSITION IN BROILER CHICKENS FROM 29 TO 42 DAYS

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The aim of this study was to estimate in vivo carcass composition using BIA in broilers from 29 to 42 days of age. A total of 144 animals were used in the study arouped per gender and breed (A and B) equally distributed. Animals were fed ad libitum with a common starter (0-14d; 2875 kcal AMEn/kg, 8% EE and 22% CP), grower (15-29d; 2925 kcal AMEn/kg, 7.5% EE and 21% CP) and finisher diet (30-42d; 3000 kcal AMEn/kg, 8% EE and 20% CP). BIA was measured at different ages (29, 35 and 42 days) with a body composition analyser. Then, animals were stunned, slaughtered, bled, plucked, gutted and frozen. Afterwards, carcasses were thawed and ground in order to collect representative samples which were frozen, freeze-dried and milled for further chemical analysis of dry matter, fat, protein, ash and energy. Water (%), protein and ashes (% DM) decreased with the age (67.8 to 66.4 %, 52.8 to 51.1 and 7.91 to 7.10 % DM, respectively; P <0.05) On the contrary, fat (% DM) and energy (kJ/100 g DM) increased with the age (36.9 to 38.6 and 2605 to 2648; P < 0.05). Multiple statistical differences were observed between breeds and genders as well as an interaction between gender and breed for the protein (%) (P = 0.0069) content. Resistance (α) and reactance (Ω) were significantly (P < 0.05) correlated with most of the different variables. A multiple linear regression analysis was carried out to select the most suitable equation model to predict carcass composition. The prediction accuracy of the equations was measured calculating the relative mean prediction error (RMPE; %) through cross validation and using independent data. The coefficient of determination (R2) to estimate water (%), protein (% DM), fat (% DM), ash (% DM) and energy (kJ/100 g DM) content were 0.443, 0.284, 0.262, 0.442 and 0.334, and the relatives mean prediction errors (RMPE) were 1.46, 2.42, 5.28, 6.10 and 1.49 %, respectively. The coefficient of determination (R2) of the equations obtained to estimate water (g), protein (g), fat (g), ash (g) and energy (MJ) content were 0.973, 0.974, 0.882, 0.943 and 0.953, and relatives mean prediction errors (RMPE) were 3.11, 4.45, 8.78, 6.73 and 5.38 %, respectively. A t-test analysis was run, observing no differences in any of the parameters under study, excepting for the ashes (g). Based on these results, we can affirm that BIA can be used as a valid non-invasive technique to predict in vivo carcass composition in broilers from 29 to 42 days of age.

BAYESIAN INFERENCE OF GENETIC PARAMETERS FOR GROWTH RATE IN MAZANDARAN NATIVE CHICKENS

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Indigenous chicken breeds play an important role in the rural economy in Iran. Despite their positive characteristics such as their greater resistance to harsh environmental conditions than commercial breeds, their growth rate is slow and it takes them longer to reach the market-desired weight indicating the need for genetic improvement in these breeds. The aim of this study was to estimate the (Co) variance components of growth rate in Mazandaran native chickens using Bayesian statistical method. For this purpose, 79533 records collected from 21 consecutive generations at breeding center of Mazandaran native fowls were used. Studied traits included the growth rates (average daily gains) from hatch to eight weeks (GR1), hatch to 12 weeks (GR2), 8 to 12 weeks (GR3), hatch to sex maturity (GR4), 8 weeks to sex maturity (GR5), and 12 weeks to sex maturity (GR6). To estimate heritability, six univariate animal models were compared with and without maternal effects and the best model for each trait was determined based on DIC value. Bayesian statistical method was used for data analysis using Gibbs sampling and GIBBS1F90 software. The number of Gibbs sampling chains was 200,000 with100 sampling intervals, and first 20,000 rounds as burning period. Based on the DIC criterion test, the best model for the GR1 and GR3 included the direct genetic and the maternal permanent environment effects. For the GR2, the model including the direct genetic effect was found to be the best model. For the GR4, GR5 and GR6, the model with direct genetic effect, maternal genetic effects with non-zero direct-maternal genetic covariance was found as the best model. From DIC values, estimates of heritability were, 0.18, 0.28, 0.11, 0.32, 0.33 and 0.36, for GR1, GR2, GR3, GR4, GR5 and GR6, respectively. For traits for which maternal genetic effects were significant, a negative correlation was observed between maternal and direct genetic effects. The results showed that the use of maternal effects in statistical models leaded to a more accurate estimation of direct heritability in studied traits.

ANTIOXIDANT STATUS AND GROWTH PERFORMANCE OF CONVENTIONAL, FREE-RANGE, AND FREE-RANGE FED WITH AROMATIC PLANTS BROILERS UNDER INDUSTRIAL SCALE PRODUCTION

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Poultry production is related to several stressors, which affect poultry production efficiency and health.1 Plant extracts have attracted much attention as growth and health promoters in broiler nutrition because their increased beneficial components might improve chicken antioxidant status.2 This study reveals the influence of a free-range system and plant extract additives on growth performance and redox status in industrial poultry production. Three different industrial scale systems were examined: 1st Conventional chickens (C) (Ross 308) raised indoor (15 birds/m2); 2nd Free-range chickens (FR) (Sasso) raised both indoor and outdoor (13 birds/m2 and 1 bird/m2 of forage paddock); 3rd Free-range chickens with enhanced diet (FRE) raised as the FR. The diet of C and FR chickens was according to standard specifications. FRE fed as the FR with the addition of aromatic plants. Performance was assessed by recording the mortality, body weight (BW) and Feed Conversion Ratio (FCR). Antioxidant status was evaluated by the Total Antioxidant Capacity (TAC) and TBARS assay in plasma and muscle tissue samples, while atocopherol was determined in broiler plasma. Statistical analysis was performed according to the independent samples t-test. Two different comparisons were made:1) Comparison between C and FR groups: TAC was significantly higher for the FR chickens compared to the C ones. TBARS in plasma were significantly lower for the C group while in muscle tissue FR chickens presented significantly lower lipid peroxidation. Mortality, BW, and a-tocopherol had no significant differences between C and FR chickens. However, FCR was significantly higher for the FR group. 2) Comparison between FR and FRE groups: Results for the FRE chickens presented no significant differences to the FR group neither in growth performance nor in antioxidant status. Concluding, free-range chickens presented better antioxidant status and lower oxidative stress than the conventional. Mortality and BW weren't affected by the rearing system, but FCR was higher for the FR chickens. Furthermore, plant extracts as feed additive did not influence the chicken growth performance and the antioxidant status. These data are important for large-scale poultry production due to the industrial scale experimental conditions.

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GROWTH PERFORMANCE, MEAT QUALITY AND ORGANOLEPTIC CHARACTERISTICS IN CHICKENS RAISED UNDER COMMERCIAL PRODUCTION SYSTEMS

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Poultry meat has high nutritional value, but also it is a low-cost product for consumers and industry. [1,2] Nowadays, there is also an increased interest to improve animal welfare, meat quality and productivity. The purpose of this study was to evaluate the growth performance, meat quality, and organoleptic characteristics for conventional (C), free-range (FR), and free-range chickens fed with plant extracts (FRPE). C (Ross 308), FR (Sasso) and FRPE (Sasso) chickens were raised under the same industrial conditions. FR and FRPE chickens were also left for outdoor grazing. Diet was designed for each group according to the age and the genotype. Growth performance was estimated by recording the mortality, body weight (BW) and Feed Conversion Ratio (FCR). Meat chemical analysis was performed with standard methods. Organoleptics were estimated in roasted chicken thighs. Comparison of C vs FR: FR chickens presented higher protein and lower fat-content, lower moisture and water holding capacity (WHC). Ash and pH were almost the same for both groups. Also, FR displayed higher FCR but lower mortality and BW. Organoleptic characteristics were overall better in the FR chickens. Comparison of FR vs FRPE: FRPE chickens exhibited higher protein content, while the fat-content, moisture, ash, pH, and WHC were almost the same for both groups. FRPE exhibited higher FCR, mortality, and BW. Organoleptics were almost the same for FR and FRPE chickens. Concluding, the results of this study suggest that free-range system: 1) improves growth performance; 2) positively affects meat quality; 3) provides better organoleptics except for tenderness, which was expected because FR chickens exerted more physical activity and were more mature. Also, results imply that the addition of aromatic plants in the free-range chickens improves further the growth performance, while does not affect organoleptics. All the above findings are important for exploiting them in industrial poultry production systems.

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Infectious diseases

Selected short communications

AUGMENTING TRADITIONAL SURVEY-BASED METHODS WITH SOCIAL NETWORK ANALYSIS, SOCIAL MEDIA ANALYSIS AND MAPPING TO BETTER UNDERSTAND NON-COMMERCIAL POULTRY MOVEMENT

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Introduction. The 2018-2020 outbreak of virulent Newcastle Disease (vND) in Southern California was primarily driven by backyard chickens (BYC) and game fowl (GF) transmitting the virus to commercial operations in the region. While there have been studies that surveyed BYP and GF in the US, they were performed over two decades ago and lack granular location and movement data. Data that is paramount for disease modeling and targeted outreach efforts.

Objective. Characterize BYC and GF movement with survey and classified advertisements data to improve vND modelling Methods. The target populations for the survey were BYC owners and GF breeders from California. BYC were defined as chickens primarily kept for eggs and/or meat at residences. GF were defined as breeds of chickens, such as Kelso, Hatch, Claret, and Roundhead, intended primarily for exhibition/competition and bred for beauty, strength, health, vitality, and longevity. An online survey was created in English using the survey instrument Qualtrics XM. The main sections were: 1. Information about the BYP owner 2. Biosecurity practices 3. Live bird movement. The movement networks were visualized using R 4.1.2 and R Studio 2021.09.2 with the statnet, network and maps packages. Craigslist.org (classified advertisements website) posts that were selling chickens in the Los Angeles, CA, USA region were collected once a month from June 2021 to January 2022. Posts were collected using R and the tidyverse, rvest, DataExplorer, and data. table packages.

Results and conclusion. In total, there were 104 survey participants. The survey results showed connectivity between Northern and Southern California, which suggests expanding the quarantine region might be beneficial during outbreaks. Although the movement data was mapped at the county level, the sales posts were mappable at a more granular level using zip codes. Moreover, monthly sales data was captured with data mining which would otherwise be difficult to capture with traditional survey methods. These preliminary results indicate that traditional surveys and data mining can be used to identify regions that import and export the most BYC and GF.

REASSESSING THE EFFECTS OF EIMERIA SPP. INFECTION ON BROILER PERFORMANCE

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Eimeria infections compromise animal welfare and reduce the productivity of poultry sector. This meta-analysis was developed to reassess the effect of Eimeria spp. on broiler growth performance. Methods of systematic review were used to access studies published in scientific journals from 2000 to 2020 reported performance of broilers from 1 to 50 days old subjected to an experimental challenge by Eimeria spp. (excluding vaccines). Data of 131 articles (162 experiments and 46,354 birds) were included in the database (1,970 lines) and coded to limit the studied effect to the Eimeria inoculation (excluding any special condition, such as medication or feed additive supplementation). Results were analyzed as raw data, when control and challenged groups were compared by variance analysis using mixed models with fixed effect of challenge and random effect of study. The percentage of response to the challenge was calculated (Δ %) and the relationship between the variations in average daily feed intake (ADFI) $(\Delta\%FI)$ and average daily gain (ADG) $(\Delta\%G)$ was studied by correlation and regression analysis. Broilers challenged by Eimeria spp. reduced ADFI by 6%, ADG by 20%, and gain to feed (GF) by 11% (P<0.001). Whatever the type of challenge (whole database, type of Eimeria species, or pool of species) showed highly significant effects. Only the challenge by E. maxima did not influence ADFI. The greatest impairment in broiler performance was found for pool challenge in case of ADFI (-9%) and for E. maxima in ADG (36%) and GF (15%) responses. The Δ %G showed a linear relationship with Δ %FI. The correlation between Δ %G and Δ %FI caused by Eimeria challenge was high (r=0.705; P<0.05). When simulating a scenario without changes in ADFI (i.e., Δ %FI was set to zero in the equations), the Δ %G was estimated to be 6% for the overall database, 4% in challenges caused by E. acervulina, 17% for E. maxima, 4% for E. tenella, and 5% for pool of Eimeria. These reductions represented 32% of total ADG impairment for the overall database, 23% for E. acervuline, 65% for E. maxima, 18% for E. tenella, and 26% for pool of Eimeria spp. These results are very important since the relationship between ADFI and ADG impairment are very difficult to quantify in conventional experimental designs. The provided information is also useful to further understanding the effects of health challenges in the nutritional requirements used for animal maintenance.

HIGH SEQUENCE SIMILARITY OF AVIAN PATHOGENIC E. COLI WITHIN BIRD AND FLOCK IN COLIBACILLOSIS OUTBREAKS WITH HIGH FIRST WEEK MORTALITY

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Avian Pathogenic E. coli (APEC) are a cause of high first week mortality (FWM) in broiler chickens worldwide. To investigate the epidemiology of colibacillosis in broiler chicken flocks it is important to develop reliable and economically efficient sampling regimes, and to define the minimum number of required samples that represent flock studies on outbreaks of colibacillosis.

In this study the diversity of E. coli isolates from flocks with colibacillosis were analyzed by whole genome sequencing. Each flock was represented by five animals with typical signs of colibacillosis; spleen, liver and one other organ from each animal was sampled for E. coli. Altogether, 47 E. coli isolates were sequenced and compared by Multilocus sequence typing, serotyping and phylogenetic analysis.

We found little or no diversity between APEC isolates from different organs within individual birds. Within flocks, isolates with the same sequence type (ST) and serotype were highly similar with few differences in single nucleotide polymorphisms (SNPs) covering more than 95 % of the genome based on phylogenetic analysis. Differences in APEC isolates from samples taken within an individual bird differed on only three of 47 occasions. Samples from the liver were in all cases consistent with other E. coli identified within an individual bird and seem to represent the variant causing systemic infection. Two flocks were identified with ST429 as the main ST; the third flock was represented with three STs: ST10836, ST457 and ST95. Phylogenetic analysis of ST429 revealed two distinct clades (A and B), separated by flock. The total average genome coverage of all included ST429 genomes was 97.5%. Clade A represented flock 1 with a mean SNP distance of 6.2 and a SNP-range of 0-21. Clade B representing flock two had a mean SNP distance of 4.45 and a SNP-range of 0-11. The mean SNP distance between clade A and B was 36.0. Phylogenetic analysis of ST95 revealed smaller clades representing individual birds. The mean SNP distance was 18.2 and SNP-range was 6-29 based on a total average genome coverage of 96.7%.

Results indicate that one sample from the liver per bird would represent the diversity of E. coli types within a flock, as long as several birds from the same flock were included in the sampling regime.

MECHANISMS FOR VIRAL ADAPTATION TO NEW HOSTS OF HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUSES USING VIRAL QUASI-SPECIES ANALYSIS

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Avian influenza (AI) is a viral disease caused by a single-stranded segmented RNA with viral RNA polymerase but without proofreading capacity. These characteristics give the virus a great potential for genetic variability. Fuelled by high mutation rates, viral mutants arise continually and change in relative frequency as viral replication progresses. The objective of this study was to apply this paradigm to understand the mechanisms involved in viral adaption following highly pathogenic AI virus (HPAIV) infection and the possible compartmentalisation effect on viral replication. Chickens (Gallus gallus domestica) experimentally infected with HPAIV strains A/Chicken/Italy/5093/1999 (H7N1) an A/Goose/Spain/IA17CR02699/2017 (H5N8 clade 2.3.4.4 group B) were used. Original inoculums, oral swabs, and lung and spleen samples from birds at 3 days post-inoculation (dpi) were deep-sequenced, and viral quasi-species populations were constructed after RNA extraction. To understand viral adaptation during the infection process, nucleotide positions that could be differentially selected between the original inoculum and the oral swabs were identified. The average frequencies of each nucleotide for each position and sample were compared using an analysis of molecular variance (AMOVA). The nucleotide changes were determined as synonymous or nonsynonymous at the codon level. Compartmentalisation was studied by estimating nucleotide diversity among different samples (oral swab, lung, and spleen) of the same individual. The results showed different quasi-species populations of the virus between viral strains and different viral quasi-species diversity levels among samples of the same individual. The segments with the higher number of amino acid changes were PB2 and PA for the H7N1 strain (chicken origin), and HA, PB1, and NS for the H5N8 strain (goose origin). Our results suggest that HPAIVs have a different strategy for adaption to the new host depending on the origin of the strain. Finally, the viral diversity among samples of the same individual suggests some degree of independent viral replication among tissues.
BIOFILM-FORMING ABILITY, ANTIMICROBIAL SUSCEPTIBILITY AND PROTEOMIC PHENOTYPES OF KLEBSIELLA PNEUMONIAE STRAINS ISOLATED ON A TURKEY FARM

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Klebsiella pneumoniae is often recognized as a multidrug resistant species which represents a growing clinical problem in human and veterinary medicine. Although it is usually an environmental contaminant, it has an important role in spreading antimicrobial resistance genes to clinically important species. We investigated the biofilm-forming ability, antimicrobial susceptibility and proteomic phenotypes of thirteen K. pneumoniae strains isolated on a turkey farm. The study included strains isolated from six flocks during the routine health monitoring. Ten strains were isolated from the birds, two from the cardboard transport boxes of 1-day old poults, and one from the feeding system. The birds were submitted to necropsy and sampled upon indication, the feeding system tray was swabbed, and the transport boxes were sampled by scraping and collecting the feces. Bacterial identification was based on morphology, biochemical properties, and Bruker Microflex LT MALDI-TOF Mass Spectrometry. Biofilm-forming ability was tested using quantitative adherence assay on microtiter plates. Strains were tested in quadruplicate and the assay was performed twice. Antimicrobial susceptibility was determined using disk diffusion assay on Mueller-Hinton agar. The assay was performed with fourteen antibiotics and the inhibition diameters were interpreted according to EUCAST standards. The proteomic phenotypes of the strains were compared using principal component analysis and the minimum spanning tree generated with MALDI Biotyper. All strains were multidrug resistant, with the highest resistance rates to amoxicillin and lincomycin (100%), and enrofloxacin, flumequine and norfloxacin (76,92%). The proteomic analysis showed nine lineages, with the cut-off value set at a 100-distance level. There was no significant link between the tissue and flock of origin with the proteomic similarity of the strains. The results of the biofilm quantification showed that 23,08% and 76,92% of the investigated strains were moderate and strong biofilm producers, respectively. Although the farm is successfully implementing the antibiotic reduction strategy, the detected levels of multidrug resistance were high, while the biofilm-forming ability of the strains additionally reduces the efficacy of antimicrobials and disinfectants. The results confirm the important role of K. pneumoniae as a reservoir of resistance genes and indicate a need for continuous surveillance.

EARLY SALMONELLA TYPHIMURIUM INOCULATION ALTERS SYSTEMIC IMMUNOMETABOLISM 24H POST-INOCULATION WITH EIMERIA MAXIMA

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Coccidiosis due to Eimeria spp. infection is a significant source of production and economic losses in the global poultry industry. While many factors underlying Eimeria-induced performance losses are well-documented, the impacts of infectious dose and early microbial challenges on immunometabolism during coccidiosis are relatively uncharacterized. The study objective was to examine the effect of early Salmonella Typhimurium inoculation followed by varying Eimeria maxima live oocyst doses (Hargis lab, University of Arkansas-Fayetteville) on broiler immunometabolism 24h post-inoculation (pi). Ross 308 broilers were fed a commercial diet and housed 7/cage in brooder batteries to ensure a synchronized model. Chicks were orally gavaged with PBS or 1x105 CFUs Salmonella Typhimurium on d1 (42/ group). On d14, Salmonella-inoculated birds were gavaged with 15,000 sporulated E. maxima M6 oocysts or PBS. Separately, on d14 168 Ross 308 broilers were orally gavaged with PBS, 5-, 15-, or 25,000 sporulated E. maxima M6 oocysts (42 birds/ dose; no d1 Salmonella). Blood was collected for peripheral blood mononuclear cell (PBMC) isolation at 24hpi from 8 birds/challenge. Agilent real-time ATP and glycolytic rate assays (Santa Clara, CA) determined dominant pathways underlying ATP production and glycolysis as a marker of immune activation in collected PBMC, respectively. Data were analyzed using proc mixed (SAS 9.4) with Eimeria dose and challenge effects (unchallenged or Salmonella + Eimeria; P≤0.05). Eimeria dose did not affect oxidative or alycolytic contributions to PBMC ATP production or alycolytic rate in the first 24hpi compared to unchallenged controls. Early inoculation with Salmonella Typhimurium increased overall ATP production 38.1% and glycolytic rate 31.9% at 24hpi with E. maxima compared to unchallenged controls ($P \le 0.01$). Additionally, PBMC from Salmonella-inoculated birds challenged with E. maxima had 32.5% increased ability to utilize glycolysis when oxidative pathways were inhibited during the glycolytic rate assay (P=0.01). Changes to peripheral immunometabolism may indicate immune activation and recruitment; therefore, these outcomes suggest that E. maxima doses up to 25,000 sporulated oocysts require >24h to induce a metabolic shift in the systemic immune response. However, early inoculation with Salmonella Typhimurium may have altered intestinal immune responses requiring systemic immune cell recruitment as early as 24hpi with E. maxima.

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Selected short communications

COMBINED SUPPLEMENTATION OF FUNCTIONAL AMINO ACIDS AND POLYPHENOLS SUPPORTS GUT HOMEOSTASIS IN BROILERS SUBJECTED TO A CORTICOSTERONE CHALLENGE

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To address the overuse of antimicrobials in poultry production, new functional feed ingredients can play a crucial role. Among the alternatives, dietary functional amino acids (AA) and polyphenols extracts were shown to promote gut health when used as single additives. Data regarding the beneficial effects of their combination remain, however, scarce. This study thus aimed at evaluating the efficiency of the supplementation of arginine, threonine and glutamine together with grape polyphenols on the gut integrity and functionality of broilers facing a physiological stress. A total of 108 straight-run one-day-old PM3 chicks were randomly allocated to 3 treatment groups in 4 replicates of 9 birds each until day 35. Broilers in the control group were fed a basal diet in standard conditions. In experimental groups, birds were administered with corticosterone (CORT) in drinking water (20 mg/L, from day 11 to day 13) to mimic a physiological stress. The broilers in experimental groups were fed either a basal diet or supplemented with a mix of functional AA together with grape seed and skin extract (1 g/kg of)feed – MIX group). Gut permeability was assessed in vivo on 12 birds per treatment on day 32 by measuring the amount of fluorescently labelled dextran (FITC-d) in plasma after oral gavage. On days 16 and 35, 8 birds per treatment were slaughtered to collect ieiunum tissue and caecal digesta. Data were analysed with ANOVA or mixed model procedures (pen as a random variable). A P-value of \leq 0.05 was considered significant. CORT stress induced a significant increase in FITC-d plasma levels in non-supplemented birds. This stress effect was alleviated in MIX-supplemented birds with similar gut paracellular permeability than in controls. MIX supplementation also helped broilers to cope with the reduction of villi crypt depth observed on day 35 after a CORT challenge. The protective effect of the MIX supplementation in response to CORT stress could be explained by early modulation at gene transcript levels. Indeed, on day 16, the gene expression of MUC2 and TNF-a was up-regulated in the MIX group compared to the challenged and non-supplemented birds. Caecal microbiota remained similar between the 3 groups. It can be therefore concluded that a balanced diet supplemented with functional AA and polyphenols can help restore broiler intestinal barrier after a stress exposure.

EFFECT OF PHYTASE ON PERFORMANCE AND INTESTINAL MUCOSA PHYSIOLOGY IN YOUNG BROILER CHICKENS

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Phytic acid is an important antinutritional factor that limits the bioavailability of phosphorus (P), calcium (Ca) and further nutrients, and it is hypothesized that it could also directly affect the intestinal mucosa. The aim of the experiment was to investigate the response to graded levels of phytase (Phy) in phytate-containing (pc) and a phytate-free (pf) diet. A 21-day study with one-day-old broiler chickens was performed using six dietary treatments: a pf diet based on Hermetia meal, potato protein, fish meal and corn starch without (T1) or with (T2) 3000 FTU/kg Phy (Natuphos® E, BASF SE, Germany) and a pc diet with corn, soybean meal and sunflower meal prepared without (T3) or with Phy at levels of 500, 1,500 and 3,000 FTU/kg (T4-T6). For each treatment, 8 replicates with 8 broilers/floor pen (a) were used. Performance was weekly registered and apparent ileal digestibility (AID) of some nutrients was measured at the end of the study. Electrophysiological measurements using the Ussing chamber technique were conducted in jejunal tissue from animals of group T1, T2, T3 and T6. Histomorphometry and gene expression were performed in jejunal and caecal tissue. Statistical analysis was performed with ANOVA and Tukey test ($P \le 0.05$), linear and guadratic regressions were used for testing the effects of increasing levels of Phy. Body weight (BW) and feed intake increased linearly with Phy inclusion in pc diets ($p \le 0.001$) while performance parameters in the pf group was not impacted. Relative AID of crude ash and P increased ($p \le 0.001$) and that of Ca decreased ($p \le 0.001$) with graded levels of Phy. The short-circuit current in jejunal mucosa after glucose addition showed a trend to increase in group T6 compared to T3 (P=0.079). The expression of target genes related to barrier function and immune response in jejunal tissue showed no differences among groups. Crypt depth in jejunal and caecal tissue expressed in relation to metabolic BW decreased with increasing Phy levels in pc diets. No differences in goblet cells count were observed. The use of increasing levels of Phy showed improvements in P utilization and performance. Phy led to lower mucosal cell renewal in intestinal tissue. Different mucus markers showed no differences among treatments. Epithelial glucose transport in small intestine seems to be influenced by Phy in pc diets. The effect of Phy/phytate on intestinal physiology needs further validation.

DIFFERENTIATED SHORT- AND MEDIUM-TERM IMPACTS OF EARLY XYLANASE-XOS SUPPLEMENTATION ON CAECAL METABOLOME AND MICROBIOME IN BROILER

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The postnatal period is critical for early development. Due to the great plasticity of the digestive tract, this period constitutes a unique window to orient the digestive phenotypes. Xylo-oligosaccharides (XOS) produced from the hydrolysis of non-starch polysaccharides (NSPs) by xylanase (Xyl.) are prebiotics acting on the caecal microbiota. In this work, we compared the effects on metabolome and caecal microbiota composition of early (starter) or late (grower) supplementation with a mixture of xylanase and XOS (Xyl.XOS) with an Eimeria tenella challenge. Chicks fed a basal diet rich in NSPs (wheat/rye) were divided into four groups: without additive (D0-D22), with a supplementation of XyI.XOS during the start-up period (D0-D9), the growth period (D10-D22), or during the entire period (D0-D22). Based on this full factorial design, effects on performance as well as on the metabolome (RMN) and caecal microbiome (16S rRNA sequencing) were assessed at D9 and D22. During the start-up period, the addition of XyI.XOS has a positive effect on growth. At D9, a decrease in Short Chain Fatty Acids (SCFAs) and Lachnospiraceae, producers of SCFAs observed in the caeca of supplemented animals with XyI.XOS. In contrast, supplementation in the starter diet alone increased SCFA production until D22. We are actually evaluating the effect of this early supplementation on the microbiota at D22. In animals inoculated with E. tenella, the supplementation with XyI.XOS in grower reduced significantly the deleterious effects of infection on growth while early supplementation had no detectable effect. In conclusion, early supplementation with XyI.XOS has a shortand medium-term effect on the metabolites and growth of the animals.

EFFECT OF WHEAT-ARABINOXYLAN TREATED WITH DIFFERENT NSP-ENZYMES ON THE BACTERIAL FERMENTATION IN CHICKEN CAECUM IN AN EX VIVO FERMENTATION MODEL

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The aim of the study was to test a series of wheat arabinoxylan oligosaccharides (AXOS) generated with different NSP-hydrolyzing enzymes, in an ex vivo model mimicking the conditions prevailing in chicken caeca. In the ex vivo model all chemical elements come from authentic broiler chicken intestine and the physicochemical environment (pH, temperature, and redox potential) corresponds to that of authentic caecum. The inoculum for fermentation was fresh cecal digesta taken 2-3 hours before inoculation from broilers fed wheat-based diet. The digesta from several birds were pooled and kept under anaerobic conditions. Fermentation was initiated by mixing 3 parts of ileal extract; 7 parts of cecal extract; 10 parts of reduced phosphate buffer; 1 part of inoculum. The cocktail was filtered through a steel mesh (0.6 mm). Then, 10 mL of the cocktail was introduced in 5 replicate serum bottles containing the test products at 1 mg/mL. The test products were obtained by hydrolyzing wheat arabinoxylan with different commercially available NSP-enzymes and composed of 91% AXOS and 9% Na-Acetate, whereas the control group represents untreated wheat arabinoxylan (91% Arabinoxylan and 9% Na-Acetate). The enzymes used to treat the wheat arabinoxylan were 1) Xylanase/β-glucanase derived from A. niger (Natugrain® TS, BASF); 2) Xylanase derived from T. reesei and 3) NSP-enzymes cocktail derived from T. versatilis. The serum bottles were sealed and incubated at 37°C with continuous mixing at 100 rpm. Short chain fatty acids (SCFA) were analyzed after 5h and 10h by gas chromatography. Data were analyzed by independent t-test where each treatment was compared to the untreated arabinoxylan and differences were considered significant at P<0.05. Untreated and enzyme treated AXOS stimulated fermentation which suggests that AXOS was relatively easy for cecal bacteria to digest. Some enzyme treatments facilitated fermentation. After 5h, AXOS treated with Natugrain® TS increased total SCFA concentration in comparison to the untreated arabinoxylan (P<0.05). The acetic acid concentration was increased after 5h when AXOS was treated with Natugrain® TS or with enzyme cocktail (P<0.05). The total branched chain fatty acids concentration tended to decrease with AXOS treated with Natugrain \mathbb{R} TS (P=0.09). In conclusion, treatment of arabinoxylan with a xylanase/ß-glucanase (Natugrain® TS) showed an impact on the fermentation pattern and increased the production of SCFA in the chicken caecum.

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Posters

BUTYRIC ACID, MEDIUM-CHAIN FATTY ACIDS AND ZEOLITE MIXTURE AS FEED ADDITIVE: IMPACT ON GROWTH INDICATORS AND INTESTINAL STATUS OF BROILER CHICKS

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The growth of broiler chickens depends on, primarily, the broilers digestive processes and general state of health. Therefore, the impact on appropriate intestinal status by meeting chicks body needs should be taken into account. The aim of this study was to evaluate the impact of butyric acid, medium-chain fatty acids and zeolite mixture on broiler chicken growth performance and intestinal status. A total of 206 000 1-d-old Ross 308 broiler chicks were divided into two treatments (103 000 broilers/treatment): standard compound diet; standard compound diet supplemented with 1 kg/t butyric acid, medium-chain acids and zeolite mixture (Lumance®, INNOV AD nv/sa, Belgium). At the end of the feeding test, 10 birds (42-d-old) from each treatment were randomly selected and euthanized by using electrical stunning. Slaughter was carried out at a commercial slaughterhouse in accordance with the established procedures which complied with the laws of the Republic of Lithuania. The weights of glandular and muscular gizzard, pancreas, heart and liver were recorded. The length of the intestine was measured with a flexible tape on a glass surface; after that the intestinal walls were washed with saline, drained with filter paper and weighed. The SCFA profile of the duodenum and ileum were determined using the HPLC system (Varian, Inc., Palo Alto, CA, USA). Samples for histomorphological analysis were taken from the middle of the duodenum and ileum intestinal segments and examined using an Olympus BX63 microscope, Olympus DP72 digital camera (Olympus Corp., Tokyo, Japan) and Image-Pro Plus programme system (Media Cybernetics, Inc., Bethesda, MD, USA). Data analysis was performed by SPSS for Windows, version 25.0 (IBM SPSS Inc., IL, USA, 2017). Our study results show that feed supplemented with butyric acid, medium-chain fatty acids and zeolite can significantly increase FCR by 6%, intestinal length by 4%, acetic and butyric acids contents, respectively by 21% and 23%, ileum villus height and crypt depth ratio by 2%, compared to standard compound diet (p<.05). We can conclude that the mixture of butyric acid, medium-chain fatty acids and zeolite can improve the development of the gastrointestinal tract, can increase SCFA's concentration in the intestine by enhancing the general broiler chicken intestinal status; it may also have a positive impact on the nutrient absorption activity by increasing suction area of the gastrointestinal and intestinal mucosa.

GAA IMPROVES BREAST WEIGHTS WITHOUT EXACERBATING BREAST MYOPATHIES THROUGH CHANGES IN ENERGY METABOLISM

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Breast muscle myopathies (BMM) are a common issue in fast-growing broilers. The feed additive guanidinoacetic acid (GAA) increases growth rate and final breast weights of broilers1-3, which are characteristics strongly associated with BMM. A high BMM incidence of \sim 85% is common in broiler production; however, at such a high incidence it is difficult to see whether a feed additive treatment may increase BMMs. This study was designed to test GAA in very low crude protein diets in in order to reduce BMM incidence in controls to a level where both an increase or decrease could be detected in the GAA treatment. We tested 340 male Ross 708 broilers with 17 birds/pen and 10 pens per treatment for 49 days. Broilers were protein levels ~2.5% fed corn-sov diets with crude below breeder recommendation, while meeting amino acid recommendations4. Pen body weights and feed were weighed on day 14, day 25, day 39 and day 49. One bird/pen was sacrificed at day 22 to take blood plasma samples from the right brachial vein for metabolomics. At day 49 birds were slaughtered in order to determine carcass, meat yield and BMM scores. On average GAA fed birds gained 91g more weight (ttest; p-value <0.05) and had 27g heavier breasts (t-test; p-value <0.05) by day 49. As expected, we found that heavier breasts had a higher BMM incidence (ANOVA, p-value < 0.01). However, breasts with same BMM score were significantly heavier in GAA fed birds (t-test; p-value <0.05) while GAA did not negatively affect proportion of BMM scores (Chi-Square test, p-value >0.05). Metabolomics of blood plasma showed no strong systemic effect of GAA (evaluated using PCA). Still, 4 out of 629 measured plasma metabolites change in response to GAA treatment: GAA, acetylcarnitine, methyl imidazolelactate and methyl thioadenosine. We conclude that GAA increased number of heavy breasts without increasing BMM scores. Therefore, GAA can increase breast weights without increasing the risk for BMM in the same proportion. We hypothesize that GAA causes these results through increased overall energy metabolism, increased methionine salvage metabolism and reduced protein degradation while not disturbing healthy normal metabolism.

Cordova-Novoa, H.A. et al. 2018. Science 97(7): 2494-2505 Poultry 1 2 2018. Poultry Science 97(7): Cordova-Novoa, H.A. et al. 2479-2493 3 Michiels, J. et al. 2012. Poultry Science 91(2): 402-412 4 AminoChick 2.0 (Evonik Operations GmbH)

EFFECT OF AGE/ADAPTATION TIME AND TWO LIMESTONE SOURCES ON RETAINABLE P IN YOUNG BROILERS FED DIETS WITH AND WITHOUT PHYTASE

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Literature shows that a coarser limestone source (LS) with low calcium solubility (Ca-sol) can result in higher phosphorus (P) absorption. Question was how a difference in LS can affect P retention (ret) after feeding diets for two different adaptation times (AT). This study determined the effect of 6 dietary treatments (trt) on apparent retention (ret) of P in broilers at two different ages, after a short (3d) and long (9d) AT with the same diets. Diets were based on a corn-sova with addition of phytate rich ingredients (rapeseed meal and sunflower meal). Negative control diet (NC) was formulated without an inorganic P source (2950 kcal/kg AME, 200g/kg CP, 3.2 g/kg phytate-P, 4.4 g/kg P and 5.1 g/kg Ca). The NC was supplemented with 1000 FTU/kg of a novel consensus bacterial 6-phytase variant (NC+PhyG) or with 0.6 g/kg P from monocalcium phosphate (used as positive control diet (PC) with 5.9 g/kg Ca). These three diets were made with a fine (<0.1mm) and a coarse LS (0.3-0.6mm) both with 38.5% Ca. Ca-sol (5 min at pH=3) was 97% for fine and 42% for coarse LS. All 6 dietary trt (+3.5 g/kg TiO2 as inert marker) were fed ad lib as pellets to Ross 308 male broilers (6 replicate balance cages/trt, 12 birds/cage), from 11-20 days of age. Excreta was collected at D13+14 and at D18+19+20 to determine ret P. Results were analysed by 3way ANOVA using AT, LS and diet as factors. There was no 3-way interaction present, but ret P was significantly affected by interaction between AT*diet (P<0.001) and LS*diet (P<0.001). Compared to 3d AT, ret P % was increased after 9d AT for PC (43 to 46%) and NC (41 to 47%), but decreased for NC+PhyG (69 to 63%, which may be due to requirement was met). As a consequence, the response of the phytase supplementation (compared to NC) was decreased from 28% units ret P at d14 to 16% units at d20. Compared to fine LS, ret P % was increased with coarse LS for PC (42 vs 47%) and NC (43 to 46%), but decreased for NC+PhyG (68 to 64%, may be due to the requirement was met). The response of the phytase supplementation (compared to NC) was increased from 18% units ret P with coarse LS to 25% units with fine LS. Results showed that a coarser LS may not result in a higher ret P, but this does not exclude a difference in ileal absorbed P. Although effect of AT is confounded with age of bird and thus their physiological status (e.g. adaptation and P requirement), it is concluded that AT and LS are important factors in phytase efficacy studies.

EFFECT OF TWO LIMESTONE SOURCES (FINE, COARSE) ON MINERAL ABSORPTION AND RETENTION IN YOUNG BROILERS FED DIETS WITH AND WITHOUT MCP AND A NOVEL CONSENSUS BACTERIAL 6-PHYTASE VARIANT

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Limestone sources (LS) with different calcium solubility (Ca-sol) can have an impact on phytase efficacy and broiler performance. This study determined the effect of two LS on mineral absorption (abs) and retention (ret) in young broilers fed diets with or without a novel consensus bacterial 6-phytase variant (PhyG) and monocalcium phosphate (MCP). A negative control diet (NC) rich in phytate-P (IP) was formulated without inorganic P (2950 kcal/kg AME, 200g/kg CP, 3.2 g/kg IP, 4.4 g/kg P and 5.1 g/kg Ca). The PhyG was added at 0, 250, 500, 1,000 and 2,000 FTU/kg to NC diets. NC was supplemented with 0.6, 1.2 and 1.8 g/kg P from MCP as positive control diets (PC1 to PC3) with 5.9, 6.6 and 7.4 g/kg Ca, respectively. These 8 diets (+3.5 g/kg TiO2) were made with fine (<0.1mm) or coarse LS (0.3-0.6mm) both with 38.5% Ca. Ca-sol (after 5 min at pH=3) was 97% for fine and 42% for coarse LS. Diets were fed ad lib as pellets to Ross 308 male broilers (6 balance cages/trt, 12 birds/cage) from 11-21 d and performance was measured. Excreta were collected at D18+19+20 to determine P and Ca retention. At D21, ileal digesta samples of all birds and tibia of 4 birds/cage were collected to determine P, Ca absorption and bone-ash content. Results were analysed via 2way ANOVA ($P \le 0.05$ considered as significant) with LS and diet as factors. Interaction effect was shown on all response parameters, except for abs Ca (q/kq). Compared to fine LS, abs Ca was lower (P<0.05) with coarse LS for all trt except NC, resulting in lower ret Ca except PC1,2 and NC. P abs was higher (P<0.05) with coarse LS for all trt. However due to low ret Ca, P ret was lower (P<0.05) with coarse LS for most of the trt except NC and PC1. As a result, tibia ash was lower (P<0.05) for all trt with coarse LS except PC1. Results for BWG were supporting the results for tibia ash and both can be related to ret Ca as limiting factor in diets with coarse LS. Maximal abs P with PhyG at 2000 FTU/kg was 3.63 and 3.36 g/kg for coarse and fine LS, respectively, but with PC3 it was 3.00 and 2.59 g/kg.Ca. MCP-P was maximal 58-60% absorbed regardless of LS, (maybe due to adaptation, the ANF effect and Ca content. It is concluded that compared to fine LS, the coarser LS resulted in higher abs P regardless of addition of phytase or MCP-P to the diets. However, to have subsequently benefit for tibia-ash and BWG this is depending on the Ca level in the diet which should be higher for coarser LS.

CONTROLLING AMINO ACIDS AT THE LEVEL OF THE RECOMMENDATIONS IN STARTER AND GROWER PHASES IMPROVES PERFORMANCE AND BONE QUALITY IN BROILER CHICKENS

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Slight deficiency in amino acids during the starter and grower phases can impair performance and bone development in broiler chickens. Among amino acids, threonine (precursor of glycine, the most abundant amino acid in collagen), arginine (involved in hydroxyapatite nucleation) and valine and isoleucine (major components of mineral transporters) seem critical for bone development. The current study aimed at evaluating the effect of controlling these amino acids at the level of the recommendations in starter and grower phases on performance and bone quality in broiler chickens. Six hundred (600) newly hatched male broiler chicks of commercial strain (Ross 308) were allocated to two treatments with 15 replications (20 birds per replication). Broiler chickens from treatment 1 (100% AA) were fed starter and grower feeds (from day 0 to day 21) where all amino acids followed the recommendations of METEX NOOVISTAGO. Broiler chickens from treatment 2 (95% AA) were fed starter and grower feeds where threonine, arginine, valine. and isoleucine were slightly deficient (95%) of the recommendations of METEX NOOVISTAGO) while other amino acids were supplied at the level of the recommendations. During the finisher phase (day 21 to day 35), broiler chickens from both treatments received the same commercial feed. Zootechnical criteria (average daily feed intake [ADFI], average daily growth [ADG] and feed conversion ratio [FCR]) were calculated. At the end of the trial bone quality parameters (tibia weight, tibia stiffness, mineral content) were assessed. Data were analyzed by ANOVA followed by post-hoc Tukey tests. When considering the total trial period, supplying amino acids to the level of the recommendations tended to improve ADG (P=0.098) without altering ADFI (P=0.550) which translated into an improvement of FCR (100% AA: 1.55 vs 95%) AA: 1.58; P=0.019). Interestingly, tibia length was numerically heavier (P=0.173), tibia stiffness was significantly higher (100% AA : 106.79 N/mm vs 95% AA : 99.53; P=0.019) and mineral content significantly lower (100% AA : 22.00% vs 95% AA : 22.68% ; P=0.027) in broiler chickens from treatment 1 (100% AA) compared broiler chickens from (95%) AA). to treatment 2 In conclusion, this study shows that controlling amino acids at the level of the recommendations improves performance and bone quality parameters in broiler chickens.

ENHANCEMENT OF INTESTINAL ANTIOXIDANT CAPACITY BY MODIFYING GENE EXPRESSION OF ANTIOXIDANT AND KEY DETOXIFICATION ENZYMES IN BROILERS FED VERNODALIN

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The present study investigated the effects of dietary vernodalin supplementation on tissue distribution of vernodalin and its metabolites, intestinal antioxidant capacity, and expression of detoxification-related genes in broiler chickens. A total of 720 male day-old Cobb500 chicks were randomly assigned to 4 dietary groups each consisting of 6 replicates of 30 birds in a single factorial design. Birds in the control group were fed a basal diet and the others were fed a basal diet supplemented with 200, 400 or 800 mg/kg vernodaline. The experiment lasted 21 days. Vernodalin was present at 11.08 to 14.15 mg/g in the caecal digesta, 64.70 to 467.21 μ g/g in the jejunal mucosa, 32.21 to 69.16 μ g/g in the liver, and 8.17 to 9.54 µg/mL in the plasma. Jejunal and hepatic vernodalin levels were significantly (P < 0.05) increased in response to supplementation with 400 and 800 mg/kg vernodalin, respectively, compared with the 200-mg vernodalin/kg group. There was a linear effect (P < 0.001) of dietary vernodalin on the relative abundance of SOD1, GPX1, CAT, HO-1, and Nrf2 transcripts, and a quadratic increase (P < 0.001) in GSH-Px and T-AOC activities in the jejunal mucosa. The expression of CYP1A4, CYP2D17 increased and CYP1B1, CYP2A6 decreased linearly (P < 0.001) with dietary vernodalin concentrations. In addition, dietary vernodalin increased gene expression of GST, MRP6, and ABCB1 in the jejunal mucosa. In conclusion, dietary vernodalin supplementation of 200 to 800 mg/kg increased the accumulation of vernodalin and its metabolites in the jejunum as well as the antioxidant capacity and detoxification potential, which play a major role in protecting the broiler gut from damage.

Key words: Vernodalin, tissue distribution, intestinal antioxidation, cytochrome P450, broilers.

POSITIVE IMPACT OF A TRIPLE STRAIN BACILLUS-BASED PROBIOTIC ON INTESTINAL BIOMARKERS AT 7 DAYS OF AGE

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A controlled study comparing 3 treatment groups of 180 male chicks (control-CTRL, lincomycin-Linco and a triple strain bacillus-based probiotic Prob_3S) was conducted. The aim was to evaluate the impact of the different additives during the early life at 7 days of age. The study was done on multiple intestinal biomarker measurements. The relative gene expression of MUC2 in duodenum and jejunum from the Prob 3S group showed higher gene expression in comparison to CTRL and Linco treatment groups (p<0,05). However, a significant difference could be demonstrated in jejunum for Prob 3S and Linco groups (p<0.05). These findings indicate that Prob_3S significantly improve the gene expression of MUC2 at 7 days old chicks. TLR4 expression in chicks from Prob 3S treatment group was significantly stronger (p<0,05) in comparison to CTRL and linco treatment group, either in duodenum or in ileum. No effect, however, could be demonstrated between linco and CTRL groups (p>0,05). The interleukin 2 (IL2) gene expression at 7days old chicks in duodenum and jejunum has shown that Prob_3S group showed lower gene expression in duodenum in comparison with control and linco group. On the other hand, Linco group had a higher IL2 expression in comparison to Prob 3S (P<0,05) and CTRL (P<0,05). However, this pattern changes in the duodenum, where the highest expression of IL2 was demonstrated in Prob 3S group (P<0,05). Interleukin 6 (IL6) expression were stronger in Prob_3S and linco groups in comparison to CTRL, either in duodenum or in jejunum (P<0,05). The gut integrity biomarkers, OCCU, JAM and VEGF for duodenum and jejunum revealed that chicks from Prob 3S group showed stronger OCCUD expression in comparison to others groups in jejunum (P<0,05). However, this effect could not be demonstrated in duodenum. Similar, it could be also seen as a stronger expression of JAM in jejunum of Prob_3S chicks in comparison to Linco (P<0,05) and CTRL (P < 0.05), but no difference between groups could be demonstrated in jejunum (p>0,05). The VEGF expression was stronger in Prob 3S group in comparison to CTRL group (P<0,05) in jejunum, but no significant difference was demonstrated in duodenum (p < 0,05). These different findings prove that supplementation of feed additives such as combination of probiotic strain can have a beneficial direct effect on key intestinal biomarkers as early as 7 days of age. Prob_3S can influence the quality of the epithelium integrity on the intestine.

EFFECT OF THE ADMINISTRATION OF HIGH DOSES OF PHYTASE IN BROILERS FROM 1 TO 21 DAYS OF AGE IN PERFORMANCE AND INTESTINAL PHYSIOLOGY

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The supplementation of feed with dietary phytases enables monogastric animals to utilize the phosphorus that is bound to phytate in plant-based ingredients. The use of high doses of phytase is reported to have a positive effect beyond the improvement of nutrient utilization. The aim of the experiment was to investigate some of the potential effects on broilers fed a basal diet supplemented with increasing doses of phytase in the period from 1 to 21 days of age. A total of 112 one-day old broilers were assigned for a period of 21 days to four dietary treatments receiving a diet based on corn, soybean meal and sunflower meal without (T1) or with the supplementation of Natuphos® E (BASF SE, Germany) at levels of 500, 1,000 and 3,000 FTU/kg (T2-T4). Body weight (BW) and feed intake were registered weekly. On day 21, birds were slaughtered and ileum digesta was collected for determining apparent ileal digestibility (AID) using TiO2. Left tibia bone was excised for measuring mineral levels. Jejunum and caecum tissue were collected for morphometrical measurements and count of CD3+ intraepithelial lymphocytes (IELs). Results were statistically evaluated on SPSS using a one-way ANOVA with animal as random effect and treatment as fixed effect. We observed a clear effect of the administration of phytase on overall body weight gain with increases of 14.2% (T2), 16.6% (T3) and 30.9% (T4) (p<0.001) and an improvement in feed conversion rate by 6.7% (T2), 7.3% (T3) and 11.9% (T4) (P<0.001). The AID of gross energy, crude protein, crude ash and phosphorus increased with the addition of the phytase (P<0.001) as well as the AID of most of the amino acids. Tibia mineralization increased with the addition of phytase (P<0.001). Villus length in jejunum tissue (related to BW) tended to be lower in the phytase groups (P=0.073) and crypt depth related to BW decreased in parallel to the increase of phytase application (T2: -15.8%, T3: -19.8%, T4: -30%) compared to T1. Crypt depth related to BW in caecal tissue decreased by -18.3% in T2, -16.2% in T3 and -31.7% in T4. The number of CD3+ IELs (expressed in 10,000 μ m2) in caecum crypts decreased by supplementing graded levels of phytase (T2: -27.7%, T3: -37.2%, T4: -39.2%). Thus, we can conclude that the addition of graded phytase levels to broiler feed showed a positive effect on intestinal morphology and immune response, in parallel with an improvement on nutrient efficiency, compared to the basal diet.

THE EFFECT OF 2 YEAST CELL WALL PRODUCTS SUPPLEMENTATION ON BROILER PERFORMANCE, WHOLE BODY AND MEAT COMPOSITION AND QUALITY, INTESTINAL HEALTH AND WELFARE PARAMETERS.

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In a context moving towards ATB-free livestock production, research in feed additives has largely increased to evaluate different products on animal health, besides animal productivity. Among them, yeast cell wall (YCW) extracts have been widely studied. In this study, the effect of 2 YCW products was evaluated on broiler performance, whole body and meat composition and quality, intestinal health and welfare parameters. A total of 450 chicks were allocated into 3 groups with 10 replicates each: control (C) or 2 YCW products (OptiWall, Lallemand: O or commercial product: P at 1 kg/ton feed). Body weight (BW), average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) were recorded for starter (1-10d), grower (11-24d), and finisher periods (25-35d: F). At the end of the study, intestinal samples were collected for histomorphometry (villus height: VH, crypt depth: CD, VH/CD ratio) and immune organs were weighed. Whole body and meat (breast, thigh) chemical composition (protein, fat, dry matter (DM), ash) and carcass yield (CY) was evaluated, as well as meat quality through lipid oxidation (MDA level) after 4d of refrigeration storage. Additionally, welfare parameters were investigated: litter DM, fecal DM, litter ammonia (NH3) and litter pH. O tended to improve BW and ADG, compared to control group, P being intermediate (P<0.1). Differences between groups were mainly observed in F period. FI was reduced in O and P compared to C in F period (P<0.05). FCR was thus improved for O and P compared to C in the total period (P being intermediate in the finisher phase) (P=0.001). CY was increased in O and P compared to C (P<0.05). Interestingly, the better zootechnical performance of O translated also into higher breast protein (P<0.1) and DM content (P<0.001). Meat MDA level was reduced in O and P compared to C (P<0.001). The absorption capacity of the nutrients in the GI tract (VH/CD ratio) and the number of goblet cells was mainly improved in duodenum and ileum by O and P, respectively (P<0.1). O and P stimulated the peripheral immunity with an increase of spleen weight compared to C (P<0.05). O and P had positive effects on litter DM at d35 and fecal DM at d10 (P<0.1), while decreasing litter NH3 at d24 and 35 and increasing litter pH at d35, the strongest effect being observed with P (P<0.05). O and P supplementation can therefore support broilers performance likely through positive effects on gut health, muscle quality and welfare parameters.

MYO-INOSITOL CONCENTRATIONS IN THE DIGESTIVE TRACT AND BLOOD OF BROILERS AND TURKEYS AS INFLUENCED BY MCP AND PHYTASE SUPPLEMENTATION

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Myo-inositol (MI), an end product of phytate (InsP6) dephosphorylation, is involved in many metabolic processes and might affect performance of broilers. An increase of MI concentration in the digestive tract and in blood due to phytase supplementation has been found in broilers. Data for turkeys, however, is very limited. Thus, the aim of this study was to compare broilers and turkeys regarding MI concentrations in ileal digesta and blood as influenced by phytase, P, and Ca content of the diet. The experiment had a 2x2x2-factorial arrangement of treatments with 2 poultry species, 2 Ca and P levels, and 2 phytase levels. Diets were formulated to meet recommendations for turkeys (except for P and Ca) and were supplemented without (CaP-) or with (CaP+) monocalcium phosphate, and without (Phy-) or with (Phy+) 1500 FTU phytase/kg. At 15 days of age, birds were allocated to 24 pens per species and fed the experimental diets for 7 d. Following decapitation on d 21, trunk blood, mucosa samples of the jejunum, and digesta from the terminal ileum were obtained. Blood was analysed for MI, digesta was analysed for InsP6, MI and TiO2. Mucosal phosphatase activity was assayed after brush border membrane enrichment. Data were subjected to three-way ANOVA. Mucosal phosphatase activity was only affected by phytase, being higher in Phy+ than Phy- (P = 0.043). Prececal InsP6 disappearance did not differ between broilers and turkeys in CaP-Phy- but was higher in turkeys than broilers in CaP+Phy-. In all Phy+ treatments, broilers had higher prececal InsP6 disappearance than turkeys, with an overall lower level in CaP+ (P = 0.013). MI in ileal digesta of broilers was higher in Phy+ than Phy-, with highest concentration in CaP-Phy+. In ileal digesta of turkeys, MI concentration overall was low and only slightly higher in CaP-Phy+ than in other treatments (P < 0.001). MI concentration in the blood of both species was lowest in CaP+Phy- and increased in CaP-, Phy+, or both (P < 0.001). With added phytase more InsP6 was degraded and resulting lower InsP in the anterior digestive tract might have triggered mucosal phosphatase activity and thus explain the observed higher activity in Phy+. Blood MI concentrations did not differ between species to the same extent as MI concentrations in the ileum. One explanation is a potentially higher absorption and use of MI in enterocytes of turkeys or a faster transport of MI to organs and tissues of turkeys.

INVESTIGATING THE POTENTIAL OF REDUCED PARTICLE SIZE WHEAT BRAN AND ENDOXYLANASE AS BROILER FEED ADDITIVES TO INCREASE ARABINOXYLAN HYDROLYSIS AND FERMENTATION IN BROILERS

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With the ban of prophylactic antibiotics in feed, scientists are searching for new strategies to address the challenges it brings. Stimulating the intestinal microbiota by increasing its fermentation capacity toward the dietary fibre fraction of the feed is an emerging strategy to improve animal health and performance. Feed additives such as enzymes and prebiotics contribute to this. However, the majority of these additives fail to increase the proportion of dietary fibre that enters the caecum as the majority of insoluble fibre is too large to enter. Reduced particle size wheat bran (RPS-WB) is in this sense a potentially interesting and rich source of easily accessible arabinoxylan (AX). The objective of this study was to investigate the potential of RPS-WB and endoxylanases to trigger AX hydrolysis and fermentation along the hindgut of broilers. To this end, RPS-WB and endoxylanase supplementation were evaluated in a 2×2 factorial broiler trial using 256 male 1day-old chicks (Ross 308). The 4 dietary treatments consisted of a basal wheatbased diet with (1) no feed additives (control, CNT), (2) 100 ppm of Econase XT25 (an endoxylanase product from AB Vista, Marlborough, UK) (XYL), (3) 1% wheat bran with an average reduced particle size of 300 µm (RPS-WB) and (4) 100 ppm of Econase XT25 and 1% RPS-WB (XYL+RPS-WB). On d 10 and 28, 24 and 16 broilers per treatment, respectively, were euthanised to follow up AX degradation and short-chain fatty acid production in the GI tract. Data were statistically analysed using a three-way ANOVA with broiler age, XYL and RPS-WB and their second-order interactions as model effects. Compared to the CNT group, broilers receiving XYL in their diet showed increased AX solubilisation and fermentation throughout the broiler's life (P < 0.05). Adding RPS-WB to the diet stimulated AX digestion at the level of the caecum at 11 d of age compared to the CNT group, possibly illustrating a better accessibility of AX in RPS-WB compared to ground wheat (hammer milled) to the primary AX degraders (P < 0.05). At 28 d, RPS-WB addition seemed to affect body-weight gains negatively but increased butyrate levels compared to the XYL and CNT group (P < 0.05). Although no synergistic effect for RPS-WB+XYL broilers was observed for AX hydrolysis and fermentation, these findings suggest that both additives can benefit the broiler as a butyrogenic effect and improved AX degradation along the hindgut were observed throughout the broiler's life.

THE IMMUNOMETABOLIC EFFECTS OF BUTYRATE IN CHICKEN SMALL INTESTINES AND MACROPHAGE-LIKE CELLS.

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Butyrate and other short chain fatty acids (SCFA) are produced by many bacteria in the gut and these SCFA influence energy metabolism of a variety of cells. Studies have shown that SCFAs, like butyrate, aid in the regulation of inflammation and immunity. Past research has shown that the incorporation of these SCFA in feed can help boost immune responses to fight off pathogens. The objective of this project was to examine the immunometabolic impact of butyrate in chicken small intestines and macrophage-like cells (HD11). Kinome peptide array analysis was performed on jejunum and ileum samples of broiler chickens at d 21 and d 35 supplemented with 0.1% of sodium butyrate (SB) in the total feed. The results showed significant changes in the phosphorylation of HIF1-alpha, T cell receptor, mTOR and other immunometabolic pathways (p-value ≤ 0.05). Trypan blue exclusion assay showed that treatment of HD11 with 16 mM of SB for 6 hours induced significant changes in cell viability. Using the Seahorse XFp analyzer, a metabolic flux assay measuring the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) was performed on HD-11 cells with and without pretreatment with 16mM butyrate for 6 hours. These cells were later infected with Salmonella Enteritidis (SE) or Salmonella Heidelberg (SH) at a MOI of 1:100. The results showed no difference in OCR of uninfected cells treated with SB compared to control. However, the ECAR of HD11 cells treated with SB was significantly higher than control. The increase in ECAR in butyrate treated cells is a sign of a pro-inflammatory response. Metabolic flux results of SE and SH infected cells post-butyrate treatment showed an increase in ECAR and OCR of uninfected cells compared to infected cells. SH infected cells post-butyrate treatment showed a significant increase in ECAR compared to SH infection alone. Using an alamarBlue cell viability and proliferation assay, we saw clearance of infected cells via decrease in absorbance at 570 nm when cells were treated with 4 mM (p=0.0443) or 16 mM (0.0758) of SB before SH infection. Anova and t-tests with a p-value cut off of 0.05 were used in this study. Here we show data that indicates butyrate increases activity of proteins in immunometabolic pathways which increases inflammatory responses and aids the host in fighting off infections.

THE INFLUENCE OF HYDROLYSABLE CHESTNUT TANNINS ON GROWTH AND INTESTINAL DEVELOPMENT IN BROILERS

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It has been known that high doses of various tannins could impair broiler growth, mainly linked to a lowered protein availability. However, effects on protein digestion under the influence of hydrolysable tannins (HT) were minimal in previous research and literature. Other possible proposed reasons to explain reduced growth are scarce. In this experiment we studied the effect of adding HT to the feed in different combinations of growth phases on body allometry. In total 112 individually reared male Ross 308 broilers received a three-phase basal diet with chestnut wood extract (+: 2000 mg/kg) or not (-: 0 mg/kg) (Tanno-SAN®, Sanluc International NV, Belgium). This resulted in 2 groups during the starter period (S+, S-), 4 groups in the grower period (G++, G+-, G-+, G--) and 8 groups in the finisher period (F+++, F++-, F+-+, F+--, F-++, F-+-, F--+, F---) each including 8 animals. Similar to literature, growth reduction was also confirmed in this study. Effects were the largest in animals that were given the tannins during the grower phase at the end of the grower and finisher. At the end of each phase 8 animals per group were euthanized and sampled. Liver weight, pancreas weight, intestinal weight, intestinal length and pectoralis muscle weight were recorded. The largest effects were seen on intestinal size. Animals that received tannins during the grower phase, had longer intestines at the end of the finisher period. Furthermore, histological differences between treatment groups were observed at the end of the grower. Addition of tannins in the grower phase (G_{+}, G_{+}) resulted in longer villi, whereas addition of tannins in the starter (G+-, G++) caused deeper crypts at the end of the grower phase, with the group (G -+) having the highest villi crypt ratio. Little effects were observed at the end of the starter and finisher phases. These results tentatively prove that tannins influence intestinal growth, both macroscopically as well as histologically. We hypothesize that the observed growth reduction with tannins could be the result of a changed energy and nutrient partitioning i.e. more nutrients are invested in intestinal growth than for muscle growth.

REDUCING CALCIUM LEVELS WHILE USING A MARINE MINERAL COMPLEX ENHANCES GROWTH PERFORMANCE OF BROILERS

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Reducing calcium (Ca) levels in broiler diets have been well investigated, showing an enhanced performance but also bone strengths issues. Novel strategies are required to ensure optimal Ca supply and bone formation, while improving growth performance. One of these strategies could be using a marine mineral complex (MMC) derived from the red seaweed Lithothamnion glaciale. The high bioavailability of this product compared to traditional Ca sources such as limestone performance gives an opportunity to enhance growth of broilers. The objective of this study was to investigate the effect of reducing Ca levels of the diet on bird performance, based on limestone or partial replacement of this with MMC. A total of 2240 male broilers (Ross 308) were randomly assigned to 4 treatments (n=16): 1) Positive control (PC, Standard Ca); 2) Negative control (NC, low Ca); 3) Low Ca + 0.2% MMC; 4) Low Ca + 0.4% MMC. The PC treatment was based on Ross 308 recommendations of 0.9% Ca in the starter phase. Treatments 2 and 3 had a Ca level of 0.8% Ca in the starter phase, while treatment 4 had a Ca level of 0.70% in the starter phase. For all treatments, grower and finisher Ca levels were reduced by 0.1%. The MMC was included at the expense of limestone. Total tract nutrient digestibility was determined during the grower phase (day 21) for all pens. Results were analysed as a Complete Randomized Design using the GLM procedure of the Statistical Analysis System (SAS; SAS Inst. Inc. Cary, NC). The dietary treatments did not affect feed intake (P>0.05). The 0.4% MMC treatment group had an improved body weight gain (BWG) between day 29-42 compared to the NC group: 1667 g vs. 1597 g (SEM 18.5 g; P=0.038), but this was not different from the PC or 0.2% MMC. Birds fed either 0.2% or 0.4% MMC had significantly (P=0.037) better overall FCR compared to the NC treatment: 1.471 and 1.473 for the 0.2% and 0.4%, respectively, compared to 1.492 for the NC treatment (SEM 0.0005, P=0.007), whilst this was not different from the PC treatment. Birds fed 0.4% MMC had higher apparent retention of calcium compared with other treatments (68.3%); birds fed 0.2% MMC (59.4%) and NC (57.0%) had higher apparent retention of Ca than birds fed the PC diet (47.4%; SEM 2.510; P<0.0001). The results of this study indicate that reducing of Ca levels and including MMC will improve performance and feed efficiency of broilers, while increasing Ca retention in comparison to traditional Ca levels.

THE COMPARATIVE EFFICACY OF TWO DIFFERENT NEW PHYTASES AT VARYING DOSES ON 12-DAY OLD BROILER PERFORMANCE AND BONE ASH

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Phytases are supplemented to poultry diets to hydrolyse phytate, improve digestibility of P and thereby decrease P excretion and feed cost. It has been shown that the in vivo efficacy can differ between 6-phytases, leading to differences in the slope and asymptote in the response in digestible P with increasing phytase dose. Therefore, the objective of this study was to compare the efficacy of two phytase enzymes in young broiler diets on performance parameters (body weight (BW), body weight gain (BWG) and FCR) as well as tibia ash weight (mg/g bone). A novel consensus bacterial 6-phytase variant expressed in Trichoderma reesei (PhyG) was compared against hybrid phytase expressed in Aspergillus niger (PhyH). A total of 1246, 4-day old Ross 308 male broilers were randomly assigned to one of 12 treatments (14 birds/cage, 12 replicates for treatments 2-14 and 12 replicates for the negative control (NC)). A crumble corn-soy-based NC diet, free of supplemental inorganic P (1.2 g/k rP (retainable P), 3.4 g/kg P and 0.70% Ca) and supplemented with each phytase product based on analysed phytase units (FTU) at the five dose levels (500, 1000, 1800, 2500 and 3500 FTU/kg) to determine a dose response. The results were then compared to a positive control (PC) diet, which consisted of the NC diet + monodicalcium phosphate (3.1 g/kg)rP, 5.5 g/kg P and 7.2 g/kg Ca). BW, BWG and FCRm were determined for the trial period (4-12 days). On day-12, the right tibia from 10 broilers per cage were sampled and pooled to determine fat free bone ash weight (mg/g bone). Bone ash, BW, BWG and FCRm were plotted against analysed phytase dose using JMP statistical package according to the equation: y=a+b*cx. The PhyG phytase exhibited a higher asymptote (270.18 mg/g bone) compared to the PhyH phytase (254.69 mg/g bone) for tibia ash weight. Both products exhibited similar asymtotes for BW (352.52 vs 356.10 g) and BWG (246.81 vs 246.80 g). However, PhyG phytase was able to achieve its asymptote at a lower phytase dose compared to PhyH (give FTU) due to its steeper gradient. Furthermore, PhyG exhibited a 7 points improvement in its asymptotic value for FCRm 1.68 vs 1.75). The results from this study suggest large differences in the in-vivo efficacy of newly developed phytases based on differences in the gradient and asymptote of the phytase dose response in bone ash, BW, and FCRm.

EFFECTS OF VARIOUS FIBRE SUPPLEMENTATIONS ON THE ILEAL MORPHOLOGY AND THE IMMUNOLOGICAL PROFILE IN THE CAECA OF BROILERS

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Depending on fibre quantity and physicochemical characteristics, even moderate amounts of dietary fibre can influence the functionality of the intestinal tract of broiler [1]. The epithelial mucosa may be positively affected especially by the insoluble proportions of fibre, whereas the expression of inflammation related cytokines may be modulated by the soluble ones. Both effects might further lead to improved performance. The aim of this study was to examine the effect of fibre sources with different physicochemical properties added to low-fibre diets and their impact on the ileal morphology and the expression of NF-kB and TNFa in the ceca of broilers. A total of 5,040-day-old broiler chickens (Ross 308) were reared for 36 days. The animals were randomly allotted to one of four iso-caloric and isonitrogenous treatments. The control group received a commercial diet without supplemented fibre source. Experimental diets either included 0.8 % of a standard lignocellulose product, 0.8 % of a new generation lignocellulose product or 1.6 % of soybean hulls as fibre source. Distal ileal tissue samples were excised, paraffin embedded, sectioned via microtome (5µm) and stained with Alcian-Blue and periodic acid–Schiff. Six villi, crypts and muscular layers of each sampled animal were examined with computerized light microscopy. Statistical analysis of the gathered data was conducted using Statistical Analysis Software (SAS ® version 9.4.). Caecal tissue samples were collected for the evaluation of the expression of NF-kB and TNFa. After RNA extraction (TriReagent®) and cDNA synthesis (QuantiTect® Reverse Transcription Kit) quantitative PCR was performed on Rotor-Gene® 0 with SYBR® Green PCR Kit following manufacturer recommendations. The supplemented dietary fibre showed a significant influence on the ileal morphology. The experimental diets with additional fibre showed significant higher villi and deeper crypt compared to the control treatment (p < p0.05), as well as significant higher body weight in grower phase (p < 0.05). The NF-kB and TNFa expression of remained unaffected. In conclusion, positive effects on performance in grower phase as well as on ileal morphology were observed when dietary fibre was supplemented to a low-fibre diet regardless of their physicochemical properties.

1. Mateos, G., Jiménez-Moreno, E., Serrano, M., Lázaro, R. (2012) Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. JAPR 21, 156–174.

THE EFFECT OF PROBIOTIC SUPPLEMENTATION ON CALCIUM METABOLISM AND BONE RESISTANCE AND MINERALIZATION IN OLD LAYERS.

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The close link between intestinal microbiota and bone health ('gut-bone' axis) has recently been revealed: the modulation of the amount and nature of bacteria present in the intestinal tract has an impact on bone health and calcium (Ca) metabolism (Li et al., 2021; Tu et al., 2021). The objective of this study was to investigate the effect of a probiotic (P. acidilactici, CNCM I-4622, Lallemand: PA) on laying performance, egg quality, Ca metabolism and bone strength in old layers (50 wks old) during 14 wks, 480 Hy Line brown layers were divided into 2 groups (3 layers/rep, 80 rep/group) which were fed with a diet formulated to be suboptimal in Ca and phosphorus (P) (-10% of the requirements), containing PA at 100 g/ton feed or no probiotic (control: C). The laying rate (LR), the weight of marketable eggs, the feed conversion ratio (FCR), the % of downgraded eggs (dirty, broken, soft, bloody eggs) were recorded weekly, while egg quality (Haugh units: HU and eggshell weight %) was measured every 2 weeks. The Ca and P digestibility, the parameters related to bone (hardness, cohesiveness, Ca, P) and blood biomarkers related to Ca metabolism (Ca, P, calcitriol, osteocalcin, cortisone, PTH, ALP) were measured at start and end of the study. Parameters were analyzed by a mixed repeated-measures statistical model with the initial data entered as covariate. There was no difference between the 2 groups for the laying rate, while PA increased the weight of marketable eqgs by 0.9% (P<0.001), improved FCR by 1% (P<0.05) and reduced the % of downgraded eggs by 0.4 pts (P<0.1). HU and the eggshell weight % were increased respectively by 6.3% (P<0.05) and 0.3 pts (P<0.1) with PA. Ca and P retention were increased by 4 pts following PA supplementation (P<0.05), translating into increased bone hardness (+19%, P=0.1), bone cohesiveness (+43%, P<0.05) and bone Ca and P (P<0.05) for PAsupplemented layers. Blood Ca and P were respectively increased by 5% and 12% with PA (P<0.05). Blood calcitriol (promoter of Ca absorption) and osteocalcin (biomarker of osteoblast activity) were increased respectively by 83% (P<0.05) and 3% (P<0.1) with PA, while there was no difference between the 2 groups for PTH, ALP and cortisone. Overall, the improvement of Ca and P absorption with PA resulted in better bone mineralization, illustrated by better bone resistance, higher eggshell weight and reduction of downgraded eggs, which can positively impact the longevity of the production cycle.

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Protein nutrition

Selected short communications

CORRELATION OF 7-14 D PERFORMANCE METRICS WITH D 15 ILEAL AMINO ACID DIGESTIBILITY (AAD) WHEN BIRDS ARE FED STARTER FEED VARYING IN AVERAGE PARTICLE SIZE (APS)

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A 0-14 d companion study utilized starter feed that varied in average particle size (APS). Complete feed particle sizes were 1174, 1423, 1883, 2049, 2257, 2800, 3456, and 3736 µm. The feed form treatments were also defined by their percent Pellet:Crumble:Fine (P:C:F) ratios and consisted of 0:54:46, 0:65:35, 2:71:27, 10:73:17, 31:58:12, 67:23:10, 70:18:12, and 76:15:9. From d 0-14, FCR improvements were found when broilers were provided an APS >2257 µm (31:58:12, P:C:F); BW and BW gain benefits were also observed for broilers fed >2800 μ m (67:23:10, P:C:F). The current objective was to determine the effects of APS on ileal AAD and its relationship to performance and intestinal metrics realized in the companion study. A sub-sample of pens were used, where 960 Ross x Ross 708 male broilers were equally allocated to 48 pens (0.06 m2/bird). The nutritionally common diet (only varying in APS/P:C:F ratio) contained TiO2 (3.0 q/kq) and was fed from d 0-14. On d 15, one bird/pen was randomly selected for ileal digesta collection for determination of AAD. Ileal digesta was lyophilized and sent to a laboratory for amino acid (Lys, Met, Thr, Phe, Cys, Phe, Trp, Val, Ile, Leu, His, Arg, Asp, Ser, Glu, Ala, Pro, and Tyr) and TiO2 analysis. Data demonstrated similar AAD among all APS/P:C:F ratios except for birds fed 2049 µm (P:C:F ratio of 10:73:17), that had decreased AAD. Day 7-14 data from the companion study may help explain these differences. As such, there was a negative correlation of AAD with d 7-14 FCR for Ile and Leu (P=0.05, r=-0.352 and -0.353), in which AAD increased as FCR decreased. In addition, there were negative correlations of AAD with Ileum weight and pH for Ala, Glu, and Ile (P=0.04, r=-0.441 to -0.482), with there being an increase in AAD as Ileum weight and pH reduced. We hypothesize that due to the P:C:F ratio (10:73:17) of the 2049 µm APS treatment, a breakpoint occurred for birds from 7-14 d, whereas that distribution (specifically the % C) reduced AAD via possible nutrient segregation and/or passage rate/rate of digestion. Perhaps, the smaller particle feed had enhanced digestion due to increased surface area for enzymes and larger particle feed had increased digestibility due to increased feed retention time in the gastrointestinal tract. Future research should investigate the passage rate, AAD, gastrointestinal tract effects and resulting performance of birds fed complete feeds that are sieved by complete feed particle size.

SEVERE DIETARY CRUDE PROTEIN, SOYBEAN MEAL AND ENERGY REDUCTIONS AND THEIR INFLUENCE ON PERFORMANCE & CARCASS PARAMETERS OF BROILERS

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The formulation of new nutritional strategies for broilers with strongly reduced crude protein (CP) and soybean meal (SBM) is yet to be explored, especially in order to achieve a strong improvement in nitrogen efficiency and to meet sustainability targets. In general, reduced dietary CP levels result in increased body fat deposition, and one of the potential ways to counteract this is to reduce dietary energy in low CP diets. In an experiment from 14 to 34 days of age, 324 Ross 308 male broilers were randomly allocated into 6 dietary treatments with 6 pens per treatment. Experimental diets were T1 = control at 20.6% CP with 24%-SBM; T2 = 18.6% CP & 0%-SBM and extra L-Thr to compensate low Gly+Ser; T3 = 16.6% CP & 0%-SBM with addition of L-His, L-Leu, L-Phe, L-Cys and Gly to the level of the control treatment; T4 = T3 with removal of L-His, L-Leu, L-Phe, L-Cys and Gly; T5 = T3 with a decrease of energy by 50 kcal/kg and T6 = T3 with a decrease of energy by 100 kcal/kg. All diets were iso-digestible lysine (TFD Lys =1.13%) and T1 to T4 were iso-energetic (AMEn = 2,875kcal/kg). All indispensable amino acids (AA; and some conditionally indispensable) were at least at the level of the assumed requirements. SBM was replaced by mainly sunflower, rapeseed and some corn gluten meals. Average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR), final body weight (BWf), relative abdominal fat (AF) and breast meat yield (BMY) in ratio to body weight were evaluated. There was a significant treatment effect on all performance indicators (p<0.01). ADG and BWf were significantly lower in T4 compared to T1 (-12g of ADG and -252g of BWf) and FCR was significantly higher in T4 compared to T1 (+0.08 FCR). Reduction of energy by 100kcal (T6) showed a similar BWf than T1 but a numerically increased feed intake and a significantly increased FCR (+0.05). In terms of carcass characteristics, T4 resulted in a significantly lower BMY (-2.5%) and higher AF (+0.25%) compared to control. The study demonstrated that lowering dietary CP by 2 or 4%pts from 20.6 down 16.6% together with a full removal of SBM did not affect growth performance or carcass characteristics when all AA were supplemented. Moderate reduction of dietary energy content in a severely reduced dietary CP was able to maintain growth performance, indicating that a reduction in dietary level is needed in diets with strongly reduced protein levels in order to maintain body weight gain.

EXPERIMENTAL VALIDATION OF A PRECISION FEEDING STRATEGY TO REDUCE FEEDING COST AND NUTRIENT EXCRETION IN BROILERS

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In broiler production systems, precision feeding is an alternative solution to the classical phase feeding strategy to reduce feeding cost and nutrient excretion without impairing animal performance. This strategy consists in the daily adjustment of diet composition to meet the nutritional requirements of the flock (energy, protein, and lysine). To this purpose, a decision support system (DSS) based on several mathematical models was developed to evaluate the appropriate nutrient supply for the next day, using actual body weight (BW) data of the flock [1]. An experimental trial was conducted to demonstrate the relevance and benefits of this tool. The trial involved 912 as-hatched Ross 308 broilers (sex ratio 50:50) randomly distributed in 24 pens at 10 days of age. Three feeding strategies were tested (8 pens/strategy): 1/ a classical growing-finishing diet (Control, C), 2/ a daily-multiphase strategy (DMP) composed of a blend of two pre-diets changing every day and calculated prior to the trial, and 3/ a precision feeding strategy (PF) composed of a blend of two pre-diets daily adjusted according to the DSS using actual growth performance of the flock. Eight animals per pen (four males, four females) were individually weighted every day to assess the average BW for each strategy. Feed intake per pen was also measured each day. Using the average body weight of the PF birds, "most-probable" body weight gain and nutritional requirements for the next day were estimated by the DSS to prepare the optimal blend for the next day. No significant difference was found in growth or feed intake, but feed conversion ratio over the 10-31 day period was increased in DMP and PF strategies compared to C one (+3.3 and +2.2%, respectively). Despite this increase, DMP and PF strategies appear to improve economic and environmental performance. Significant lower feeding cost per kg BW gain was indeed observed in these strategies (-6.8 and -7.9%, respectively) as well as a decrease in phosphorus excretion (-25.6 and -11.8%, respectively). The validation trial confirmed the interest of applying precision feeding strategy to broiler production using modelling tools such as the tested DSS. These promising results should now be confirmed in commercial conditions.

[1] Méda et al. (2020). From measuring average body weight of the flock to precision feeding in broilers: A modelling approach to adjust daily feed composition, World Poultry Congress, 16-20 August, Paris, France

EFFECT OF SUPPLEMENTING PAPAYA (CARICA PAPAYA) LATEX ON PERFORMANCE, CARCASS TRAITS AND NUTRIENT DIGESTIBILITY IN BROILER CHICKEN FED RECOMMENDED AND SUB-OPTIMAL LEVELS OF DIETARY PROTEIN

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Three experiments were conducted to study the effects of supplementing papaya (Carica papaya) latex (papain) (PL) on performance, carcass variables and Nutrient digestibility in broilers fed diets containing varying levels of protein. The PL (4000 TU g-1) was supplemented to maize-soybean meal-based control diet (CD) to provide four concentrations of papain (0, 600, 800 and 1000 TU kg-1 diet) in experiment 1. In experiment 2, the CD (100% protein) and a low-protein basal diet (95% less protein i.e., about 1% of diet) were fed with and without PL (0 and 1000 tyrosine units - TU kg-1 diet) in a 2 x 2 factorial design. Three graded levels of dietary protein (88, 94 and 100% of the recommended level) with three concentrations of PL (0, 1000 and 2000 TU kg-1) were fed in experiment 3 to find the possibility of reducing dietary protein with higher concentrations of papain in the diet. Each diet was fed ad libitum to 10 replicates of 25 broiler chicks in each replicate from d 1 to 35. The data of experiment 1 were subjected to One-way analysis of variance, while the data of experiments 2 and 3 were subjected to 2x2 and 3x3 factorial analysis, respectively. In experiment 1, supplementation of papain at 1000 TU kg-1 to the CD significantly improved the feed efficiency (FE) compared to the control group (1-35d). In experiment 2, by reducing the dietary protein by 1%, the body weight gain (BWG) and FE reduced (1-35 d). However, supplementation of 1000 TU papain significantly improved the BWG and FE, which were similar to the control. The interaction between dietary protein and PL did not influence the performance variables in experiment 3, except the FE during 1-21 d. At 88% protein, PL supplementation significantly improved the FE and such improvement was not observed at 94 and 100% protein during d 1-21. BWG and FE improved significantly with papain at 2000 TU kg-1 diet compared to the CD. An increase in dietary protein progressively reduced the relative weights of abdominal fat and liver. Papain supplementation reduced the ready to cook yield and breast weight. Papain supplementation significantly increased the digestibility of energy and nitrogen compared to the control group. Based on the results, it is concluded that papain supplementation (1000-2000 TU kg-1) in the form of papaya latex could able to reduce the requirement of protein (about 95% of the recommendation) in corn soybean meal-based diets without affecting the performance.

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Protein nutrition

Posters

INFLUENCE OF BROILER AGE ON THE STANDARDISED ILEAL AMINO ACID DIGESTIBILITY OF WHEAT AND SORGHUM

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The current study was carried out to determine the standardised ileal digestibility coefficients (SIDC) of nitrogen (N) and amino acids (AA) at six different ages (d 7, 14, 21, 28, 35, and 42) of broilers, following the feeding of wheat- and sorghumbased diets and using the direct method. Day-old male broilers (Ross 308) were obtained from a commercial hatchery and raised in floor pens. The assay diets were formulated to contain 938 g/kg of each grain as the sole source of AA in the diet. Titanium dioxide (5 g/kg) was added as an indigestible marker. Each assay diet was fed to six replicate cages housing 14 (d 7), 12 (d 14), 10 (d 21), 8 (d 28), 8 (d 35), and 6 (d 42) birds per cage for four days prior to digesta collection from the lower half of the ileum. The apparent values were corrected by using the specific age-related basal endogenous AA flows determined in a previous study using the N-free diet. Data were analysed by using the General Linear Models procedure with cage means as the experimental unit. Orthogonal polynomial contrasts (linear and quadratic) were used to compare the treatment means. In case of wheat, no age effect was observed on the SIDC of N (P > 0.05). A guadratic effect (P < 0.05) was observed on the SIDC of methionine. The SIDC of tryptophan, aspartic acid and cysteine increased linearly (P < 0.001) with age of birds. In sorghum, the SIDC of N was higher (quadratic, P < 0.05) on d 7 (0.933). Among the indispensable AA, a quadratic influence (P < 0.05) was observed on the SIDC of arginine, histidine, threonine and valine. A linear reduction (P < 0.05) was observed in the SIDC of isoleucine, leucine, lysine and the average of indispensable AA with increasing age of broilers. The SIDC of all dispensable AA, except for cysteine, was generally higher (quadratic or linear, P < 0.05 to 0.001) on d 7. The average SIDC of all AA was higher on d 7, decreased linearly (P <0.05) and plateaued from d 14 to 42. Overall, these results suggest that the age effect on the AA digestibility in broilers is variable depending on the grain type and specific AA. This is the first study exploring the age effect on the SIDC of AA in broilers from hatching to the end of growth cycle. Key words: age, broilers, amino acid, digestibility

Acknowledgement: We acknowledge the "AgriFutures Australian Chicken Meat Program" for funding the project.

POLYPHENOLS AND FUNCTIONAL AMINO ACIDS ACT IN A SYNERGISTIC WAY TO IMPROVE PERFORMANCE OF BROILERS CHALLENGED WITH A COCCIDIAL VACCINE

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In broiler chickens, coccidiosis decreases growth performance and is a predisposing factor of necrotic enteritis. It is proven in literature that the supplementation of functional amino acids (FAA) such as arginine, threonine, glutamine or polyphenols can exert a positive influence on broiler chicken performance and health when affected by coccidiosis. However, the interaction between these two nutritional strategies has never been investigated.

In total, 1,200 newly hatched male chicks (Ross 308) were allocated to four treatments (10 repetitions of 30 broilers). At day 14, all birds were challenged with a Paracox vaccination 10x dose by oral gavage. Experimental diets were formulated following Ajinomoto recommendations and were fed from day 0 to day 42 with a 3-phase feeding program. Dietary treatments consisted in a factorial 2 x 2 design with addition or not of a combination of functional amino acids (FAA; 1kg/T) and catechin-derived polyphenols (CAT; 100g/T). FAA were supplemented beyond requirements estimated for optimal growth. ADFI, ADG and FCR were assessed at 14, 21, 28, 35 and 42d. Uniformity, carcass & breast meat yields were evaluated at 42d. Oocyst counting was evaluated at 21d. Oocyst counting was analyzed using Kruskal-Wallis test followed by Conover post-hoc tests. All other data were subjected to a 2-way ANOVA and differences among means were detected by Fisher post-hoc tests. Interaction was found between FAA and CAT supplementation on ADG during the post-challenge period (14-21d; p = 0.019) and on FCR (14-21d; p < 0.001). Only when fed the combination FAA+CAT broilers had a higher ADG (+3.3g/d) and a lower FCR (-3.5pts) compared to control. There was no interaction on ADFI, indicating that the growth improvement was driven by feed efficiency. A trend for an interaction (p = 0.093) and a significant effect of FAA was observed for BW at 21d (+23g). No other significant effect was observed for performance, cut-up yields or uniformity. Significant effect of the treatments was found for total (p = 0.012) and Eimeria acervulina (p = 0.019) oocysts counting. Supplementing the combination FAA+CAT decreased oocysts counts compared to FAA and CAT alone. This study revealed the synergistic effect of FAA and CAT on performance around the critical period of the challenge. Combining FAA & CAT was indeed more efficient than supplementing each component separately. The mode of action of this interaction still needs to be elucidated but does not seem to be linked to a direct effect on oocysts.

INFLUENCE OF DIETARY ISOLEUCINE AND LEUCINE LEVELS ON 29 TO 42 D VALINE NEEDS IN COBB 500 MALE BROILERS

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The negative effects of branched-chain amino acid (BCAA) antagonisms have been known for over half a century, but how they impact individual BCAA requirements is not. Therefore, an experiment was conducted to determine if dietary levels of isoleucine and leucine influence 29 to 42 d valine needs. A valine deficient corn/soybean meal/peanut meal diet was formulated with valine, isoleucine, and leucine to lysine ratios (Val/Lys, Ile/Lys, and Leu/Lys, respectively) of 60, 62, and 115, respectively (1.00% digestible lysine). Aliquots of L-valine were added to produce Val/Lys ratios of 60, 67, 74, 81, and 88. Additional treatments were created by adding L-isoleucine (62 and 70 Ile/Lys) and L-leucine (115 and 145 Leu/Lys) resulting in a $5 \times 2 \times 2$ factorial array of treatment. Diets were fed to 7 replicate pens of 12 broilers. Body weight gain, feed intake, and feed conversion ratio were determined for the 29 to 42 d period, and carcass traits were assessed on d 44. All data were analyzed by ANOVA to identify interactions, and in the event of significant interactions ($P \le 0.05$), data were subjected to regression analysis to determine impacts on the valine requirement. Requirements were reported as 95% of the quadratic response. Digestible valine level significantly (P<0.05) impacted all live performance parameters and valine by leucine interactions were determined for BW gain (P=0.026). At low dietary leucine levels (115 Leu/Lys), the valine requirement was determined to be 73 Val/Lys for BW gain, whereas at high dietary leucine levels (145 Leu/Lys), the valine requirement was determined to be 81 Val/Lys. For carcass traits, digestible valine significantly influenced fat pad percentage (P=0.013) and leg yield (P<0.001). No significant interactions (P>0.05) between valine and isoleucine or leucine were observed for any carcass trait. Therefore, when determining BCAA requirements for broilers, all three should be taken into account in test diets to ensure that industry applicable requirements are identified and are not underestimated due to test conditions.

EFFECTS OF EXCESS LEUCINE IN MALE AND FEMALE COBB 500 BROILERS DURING THE 15 TO 32 D GROWER PERIOD

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High dietary leucine is known to cause detrimental effects in broiler diets (D'Mello and Lewis, 1970), but corn-soybean meal diets contain levels above the leucine requirement due to corn's high leucine content. Therefore, two leucine titrations were conducted in male and female Cobb 500 broilers using superoptimal leucine levels. Low and high leucine diets were formulated to contain leucine to lysine (Leu/Lys) ratios of 115 and 170, respectively. These diets were subsequently blended to produce 6 experimental diets containing Leu/Lys ratios of: 115, 126, 137, 148, 159, and 170. The pelleted treatment diets were fed to 8 replicate pens of 21 broilers in Experiment 1 (males) and Experiment 2 (females). Body weight gain, feed intake, and feed conversion were monitored for the 15 to 32 d grower period, and carcass traits were assessed on d 33. All data were subjected to regression analysis and statistical significance was considered at P≤0.05. No differences (P>0.05) were observed for performance or carcass traits in Experiment 1. In Experiment 2, a linear (P=0.029) and guadratic response (P=0.020) was observed for feed intake where feed intake was maximized at the highest Leu/Lys ratio. No other differences (P>0.05) were observed in Experiment 2. Previous experimentation by Edmonds and Baker (1987) found no effects on live performance when excess leucine was fed, but Baker (2004) noted that chicks consumed more feed when diets contained excess leucine. Therefore, this data indicates that leucine levels do not impact growth, but negative effects associated with leucine may be the result of antagonism among the branched-chain amino acids and in agreement with Maynard et al. (2020).

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SUPPLEMENTING A COMBINATION OF FUNCTIONAL AMINO ACIDS AND CATECHIN-DERIVED POLYPHENOLS IS ABLE TO IMPROVE GROWTH AND FEED EFFICIENCY OF BROILERS FACING A COCCIDIOSIS CHALLENGE.

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In broiler chickens, coccidiosis is the main challenge for gut health, generating important economic losses every year in the poultry industry. This coccidiosis condition decreases feed intake and growth and increases the susceptibility of necrotic enteritis. Literature reports that the supplementation of functional amino acids (FAA; arginine, threonine, glutamine) as well as polyphenols are beneficial in broilers facing this condition. The current study aimed at evaluating the efficiency of the supplementation of two types of polyphenols together with FAA on performance, lesion scores, oocyst count in broiler chickens facing coccidiosis. Five treatment groups including six replicates were followed in this trial: an uninfected untreated control (UUC), an infected untreated control (IUC), an infected treated control (ITC) with addition of Maxiban (625 g/T), and 2 infected dietary supplemented groups. These two groups received a supplementation of 1kg/Ton of FAA starting from d0 combined with 100 g/Ton of catechin-derived polyphenols (FAA+CAT) or 1,200 g/T of gallic acid-derived (FAA+GAL). Infected birds were inoculated at 16 days of age with 45 000 Eimeria acervulina, 3000 Eimeria maxima and 63 000 Eimeria tenella oocysts isolated from a recent field strain. Mortality, average daily gain (ADG) and feed conversion ratio (FCR) were studied from 14 to 22 days of age. Birds were euthanized for intestinal lesion scoring and oocyst excretion on day 22. All data were subjected to an ANOVA and differences among means were detected by post-hoc tests for Fisher's exact test. Coccidiosis challenge (IUC vs UUC) reduced significantly ADG (-11 g/d) while FCR was numerically increased (+ 7 pts). The ITC treatment improved significantly and fully restored FCR back to the level of UUC. Feeding broilers with FAA+GAL improved significantly ADG but failed to improve FCR. Only broilers fed FAA+CAT had a significantly higher ADG and FCR compared to IUC, and both were restored to the level of the ITC treatment. Coccidiosis challenge increased Eimeria lesion scores and oocyst counts. All treatments reduced Eimeria tenella lesion scores but did not alter lesions from other species, except ITC group which also exhibited reduced Eimeria maxima lesions. Oocyst count was numerically decreased in the ITC group but was not altered by the two dietary treatments. This study reports that the supplementation of a combination of functional amino acids and catechinderived polyphenols can improve growth and feed efficiency and reduce Eimeria tenella lesion scores in coccidiosis-challenged birds. The mode of action still remains to be determined.
COMBINING FUNCTIONAL AMINO ACIDS AND CATECHIN-DERIVED POLYPHENOLS CAN RESTORE PERFORMANCE AND AMINO ACID DIGESTIBILITY IN BROILERS CHALLENGED WITH COCCIDIOSIS

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In broiler chickens, commercial intensive practices exacerbate the pressure of gut heath challenges such as coccidiosis. Coccidiosis impairs nutrient digestion, performance, livability of broiler chickens and reduces overall farm profitability. Literature reports that functional amino acids (arginine, threonine, glutamine) and polyphenols could exert beneficial effects in broilers facing this challenge. The current study aimed at evaluating the efficiency of the supplementation of functional amino acids (FAA) together with catechin-derived polyphenols (CAT) on performance, AA digestibility and gut morphology in broiler chickens facing coccidiosis. Three hundred eighty-four (384) newly hatched male broiler chicks of commercial strain (Ross 308) were allocated to 4 treatments with 8 replications. For all except one treatment (Uninfected Untreated Control - UUC), birds were artificially challenged with ADVENT® coccidiosis vaccine 10x dose at day 14. Broiler chickens from the three challenged treatments received either no supplementation (Infected Untreated Control – IUC), an anticoccidial drug (Maxiban at 625 g/t; Infected Treated Control – ITC) or a mix of FAA and CAT at a dose of 1 kg/Ton (glutamine, arginine, threonine, CAT – IUC+MIX). Performance and livability were followed during the 35 days of the trial. Lesion scoring and fecal oocvst count were assessed at day 21. AA digestibility and gut morphology were measured in the UUC, IUC and IUC+MIX treatments at day 25. All data were subjected to an ANOVA and differences among means were detected by multiple range tests. During the course of the trial, coccidiosis challenge reduced growth (3 q/d) and feed conversion ratio (-3 pts) without altering feed intake. It also increased total and species related lesion scores and oocyst counts in feces at d 21. These effects were fully reversed by the supplementation of the anticoccidial drug. The supplementation of the mix restored performance but had no effect on lesion scores and fecal oocyst count. Interestingly, the coccidiosis challenge reduced villous height in duodenum which translated into lower digestibility of all AA, except phenylalanine. The addition of the mix restored partially digestibility but did not mitigate the effect of the challenge on gut morphology. This study reports that the supplementation of a selected combination of functional amino acids and catechin-derived polyphenols can fully restore performance of coccidiosis-challenged birds with an improvement of AA digestibility. The mode of action of the mix does not rely on a direct effect on lesion scores, oocyst count or gut morphology and still needs to be determined.

EFFECTS OF AMINO ACID LEVELS DURING REARING ON FEATHERING OF BROILER BREEDERS

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Crude protein and amino acid (AA) levels have essential effects on feather growth and quality. However, only a few studies have reported the effects of these nutrients during rearing on feathering after the onset of egg production. This experiment evaluated the effects of four dietary AA levels during rearing on the feathering of Cobb 500 slow-feathering (SF) broiler breeders during the laying phase. Dietary treatments contained balanced protein levels with 80, 90, 100, and 110% AA from the Cobb 500SF guidelines. At 22 wk of age, 1,040 females and 112 males were distributed in 16 floor-pens with 65 hens and 7 roosters. Feathering (FS) and fleshing (FLS) scores, BW, laying status, and hen housed egg production (HHEP) were recorded in all hens at 32, 36, 40, 46, 50, 56, and 60 wk. Female: male (F:M) ratio and differences in BW between sex were calculated. A scoring scale from 1 to 5 was used to evaluate FS, where 1 represented normal smooth feathers and 5 naked spot areas with scratches. FLS was assessed from 1 (Under-desired) to 7 (Oversized) and laying hen status was determined by physical characteristics. Data were analyzed in a CRD with AA level as treatments and four replicate pens per treatment. Heavier breeders (P<0.05) resulted from 110%AA diets at 32, 36 and 40 wk. Breeders fed 90, 100, and 110%AA levels had higher FLS (P<0.001) at 32 and 46 wk compared to 80%AA. Dietary treatments affected (P<0.05) FS from 32 to 60 wk. Diets containing 110%AA resulted in poorer FS, with an average score of 4.03 from 46 to 60 wk. BW, FLS, laying status, and FS increased linearly and HHEP quadratically with AA (P<0.05), while quadratic effects of age (P < 0.05) were observed in the response surface analyses. FS was positively correlated (P<0.05) with BW, FLS, HHEP, age, BW difference between sex, and negatively with laying status (r=-0.54). No correlations between FS and AA nor F:M ratio were detected (P>0.05). Multiple linear regression (MLR) indicated (R2=0.90) a small contribution of AA in the FS variability (11.1%), while age, FLS, and F:M ratio accounted for 88.9% of the variation. Collinearity in this model was reduced using principal components (PC) analysis. The first PC represented 79.2% of FS variability according to MLR (R2=0.86), with the participation of age, BW, HHEP, FLS, and laying status. In summary, AA levels during rearing had minor effects on FS but affected the body composition and reproductive parameters that had a great impact on feather coverage.

META-ANALYSIS ON APPARENT AMINO ACID DIGESTIBILITY IN EIMERIA-CHALLENGED BROILER CHICKENS

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Avian coccidiosis is a parasitic disease caused by the protozoa Eimeria, responsible for major economic losses in the poultry industry. The objective of this study was to use a meta-analysis approach to evaluate and quantify the effect of an Eimeria challenge on apparent AA digestibility (AID and ATTD) in broiler chickens. A database composed of 6 articles with a total of 21 experiments was built to look at effect of challenge and type of challenge (a mix of Eimeria spp. vs E. acervulina), while a sub database of 3 articles with a total of 15 experiments was used to evaluate the effect of dose of E. acervulina-challenged birds. For the overall database, regression models were fitted with the GLM procedure in Minitab 19 with fixed effects of challenge, species, and their interactions. For the sub database, the mixed model procedure was used to fit regression models and identify a linear or quadratic response to dose. Challenge affected digestibility (P<0.05) of both dispensable and indispensable AA except for Trp (P=0.066) such that digestibility decreased, with the largest effects seen in Cys, Thr, Ala, Val, and Ser (11.3%, 7.5%, 6.9%, 6.6%, and 6.2% magnitude of difference respectively). Type of challenge affected (P<0.05) AA digestibility with exception of Cys, Ala, Ser, Asp, Gly, Pro, and Tyr, while an interaction (P<0.05) was seen for Trp showing a larger effect with the mixed Eimeria challenge. E. acervulina dose linearly decreased (P<0.05) digestibility of dispensable and indispensable AA except for Trp and quadratically (P<0.05) in all AA except Met, Arg, and Trp. Similarly, with dose response, the largest effect was seen in Cys, followed by Ala, Thr, Ser, and Leu. The highest effect on Thr and Ser is supported by the increased secretion of mucin associated with an Eimeria infection while Cys plays a role in the sporulation process of oocysts. Interestingly, Trp digestibility was not affected by an E. acervulina challenge, however, decreased when challenged with a mix of Eimeria spp., suggesting a different response of Trp digestibility to other Eimeria spp. than acervulina. The results from the currently study confirmed that an Eimeria challenge negatively impacts AA digestibility and established a ranking from most affected to least, which may be indicative of the limiting AA that can be potential candidates in the mitigation of an Eimeria challenge.

Key words: broilers, amino acids, meta-analysis, digestibility, Eimeria challenge

EVALUATION OF SPIRULINA PLATENSIS IN LOW PROTEIN DIETS ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND BLOOD BIOCHEMICAL PARAMETERS OF MALE BROILERS.

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Multiple feed formulation strategies will need to be implemented to meet the anticipated increase in protein demand for animal feed. The objective of this study was to examine how male broilers responded to a low crude protein (CP) diet formulated with Spirulina Platensis. One hundred and eighty, one-day old chicks (Ross 708) were fed a common starter diet to 14 days of age then randomly assigned to an experimental treatment until 37 d. The experimental diets were a corn-soya meal based (CON) positive control (201 g/kg CP), corn-soya meal (LCP) based negative control (170 g/kg CP), and corn-sova meal-Spirulina (SLCP) diet (170 g/kg). Spirulina replaced half of the soya meal compared to the LCP treatment and was included at 10%. All diets were isocaloric and met all nutrient recommendations from the primary breeder guide with water and feed being offered ad libitum. Performance parameters, carcass yields, meat quality, meat colorimetric, and footpad scores were calculated with pen (n=5) as the experimental unit at 37d. Birds from each treatment (n=8) were randomly selected for blood and liver collection at 36d for target molecular markers and hematologic profiles, and bacterial translocation, respectively. Mean separation of $P \le 0.05$ was considered significant. Broilers fed LCP had reduced (P < 0.05) live body and processing weights, and increased feed conversion ratio and fat deposition. The LCP treatment also increased basophil percent, interleukin (IL)-6, IL-3, IL-4, IL-10, tumor necrosis factor alpha, circulating chemokine C-C motif ligand 20, -like receptor, and family pyrin domain containing 3 expression (P < 0.05) compared to the CON treatment. However, the SLCP treatment lowered inflammation by decreasing (P < 0.05) basophil percent, cytokine, chemokine, and inflammasome expression levels comparable to the CON. Bacterial translocation was reduced (P=0.05) in the SLCP treatment. The SLCP diet showed an 8-point FCR decrease compared to the LCP, yet no significant performance differences were observed. The SLCP treatment had significantly more redness and yellowness in the breast, thigh, and skin compared to the other treatments. Footpad quality was also significantly improved in the SLCP treatment compared to the CON. In conclusion, the formulation of Spirulina into low CP diets lowered inflammation, bacterial translocation, improved pigment deposition, improved footpad quality without any adverse effects on live performance in 37d old male broilers.

DOES THE AMOUNT OR TYPE OF ARGININE HAVE AN IMPACT ON BROILER CHICKENS?

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Arginine (Arg) is the fifth limiting amino acid in poultry. It can be provided by using intact protein, supplementary (suppl) Arg or products with Arg sparing claim. Herein, effect of different amount of digestible arginine-to-lysine ratios (dArg: dLys) provided either through suppl Arg or guanidinoacetic acid (GAA) on broiler's growth performance was studied. A total of 1176 male Ross 308 broiler chickens were allocated (28 birds/pen) to 7 dietary treatments (6 pens each) at random: a control diet with 1.05 dArg:dLys (CTRL, without suppl Arg), CTRL diet plus suppl Arg to achieve 1.15, 1.25 and 1.35 dArg:dLys (Arg1.15, Arg1.25, Arg1.35), 3 diets achieving 1.05 dArg:dLys either with suppl Arg (0.047% L-Arg) or with 0.06% suppl GAA (GAA77 or GAA149) considering 77% or 149% Arg sparing (according to the manufacturer). Birds and feed were weighed on a pen basis at the start and end of starter (d 0 to 10), grower 1 (d 10-20), grower 2 (d 20-30) and finisher (d 30-37) phases and FCR was calculated. Breast myopathies (BM) and foot pad dermatitis (FPD) were scored on 4 birds per pen at d 35 and 42. Data were analyzed with a one-way ANOVA. Significant differences were defined at P < 0.05. BW and FCR of birds in Arg1.15 and Arg1.25 were lower than other treatments because feed analytics showed a lack of arginine, Met+Cys and other nutrients in these two groups. Although BW of birds in these two groups stayed behind, FCR was compensated from d 30 onwards. Birds in 0.047% L-Arg, 0.06% GAA77 and 0.06% GAA149 groups revealed similar performance results in all phases. At d 42, medium + severe WB incidence was higher in GAA149 fillets (42%) compared to treatments 0.047% L-Arg and GAA77 (37.5% in both cases). GAA149 birds received 0.96% analyzed Arg and the rest was speculated to be spared by using GAA. Lack of Arg seems to be related to the incidence and severity of breast myopathies. FPD was not affected. In conclusion, suppl 0.047% L-Arg and 0.06% GAA either with 77% or 149% dArg sparing to reach a dArg:dLys ratio of 1.05 increased growth performance of broiler chickens compared to a diet without L-Arg and GAA. Considering 149% Arg sparing was linked to a higher incidence of WB at d42.

EFFECT OF AMINO ACID SUPPLEMENTATION OF LOW CRUDE PROTEIN VEGETABLE DIETS ON GROWTH AND SERUM METABOLITES OF GROWER AND FINISHER BROILER CHICKENS

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The effect of supplementation of low crude protein (CP) vegetable diets with crystalline forms of the limiting amino acids: methionine, lysine and threonine, on growth performance and serum biochemical metabolites of broiler chickens in the grower and finisher phases of production was investigated. Three groups of grower and finisher broiler diets were formulated to contain 21.5 and 19% (Ideal CP), 20.5 and 18% (Less -1% CP) and 19.5 and 17% (Less -2% CP) crude protein respectively. Diets were balanced for the amino acids methionine, lysine and threonine, with the balance of these amino acids provided in crystalline forms in the test diets. A total of one hundred and eighty 10-d old broiler chicks were randomly allotted to the three treatment groups in 6 replicates, 10 chicks/replicate for the grower (11-24d) and finisher (25-42d) phases of the study. Feed consumption and weight of the chickens were monitored, and feed intake (FI), daily liveweight gain (DLWG) and feed conversion ratio (FCR) were calculated. On 35d of the study, blood was drawn from the chickens and serum glucose, total protein, albumin, globulin and uric acid were determined. Data obtained were subjected to ANOVA (p < 0.05) and preplanned contrasts elucidating the effect of the low CP plus amino acid supplementation treatments (Ideal CP vs Less -1% CP, and Ideal CP vs Less -2% CP) on the indices assessed. The contrasts, Ideal CP vs Less -1% CP, and Ideal CP vs Less -2% CP, revealed similar growth performance; FI, DLWG and FCR in chickens on the different treatments in both grower and finisher phases of production. Chickens in the different treatments also presented similar levels of serum glucose (189.29-223.33mg/dL), total protein (1.74-2.02g/dL), albumin (0.73-0.82g/dL), globulin (1.01-1. 23g.dL) and uric acid (6.60-.79mg/dL). This result affirms that chickens raised on low crude protein vegetable diets supplemented with the limiting amino acids; methionine, lysine and threonine, in crystalline form, perform as well as chickens raised on ideal crude protein diets in the grower and finisher phases, without any detrimental effects.

A COMBINATION OF FUNCTIONAL AMINO ACIDS AND POLYPHENOLS CAN RESTORE PERFORMANCE IN BROILERS CHALLENGED WITH COCCIDIOSIS: A META-ANALYSIS

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Coccidiosis is one of the main challenges of the poultry industry leading to reduced performance and welfare and increased mortality of animals. Current treatments against coccidiosis include ionophores and chemicals, but the outbreak of resistance strains of Eimeria together with the growing pressure from consumers for more sustainable products urge to find nutritional alternatives. In this work, we reviewed through a meta-analysis the efficiency of a supplementation of a combination of functional amino acids (arginine, threonine, glutamine) together with grape extract polyphenols (INNEUSTM, METEX NOOVISTAGO, Paris) in broilers challenged with coccidiosis based on five different trials. In the first week following the challenge, we observed that the supplementation of INNEUSTM was able to partially reverse the negative effects of the challenge on performance. Interestingly, when considering the total period of the trials, INNEUSTM was as potent as the anticoccidial drug to mitigate the effect of the coccidiosis on performance. This meta-analysis suggests that the supplementation of a mix of functional amino acids together with grape extract polyphenols could be a useful tool to counteract the negative effect of coccidiosis challenge on performance. The full understanding of the mode of action warrants further research.

EVALUATION OF SINGLE-CELL MICROBIAL BIOMASS AS NOVEL FEED PROTEIN SOURCE FOR MEAT TYPE CHICKENS

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The major challenge to the poultry industry is the availability as well as the high fluctuating cost of conventional feed resources especially soybean meal. To find alternatives to protein sources, the present study was aimed at evaluating the effect of single-cell protein i.e. biomass as a newer protein source in broiler chickens. Biomass is the organic material; whose crude protein content was very high (82.4%) when compared to conventional protein sources (45-50%) and also it is renewable and produced from plants and animals. Biomass is produced through various processes like direct combustion into heat, converting into solid, liquid, or gas biofuels. The technologies for biomass conversion are categorized into thermochemical conversion processes and bioconversion processes. The trial was conducted to study the effect of the inclusion of different levels of biomass (0, 1, 2.5, 5 and 7.5%) on production performance, immune responses & cost reduction in broiler chickens. Mash feed formulation was designed as per the standard of BIS (Bureau of Indian standards). Birds fed with 5% biomass (T4) had significantly (P<0.01) highest body weight (1712.7g) throughout the trial period and showed maximum overall body weight with less feed intake and better FCR. (1.77). Higher biomass (7.5%) in feed (T5) had a negative (P<0.01) effect on feed intake as well as feed efficiency. While cellular, as well as humoral immunity, was quantitatively improved in the higher biomass inclusion group (T5). The reduction in the cost of production was about 3.91, 2.25, 10.50 and 5.69% respectively for 1.0, 2.5, 5.0 and 7.5% inclusion of biomass. So, from the study, it could be concluded that basal diet with 5% biomass inclusion in the diet is an effective alternative to protein source for better growth performance, immune response with better economics of broiler production system. We also observed that, if we repeat this experiment in the future by using crumble or pellet feed instead of mash, we may further increase the biomass percentage in feed because of no settling down effect of biomass feed.

IMPROVED PERFORMANCE IN BROILER CHICKENS AT 21 DAYS OF AGE FOLLOWING INCLUSION OF A PORCINE INTESTINAL MUCOSA HYDROLYSATE

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The first 21 days are a key period in the life of a broiler chicken that demands highly digestible nutrients to enhance their development, immunity, intestinal health, and body growth. Animal protein sources have a better composition and include higher levels of available amino acids than those of vegetable origin. Moreover, soybean ingredients contain several anti-nutritional factors. Therefore, our hypothesis was that replacing soybean by a porcine intestinal mucosa hydrolysate (PIMH) could enhance the nutritional value of diets for chicken and, with that, optimize their growth performance. This study was aimed at evaluating the effects on performance of partially replacing soybean by PIMH in the diet of broiler chickens up to 21 days of age. A total of 750 1-day-old male Cobb 500 chickens were randomly distributed into three study groups and received different diets including 0 (Control), 2.5 or 5% PIMH (Palbio 50, Bioiberica SAU, Spain) as replacement for soybean from 0 to 21 days of age. Ten repetitions were used per study group resulting in 30 pens in floor rearing (25 chicken/pen). Diets were isoprotein and isocaloric and formulated based on corn/soy as main protein sources. Body weight (BW), uniformity (U), feed intake (FI), feed conversion ratio (FCR), protein and energy efficiency ratio (PER, EER) and gizzard pH (GpH) were evaluated. Data were analyzed by ANOVA using the GLM procedure of SAS v 9.1. Differences were considered statistically significant when $P \le 0.05$. The separation of means was done through the Turkey test. After 21 days, while similar mean BW were achieved in the different study groups, a significant improvement was observed in BW uniformity with 5% PIMH (100.00 %), compared to the Control group (87.80 %). On the other hand, significant reductions in FCR were achieved with 2.5% (1.224) and 5% PIMH (1.220), compared to the Control group (1.282); and a significantly lower FI was also seen with 2.5% (736.46) and 5% PIMH (730.29), compared to the Control group (776.01). No other significant changes were observed. This study reports significant beneficial effects on performance of partially replacing vegetable protein sources with PIMH in broilers during the first 21 days. This approach could allow a dilution of the antinutritional factors present in soybean meal and, hence, contribute to optimizing poultry production.

DIETARY ARGININE REQUIREMENTS FOR THE OPTIMIZATION OF GROWTH PERFORMANCE AND CARCASS TRAITS OF MALE BROILERS

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Recently, it has been reported that higher levels of arginine have improved growth performance and meat quality of modern broiler strains. However, these reports have only evaluated arginine inclusion levels during short periods of broilers grow out. Therefore, this study was conducted to establish the optimal digestible arginine (dArg) to digestible lysine (dLys) ratio of YPM × Ross 708 male broilers from 1 to 14 d of age, 1 to 25 d of age (experiment 1), and 25 to 42 d of age (experiment 2) based on live performance, feed utilization, and processing yields. A total of 1080 (experiment 1) and 1008 (experiment 2) 1 d old male chicks, were sorted in 72 floor pens in a randomized block design, and subjected to diets with 6 dArg/dLys levels, with 12 replicate pens, each containing 15 and 14 chicks, for experiment 1 and experiment 2, respectively. The experimental diets were comprised of corn, wheat middlings, corn gluten meal, poultry meal, and soybean meal, and formulated to meet limiting amino acids requirements, except for dArg. Diets were formulated to 95% of the recommended dLys requirements to prevent overconsumption of dLys, and to ensure dArg would only be biologically efficient up to the slightly suboptimal lysine level. The calculated dArg/dLys value was formulated during all feeding phases studies to have a minimum level of 77, and 10-point increments were added to the basal diet in the form of L-arginine at the expense of an inert filler. Optimum dArg/dLys values for live performance and carcass traits for each experimental period, were calculated via quadratic broken line methodology based on adequacy of the fit, following the NLIN procedure of SAS 9.4. In experiment 1, the dArg/dLys ratio for optimizing BW gain and feed conversion ratio (FCR) was determined to be 106 for both variables from 1 to 14 d of age, and 105 and 108 from 1 to 25 d of age, respectively. In experiment 2, the optimum dArg/dLys value was estimated to be 129 and 116 for BW gain and FCR, respectively, during the 25 to 42 d period. Optimum dArg/dLys values for breast weight and breast yield were estimated to be 112 and 109, respectively. Absolute and relative weights of thighs increased linearly (P < 0.001) with progressive increases in dArg/dLys. The results of this study suggest that the optimum dArg/dLys value necessary to maximize growth performance and carcass traits increases as the bird matures, likely due to considerably higher maintenance values of dArg in broilers.

EFFECTS OF DIETARY ARGININE SUPPLEMENTATION ON GROWTH PERFORMANCE, METABOLISM, AND INTESTINAL MICROBIOME OF BROILER CHICKENS

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Arginine is a versatile amino acid with proteinogenic and functional roles in the animal organism. Recently, attention has been paid to its effects on the gastrointestinal tract. Arginine has been demonstrated to have gut healthpromoting properties, like enhancement of mucosa integrity and barrier function, anti-inflammatory activity, and restoration of a desirable microbiota. Chicken is unable to endogenously synthesize arginine de novo. Consequently, chickens rely on dietary arginine to meet their requirements. Extensive research has also reported that arginine supplementation above currently recommended levels is beneficial to performance traits of broilers. Omics technologies can contribute to our understanding of how arginine utilization affects animals' metabolism. Hence, the present study evaluated the effects of dietary arginine supplementation on growth performance of broilers. Moreover, it explored the impact of arginine modulation on hepatic and systemic metabolism as well as intestinal microbiome. A total of 630 as-hatched male Ross 308 broilers were assigned to 2 treatments (7 replicate pens/treatment in a randomized complete block design) fed a basal diet (CON) or the basal diet supplemented with 1.6 g/kg of synthetic L-arginine (ARG) for 49 d. The basal diet had a total arginine to total lysine ratio of 1.08, 1.09, 1.07, 1.08 in starter, grower I, grower II and finisher phase, respectively, whereas ARG diet 1.20 throughout the trial. On a replicate basis, body weight (BW) was recorded at housing, feeding phase switches, and slaughter, while feed intake (FI) for each feeding phase. Feed conversion ratio (FCR) was calculated for every feeding phase and the entire rearing period. Performance data were analyzed with one-way ANOVA. At 49 d, 2 birds per replicate were used for blood, liver, and caecal content sampling. All samples are under analysis via proton nuclear magnetic resonance (metabolomics), while caecal content through shotgun metagenomic too. ARG showed a greater final BW than CON (3,937 and 3,778 g, respectively; p < 0.001) and a lower FCR at the end of starter and grower I phase (p < 0.05). Likewise, ARG displayed the lowest FCR during the entire feeding trial (1.73 vs 1.81; p < 0.05). These results confirm that arginine supplementation exceeding current recommendations has positive effects on broiler performance. Ongoing analyses can shed light on the molecular mechanisms behind arginine action at hepatic, systemic, and gut level.

DL-METHIONINE AND OH-METHIONINE EXHIBITED EQUAL PERFORMANCE IN ONE MILLION BROILERS CHICKENS

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The objective of this study was to compare the performance of broilers fed with either DL-Methionine (DL-Met) or OH-Methionine (OH-Met) under commercial field conditions. The study was performed in France from July 2019 to May 2020 in a poultry integration. A total of 57 farms representing 1,189,000 of male Ross 308 broiler chickens were used. Farms received alternately either DL-Met or OH-Metbased diets. The two Met sources were supplemented on equimolar basis. Animals were reared from 0 to 35 ± 1 days old, divided in four feeding phases: starter (0 to 9 days old), grower 1 (10 to 19 days old), grower 2 (20 to 28 days old) and finisher (29 to 35 days). The dietary treatments were formulated for the four feeding phases using local ingredients. Because of the duration of this study (11 months), feed ingredients varied across the study but, the nutrient composition remained equal among the different feed formulas and batches and met animal requirements. The dLys was respectively for the 4 rearing periods 1.30, 1.16, 1.06 and 1.03% and dMet+Cys:dLys ratio was of 75%. Each farm was considered as the experimental unit. Final body weight, daily weight gain, daily feed intake and Feed Conversion Ratio were collected for each farm on the 0-35 d period and analyzed using a 2-way ANOVA with the farm and the dietary treatment as main variables. After outliers' removal, a total of 24 farms (475,389 broilers) received the DL-Met treatment whereas 26 farms (562,400 broilers) received the OH-Met based treatment. No significant effect of the farm was observed on any criteria of performance. The daily feed intake (P = 0.46), daily weight gain (P = 0.61) and final body weight (P = 0.58) were not significantly different between DL-Met and OH-Met. This resulted in a similar feed conversion ratio (P = 0.84) for both DL-Met (1.568) and OH-Met (1.563). Mortality rate was 4.72 and 4.17%, respectively for DL-Met and OH-Met and was considered acceptable for the different farms. Overall, both DL-Met and OH-Met sustain equally animal growth performance as proven by more than 1,000,000 birds.

REDUCING DIETARY CRUDE PROTEIN BY THE AID OF LOCALLY PRODUCED FEED-GRADE AMINO ACIDS IS MORE BENEFICIAL TO REDUCE GLOBAL WARMING POTENTIAL OF BROILERS THAN USING NON-EUROPEAN AMINO ACIDS: LIFE-CYCLE ASSESSMENT OF A PERFORMANCE BROILER TRIAL

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Excess dietary crude protein (CP) forces the inclusion of soybean meal (SBM) that may be linked to land-use change and therefore global warming potential (GWP), as well as excess nitrogen (N) emissions into the environment. The aim of this trial was then to find strategies to reduce GWP and N excretion related with broiler production. 936 Ross 308 male broilers were fed from 21-35 days of age with 12 treatments (6 replicates of 13 birds per treatment) in a combination of 3 levels of CP (CP-19, CP-18 and CP-17, in %) and 4 levels of energy (E-3200, E-3100, E-3000 and E-2900, in kcal AMEn/kg) in a factorial design. Growth performance was not negatively affected by the reduction of dietary CP while the contrary was observed with the reduction of dietary E (see associated paper). Environmental parameters (N intake, retention, excretion and efficiency, volatilization of N and NH3 and N2O emissions, SBM intake and CO2eg kg/T feed/kg BWG) were calculated. For CO2 calculations, two amino acid (AA) sourcing scenario were tested: feed-grade AA coming from EU or from China. An interaction CP*E was tested by a 2-way ANOVA, and the main effects were reported when CP*E was not significant. The only significant interaction (p < 0.001) was found for NH3 and N2O volatilization, where the reduction of dietary CP lowered NH3 and N2O emissions, being more impactful with higher than lower dietary E content (CP-17 vs CP-19: -45% with E-3200 and -36% with E-2900, in both emissions). A trend (p<0.1) for an interaction CP*E was found for N excretion where CP-17 tended to be lower with E-3200 (-18%) and E-2900 (-7%) compared to CP-19 with E-3200. Reducing dietary CP by 1 or 2% points had a significant (p < 0.001) effect on N efficiency (+5% and +11%, respectively), and SBM intake (-8% and -25%, respectively). Regarding CO2 emissions, there was a significant interaction CP*AA sourcing scenario (p<0.001). CP-17 vs CP-19 reduced CO2 emissions by 15% (from 1241 to 1060 CO2eq/kg BWG) when using AA from EU while it only reduced CO2 emissions by 4% when using AA from China. Moreover, the CO2eg /kg BWG was significantly higher (+16%), and SBM intake tended to be higher (+2%), for E-3200 compared to the other treatments due to a higher feed intake. In conclusion, the combination of reducing dietary CP with the use of locally produced AA seems the most successful nutritional strategy to reduce GWP and N emissions associated with broiler production, with no negative effect on growth performance.

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Ratites

Selected short communications

ARTIFICIAL INSEMINATION IN OSTRICHES: PROGRESS AND FUTURE DIRECTIONS

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Artificial insemination (AI) could offer a solution to improve production in ostriches (Malecki et al., 2008). A 3-Trial study was conducted to investigate the minimum sperm dose (Trial 1), optimum inseminations frequency (Trial 2) and to compare AI and breeding pair (BP) females in chick production (Trial 3). Twenty-three South African Black ostriches (7 males; 16 females) aged between 2-10 years old were used. Semen was collected using the dummy female method and diluted 1:4 (semen: diluent) with an ostrich specific semen diluent. In Trial 1, females were inseminated three times a week every second day with various sperm doses of fresh semen from the same male, resulting in a total sperm dose of A:<2.5 billion, B:2.5-5 billion, C:5-7.5 billion and D:7.5-9.6 billion sperm/week. Fertilization status of the germinal disc of eggs produced after AI was determined using the egg breakout technique. The fertile period was calculated as the number of days fertilized eggs were laid after the last AI. The rate of sperm loss of eggs produced after AI was also estimated. In Trial 2, the minimum sperm dose along with the longest fertile period obtained in Trial 1 were used for inseminations on two consecutive days, followed by a single weekly dose for 4 weeks. Lastly, Trial 3 compared chick production of AI and BP females. Data was analysed using the Generalized linear mixed models of SAS at P<0.05. In Trial 1, 35.3±25.7% of eggs laid after the last AI were fertilized. Furthermore, dose A (P<0.05) produced a lower percentage of fertilized eggs (6.7%) than dose B (46%), dose C (37.3%) and dose D (37.8%), while no differences was observed between the latter three doses. The fertile period was 10.8±6.5 days. No differences between doses were recorded for the fertile period or rate of sperm loss (P>0.05). In Trial 2, fertility rate was 38.9±41.3% with no significant difference between AI weeks (P>0.05). Lastly, Trial 3 recorded fertility rate of 31.08±6.28% for AI females and $69.8 \pm 1.6\%$ for BP females (P<0.05). In addition, AI females produced fewer chicks per female per batch (0.1 ± 0.4) compared to BP females $(1.6\pm0.1; P<0.05)$. Hence, two consecutive AI events of 2.5-5 billion sperm/week followed by a single weekly dose maintained relatively good fertility rates of ostrich eggs. AI females produced fewer chicks than BP females. However, this indicates that chick production using AI in ostriches is possible and could eventually become indispensible for ostrich breeding.

ENVIRONMENTAL AND GENETIC EFFECTS FOR REPRODUCTION IN TWO-YEAR-OLD OSTRICH BREEDER FEMALES

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Commercial ostrich farmers traditionally start utilizing new breeders from their third season from hatching (i.e. in their third year). However, studies indicate that two-year-old birds can already reproduce at an acceptable level. This means that ostriches can start reproducing a year sooner, reducing the unproductive period of breeders. The pair-bred ostrich flock at the Oudtshoorn Research Farm in South Africa were used to study the production of two-year-old South African Black (SAB), Zimbabwean Blue (ZB) and Kenyan Red (KR) ostriches. The effects of strain, age and breed of the service sire on female reproduction was assessed with ASREML software and genetic parameters and trends were subsequently estimated. Genetic trends were only assessed for SABs as no genetic selection was done in the other strains. Two-year-old reproduction records of 734 pair-mated females hatched from 1990 to 2018 were used. Five traits were analysed, namely age at first eqg (the period from hatching to the production of the first eqg - AFE), laying interval (interval between the first and last egg during the breeding season - LI), time to lay (interval from the commencement of mating to the first egg -TTL), total egg production (TEP) and total chick production (TCP). SAB females outperformed their ZB and KR two-year-old contemporaries for TEP (respectively 24.9±2.2 vs. 15.7±3.6 and 10.4±4.9). Two-year-old SAB females mated to older males performed better for TTL (97 ± 8 vs. 121 ± 7), TEP (23.6 ± 2.5 vs. 16.1 ± 2.1) and TCP (11.7±1.6 vs. 5.2±1.3) than when mated to two-year-old males. The performance of two-year-old SAB females was independent of the service strain. Five-trait heritability estimates were 0.19±0.08 for AFE, 0.13±0.07 for LI, 0.20 ± 0.07 for TTL, 0.25 ± 0.07 for TEP and 0.21 ± 0.07 for TCP. When expressed relative to the corresponding trait means, genetic trends reflected change of -0.09% for AFE, 0.26% for LI, 0.66% for TTL, 0.84% for TEP and 1.21% for TCP. Age at first eqg has improved over time to 652 ± 13 days. This is likely an indirect result of genetic selection over more than two decades for improved reproduction by using dam records and breeding values to select replacements.

BRISTLE HAIRS, FILOPLUMES AND PINHOLES IN OSTRICHES

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Ostrich leather is deemed a luxury leather, with guality being of utmost importance. The widespread presence of small pinholes on ostrich skins has become increasingly problematic. Filoplumes and bristle hairs are implicated in the formation of the pinholes (Lunam & Weir, 2006; Weir & Lunam, 2011), and while both are deemed a natural phenomenon on avian skins, it is nonetheless regarded asa defect that retracts from leather quality. The presence of hair and hair follicles (pinholes) were consequently scored at different ages to determine environmental and genetic factors. It was found that the hair follicle scores on processed skins were heritable (±SE) at 0.40±0.06. The presence of hairson live birds was accordingly heritable at 0.51 ± 11 in juvenile birds and 0.23 ± 0.09 in adult birds assessed repeatedly. The animal permanent environmental variance ratio was also significant at 0.22 ± 0.09 for adult birds. Hair follicle score was genetically correlated to hair score on live slaughter birds (0.49 ± 0.23) while the phenotypic correlation between these traits amounted to 0.36±0.08. The genetic correlationwas lower than expected but it should be considered that only 476 hair scores were available on SA Blacks, of which only about 20% (n=97) had hair follicle scores on their skins as well. Genetic correlations of hair scores on live adult birds with other traits of economic importance were 0.64±0.30with egg production, 0.65 ± 0.35 with chick production and 0.27 ± 0.21 with adult live weight. Corresponding animal PE correlations were 0.93±0.38 with egg production, 0.78±0.32 with chick production and -0.10±0.21 with adult live weight. Phenotypic correlations amounted to respectively 0.14±0.07, 0.15±0.07 and 0.05±0.05. Hair scores on adult and juvenile birds essentially represented the same trait, as indicated by a genetic correlation of 0.93 ± 0.11 . Adult hair scores were also positively related to hair follicle scores in their slaughtered offspring, with the genetic correlation amounting to 0.61±0.27 between these traits.

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RECENT ADVANCES IN THE BREEDING OF FARMED OSTRICHES

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Previous reviews on the breeding of ostriches focused on challenges stemming from the communal breeding system of ostriches to the capturing of individual performance and pedigree records. The pair-mating breeding system, although imperfect, alleviated most of these challenges. Genetic and environmental parameters were derived for traits and traits combinations not previously reported. Genetic analyses on embryonic deaths indicated that derived heritability estimates were independent of the inclusion of the effect of evaporative water loss in the model. Updated analyses on skin traits with a non-normal distribution using threshold and linear-threshold analyses suggested that these traits were heritable on the underlying scale and not correlated with skin size. Analyses on temperament confirmed that intensive interactions with humans at a young age resulted in a more docile temperament in juvenile ostriches. Moreover, temperament traits were heritable and uncorrelated with slaughter and skin traits. Preliminary heritability estimates for monthly egg production and average egg weight in single-penned females maintained to develop a protocol for the artificial insemination of ostriches were consistent with those derived for pair-mated birds. So far, most genetic parameters were derived within trait complexes. Genetic correlations were thus derived between traits measured repeatedly in mature birds (live weight, egg production and chick production) and slaughter traits in their offspring (slaughter weight, skin size, nodule size score and hair follicle score). Adult weight was genetically positively correlated with slaughter weight, skin size and nodule size score. Reproduction traits of mature females were generally not correlated with offspring slaughter traits, barring an unfavourable genetic correlation of egg production with skin size. Further analyses across trait complexes should be prioritised for further studies. Obvious combinations for estimating genetic correlations are reproduction and temperament, embryo and chick survival with reproduction as well as repeated feather traits of mature birds with traits in the growth and slaughter bird complex. Important trait complexes not yet studied are feather traits in juvenile birds and resistance to external stressors associated with commercial ostrich farming systems. Information on all these trait combinations is needed to ensure that ostrich breeding is conducted in an ethical and sustainable way.

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Reproductive biotechnologies

Selected short communications

BUCKY BALL – AN EVOLUTIONARY CONSERVED GERM PLASM PROTEIN FOR CHICKEN PRIMORDIAL GERM CELL SPECIFICATION

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Bucky ball (Buc) acts as germ plasm organizer in zebrafish (1-3). A synthenybased approach based on two highly homologous domains identified potential Buc homologs despite low overall sequence conservation in fish, Xenopus, birds and mammals. All buc-homologs encode an intrinsically disordered protein whose biochemical activity is still unknown. To assess whether Buc could have a function firstlv in aerm plasm organization in amniotes, we localized Buc immunohistochemically in chicken follicles and uterine embryos. We found Buc located in the central cleavage furrows, aggregating the germ plasm within the central, first defining cells. Maximum binding of Buc was detected in stage III to IV embryos during zygote genome activation with strong nuclear signal. About 6-9 hours later, at stage VII, Buc was detected exclusively in the cytoplasm of the cells accompanied by nuclear labeling for Vasa. Until 48 h of incubation, Buc labeling was almost completely lost, whereas Vasa is located in PGCs consistently. To functionally analyze Buc, the coding sequence of the chicken protein was fused to a fluorescent reporter, cloned into an expression plasmid and transfected into DF1-cells. The reporter revealed a unique deposition pattern of the fusion protein, highly reminiscent of liquid phase separation. In summary, the data provide strong evidence that chicken Buc is a functional homologue of zebrafish Buc and may act as a germ plasm organizer in chickens.

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CONSERVATION OF CHICKEN MALE GERMLINE VIA PRIMORDIAL GERM CELLS FROM GENETICALLY DISTANT DONORS

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Successful derivation and cultivation of primordial germ cells (PGCs) opened the way to efficient transgenesis and genome editing in the chicken. Furthermore, implantation of male PGCs from nonchicken galliform species into the chicken embryos resulted in cross-species germline chimeras and viable offspring. We have recently improved the PGC technology by demonstrating that chicken male PGCs transplanted into the testes of adult cockerel recipients mature into functional sperms. However, the availability of this orthotopic transplantation for cross-species transfer remains to be explored. Here we tested the capacity of genetically distant male PGCs to mature in the microenvironment of adult testes. We derived PGCs from the Chinese black-bone Silkie and transplanted them into infertile White Leghorn cockerels.

Within 15–18 weeks after transplantation, we observed restoration of spermatogenesis in recipient cockerels and production of healthy progeny derived from the transplanted PGCs. Our findings also indicate the possibility of cross-species orthotopic transplantation of PGCs. Thus, our results might contribute to the preservation of endangered avian species and maintaining the genetic variability of the domestic chicken.

Chicken PGCs derived from genetically distant embryo are able to produce viable spermatozoa when transplanted into adult testes

APPLICATION OF COLLOIDAL CENTRIFUGATION TO REMOVE GLYCEROL FROM CHICKEN FROZEN-THAWED SEMEN

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Glycerol is the most used cryoprotectant to freeze chicken sperm due to an excellent protection of sperm cells against cryodamage. However, the presence of glycerol in post-thawed semen causes severe fertility reduction, leading to the necessity of removing glycerol before insemination. The main approach developed to remove glycerol is serial dilution (SD) protocol, which implies special equipment to maintain the thawed semen at 4°C and is time consuming. Therefore, we developed a simple method to remove glycerol from chicken frozen-thawed semen based on centrifugation in a colloidal gel, Percoll®, which is ordinarily used to select motile sperm in mammals as well as in fresh chicken semen. Eighteen adult T44 roosters were randomly divided between three groups and used as semen donors. Semen was collected from each group and frozen in Lake PC diluent containing 11% glycerol in straws in a programmable freezer. Frozen semen was thawed at 4°C for 3 min, layered on PBS solution containing 200 mM sucrose and isotonic Percoll® (Sucrose-Percoll® Solution, SPS) and centrifuged at 20° C, $800 \times q$ for 15 min. Different concentrations of SPS in PBS (40, 50, 60 or 70%) were tested to identify the best conditions by performing in vitro tests including glycerol concentration assay and sperm individual motility evaluation, as indicators of in vivo fertility. Artificial inseminations were performed on 36 hens with frozen-thawed semen treated by SPS and by SD protocols to compare their efficiencies. Data of in vitro and fertility tests were analysed by two-way ANOVA and Chi2 tests. Our results indicated that all concentrations of SPS efficiently decreased glycerol concentration to less than 120 mM while the concentration was 1866.3 mM before treatment. A beneficial selection of motile and progressive sperm was achieved by all concentrations of SPS, especially SPS 70% showed the best improvement of 25,1% and 15,2%. SPS 40 and 70% were two conditions selected for insemination test and reached 45.8% and 21.6% of fertility respectively, compared to 49.1% after SD treatment. Collectively, our new colloidal method can efficiently remove glycerol from chicken frozen-thawed semen with a positive selection of sperm motility. SPS 40% obtained better fertility than SPS 70% but was not significantly different from SD. Compared to the conventional SD protocol, this method can save 44% of the time while achieving similar fertility.

INTEGRATION OF RNA-SEQ AND ATAC-SEQ SUGGESTS KEY GENES IN GONAD ASYMMETRIC DEVELOPMENT OF FEMALE CHICKEN EMBRYO

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In birds, male gonads form on both sides whereas most females develop asymmetric gonads. Multiple early lines of evidence suggested that the right gonad fails to develop into a functional ovary, mainly due to the low proliferation rate of germ cells that never undergo meiosis. Despite some advances in recent years, the molecular mechanisms underlying asymmetric gonadal development remain unclear. Here, we established a relatively detailed profile of four representative stages of chicken gonadal development at the transcriptional and chromatin levels. We revealed that many candidate genes were significantly enriched in morphogenesis, meiosis and subcellular structure formation, which may be responsible for asymmetric gonadal development. Further chromatin accessibility analysis revealed that the transcriptional activities of the candidate genes were strongly regulated by nearby open chromatin regions, which mainly acted as transcription factors (TFs) binding sites and potential cis-regulatory elements. We found that LHX9 was a promising TF that bound to the left-biased peaks of many cell cycle-related genes and further enhanced mingle cell proliferation in mesonephros/gonads. In summary, this study provides distinctive insights into the potential molecular basis underlying the asymmetric development of chicken gonads.

SPERM INTACT CELL MALDI-TOF MASS SPECTROMETRY: AN INNOVATIVE PROTEOMIC PHENOTYPING METHOD FOR ACCURATE DIAGNOSIS OF MALE FERTILITY

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Currently, evaluation of sperm guality is mainly based on in vitro tests (concentration, mobility, membrane integrity...), which is poorly correlated with the male fertility observed in vivo. Thus, the development of an accurate tool for male fertility screening is still largely needed. Here, we propose the Intact cell MALDI-TOF Mass spectrometry (ICM-MS) as a new approach for spermatozoa phenotyping. We previously demonstrated that ICM-MS discriminates fertile (>70%) and sub-fertile chickens by comparing of their semen protein profiles [1, 2] and could be used as a fertility diagnostic test based on predictive mathematical models in an experimental context [3]. Here, our objective was to conduct a pilot study, to apply our standardized ICM-MS protocol, in real farming conditions, using large male populations of known fertility/hatchability from distinct breeds (one egg laying and 3 meat breeds). Qualitative and comparative molecular analyses were performed, as well as the evaluation of diagnostic tests at 3 ages. 73 layer males on one hand, and 99 broiler males distributed in 3 breeds (label, intermediate and rapid growth) on the other hand were classified according to their reproductive quality assessed by in vivo fertility/ hatchability tests. Fresh sperm cells collected at 3 age periods (23-25, 34-37, 55-60 weeks) were analyzed by ICM-MS, using a Bruker RapifleX MALDI-TOF instrument and treated by different chemometrics methods and PLS-GA modeling. Peaks were annotated with the aid of a Top-Down database house-made. Results: ICM-MS analysis revealed characteristic profiles for the layer/broiler genetically divergent breeds and a discrimination between the 3 broilers (label, intermediate and rapid growth) with specific and/or common molecular signatures. Fertile and sub-fertile males were successfully modeled, regarding fertility rates (not with hatchability rates) in the layer breed, thus confirming the suitability of ICM-MS approach to diagnose chicken fertility. Annotation allowed us to access to peptido/proteoforms corresponding to proteins already described in mechanisms implicated in fertility criteria. ICM-MS is an innovative "phenomics tool" that may help to select avian males on their reproductive capacity.

IMPLEMENTATION OF NEW METHODS IN THE HUNGARIAN IN VITRO POULTRY GENE BANK

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The part of protection of biodiversity is the preservation of those farm animal species and breeds, which were excluded from public farming due to the use of intensive, high yielding lines and hybrids. The advantage of frozen storage of the reproductive material over in vivo preservation is that it has no animal health risk and does not involve cost of the housing and feeding. While the cryopreservation of gametes and embryos is a routine procedure in many species of mammals, in birds only the sperm freezing is still the most reliable preservation practice. The establishment of in vitro gene bank of the Hungarian poultry was started in 2012 - among the first ones in Europe - by NBGK-HGI. The work started with the frozen of spermatozoa according to the FAO protocol which storage aives recommendations only for domestic fowl, therefore the sperm cryopreservation of other poultry species is still under continuous development in our institute. In avian species, the females are heterogametic (ZW) and the males are homogametic (ZZ); the ovum carrying the W sex chromosome and the embryo per se cannot be frozen. Therefore, there is a need to develop methods, which allow the female genome to be involved in the in vitro preservation. Transplantation of the ovarian tissue of day-old chicks on one hand and the primordial germ cells (PGC) on the other may be the solution. Both materials can be frozen, by which gonadal chimeras are formed and after sexual maturation donor-derived ovum can be obtained. In usage of PGCs the challenge is to establish stable cell lines moreover improve hatchability after injection of donor cells into recipient embryos. According to our previous studies on tissue grafting not all breeds are suitable as recipients, thus one of the most difficult tasks is to form appropriate donor/recipient combinations. Although the optimization of these methods is still ongoing, reconstruction of the donor genotype has been already successful, so storage of PGCs and gonadal tissues from 7 native hens has begun. Currently, there are 1034 PGC samples stored from which 517 are core, while 517 working collections. In case of ovarian tissue of the day-old chicks, 454 core collections are placed in the bank. The sperm bank currently has 500 straws/breed - altogether 3500 straws as core collection and 200 samples as working collection.

This work was supported by Horizon 2020" IMAGE" n°677353 and GÉNNET_21 - VEKOP-2.3.2-16-2016-00012.

26th World's Poultry Congress, abstracts selected in 2022

Reproductive physiology

Selected short communications

EFFECT OF NANOSELENIUM ON THE IMPROVEMENT OF CRYOPRESERVATION OF DOMESTIC FOWL SEMEN

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During semen cryopreservation, the spermatozoa undergo notable stress effect. The avian spermatozoa are extremely sensitive to the harmful effects caused by the formation of free radicals. Under natural conditions various types of antioxidant systems in spermatozoa and seminal fluid are able to bind to free radicals. Selenium with vitamin E is essential component of the antioxidant system in a multi-step biochemical process. In several animal species, antioxidants added to the semen diluent have been shown to improve the efficiency of semen freezing. Recent researches have shown that nanoparticles could be more efficiently utilized due to their increased surface area and reactivity. In the present work we investigated the effect of vitamin E and nanoselenium in various concentrations in semen diluent on the changes of qualities of rooster spermatozoa after freezing/thawing.

Semen of 20 individually placed Hungarian yellow males was collected twice weekly. The pooled semen was divided into four equal parts and diluted with Lake's diluent in 1:2 ratios. 5 µg/ml a-tocopherol was added to the diluted semen samples with the exception of control group, as well as 50, 100 and 200 µg/ml of nanoselenium solution were put to the three experimental samples, respectively. After 20 min of equilibration at 5 °C, all samples were further diluted in 1:0.5 ratios with DMA containing diluent to give a final concentration of 6% DMA. The samples were frozen 1 cm above the surface of liquid nitrogen in 0.25 ml French straws for 20 minutes and then placed in liquid nitrogen. The samples were thawed at 5°C. From the spermatological parameters motility was examined using SCA® CASA. The live/dead cell ratio and the ratio of morphological abnormalities were determined by aniline blue-eosin staining. After thawing, the proportion of motile cells in the samples with nanoselenium at different concentrations did not differ significantly from the control group ((34.6%; 26.4%; 34.5% and 29.2%, respectively). There were no differences in the survival rates of the cells (20.2%; 19.2%; 17.2%; 22.4%), and the morphological abnormalities, either. Based on in vitro studies, the use of nanoselenium and a-tocopherol in the given concentrations did spermatological not improve parameters after freezing/thawing. Despite the in vitro results further studies are planned with artificial insemination to investigate the effect of the above additives on the fertilization capacity of frozen/thawed sperm.

PROTEOME AND BIOLOGICAL FUNCTIONS OF INNER AND OUTER PERIVITELLINE LAYERS IN THE CHICKEN EGG

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The chicken egg perivitelline layer (PL), also known as the vitelline membrane, is an extracellular proteinaceous structure surrounding the egg yolk, in contact with the albumen. In avian reproduction, PL is involved in the sperm-egg binding and acrosome reaction, in the early embryonic development and in the physical and antimicrobial protection of the embryo. It thus contributes to the quality of both hatching and table eggs. It consists of two sublayers: the inner PL (IPL) and the outer PL (OPL), that are in contact with the yolk content and the egg white, respectively. About 140 different proteins were identified in the PL to date; however, the distribution of most of these proteins between IPL and OPL remains to be elucidated. The specific sites of synthesis, namely the ovary for the IPL and the oviduct for the OPL, suggest that these two sublayers possess different biological functions. The present study aims at characterizing the proteome composing IPL and OPL, and identifying the intrinsic putative functions of each perivitelline sublayer. For this purpose, PLs were harvested from ISA-Brown eggs collected on the day of lay and carefully washed to remove traces of yolk and egg albumen. IPL and OPL were carefully manually separated from isolated PLs and proteins composing these two layers were independently solubilized, fractionated by SDS-PAGE and analyzed by tandem mass spectrometry coupled to nanoliquid chromatography (GeLC-MS/MS). The proteomic analysis revealed 412 proteins in the PL including 314 and 239 proteins that were identified in IPL and OPL, respectively. Among these, 141 were common to both sublayers and encompass the most abundant proteins of the PL. The tissue origin of some of these shared proteins was investigated by the analysis of gene expression (RT-gPCR) in different reproductive tissues assumed to be involved in the formation of IPL (liver, theca, granulosa) and OPL (oviduct segments). The functional annotation (gene ontology/bibliography) of secreted proteins revealed that IPL is mainly involved in fertilization and in early embryonic development, while OPL seems to be rather involved in physical and antimicrobial defense but also in embryogenesis. Altogether, the data generated from this study give new insights into the structure and composition of the chicken egg PL and provide an integrative overview of the respective physiological functions of IPL and OPL.

CHEMERIN CONCENTRATION IN ALBUMEN AND CELL NUMBER OF GERMINAL DISC: POTENTIAL MARKERS OF THE QUALITY OF EMBRYO DEVELOPMENT FOR GENETIC SELECTION IN BIRDS

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One of the breeding companies' goal is the production of viable and robust oneday-old chicks. A major issue is the embryo mortality rate which can reach 40% according to the species. A new paradigm is necessary to identify new predictors of embryo development and to integrate them in breeding. In genetic selection, biomarkers have to be easily measurable, cheaper, reliable and inheritable. They have to be also individually stable and variable in the population. Here, we investigated two potentials molecular markers of embryo development: the chemerin concentration in albumen and the number of cells in the germinal disc at the oviposition time. We studied two breeds of layers, two breeds of broilers and one breed of duck. Eggs from 50 females from pedigree flock were collected during one week at three moments of the laying cycle. After weighing collection of eaas, albumen and yolk, and an albumen sample. the germinal disc was dissected to count cells. Chemerin albumen concentration was measured by a specific home-made avian ELISA assay kit. The different breeds of hens were compared by one-way ANOVA. Then, concentration of chemerin and cell number of germinal discs were correlated with reproductive parameters like fertility, laying and hatchability rates, number of offsprings and chick robustness. Our data showed that variability of chemerin expression in albumen is two-fold higher inter-hen compare to intra-hen for each breed. We observed significant different concentrations of chemerin in the albumen between two breeds of laying hens and two breeds of broilers. We found positive correlations: -at the time of laying peak between cell number of germinal disc and the number of chicks (r= 0.30 p-value= 0.04) and -at the beginning of the laying cycle between the germinal disc cell number and the rate of laying (r = 0.34; p-value = 0.01) for one broiler breed. Thus, we can suppose that a higher cell number of germinal discs may improve the laying and the embryo survival that's why we will study these parameters on a 2nd generation. These preliminary data need to be confirmed by other measurements on the offsprings of the animals studied. Other correlations are in progress suggesting a potential role of chemerin concentration in albumen and/or the cell number of germinal discs for eggs fertility or hatchability rate

Aknowledgement: we thank our partners Hendrix Genetics, Hubbard and Gourmaud Selection for supplying birds and ducks and valuable support.

26th World's Poultry Congress, abstracts selected in 2022

Small scale family poultry farming

Selected short communications

EGG PRODUCTION PERFORMANCE AND CONSUMPTION IN HOUSEHOLDS ENGAGED IN REARING BROWN EGG LAYERS IN THREE ALTITUDE RANGES OF SIMADA AND EBINAT DISTRICTS, NORTHWEST ETHIOPIA

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In this study, nutrition at the center (N@C), is a multi-sectoral and innovative program designed to improve the nutritional status of women of reproductive age (15-49) and children less than 2 years. N@C recognizes the impact of animal food source and is promoting poultry production to improve the nutritional status of a subgroup of participating households in its operational areas. N@C is aiming to address the challenges of poultry production by smallholder farmers through a research-based pilot intervention in two phases. Therefore, this study focuses on evaluation of egg production performances of Bovans Brown layer chicken and status of egg consumption in the households in different altitudes of Simada and Ebinat districts. One thousand farmers received five pullets and managed the pullets in semi-intensive chicken production system with provision of supplemental feeds formulated from locally available grains and disease control and health programs through supervision of agricultural extension service experts. For evaluation using structured recording formats of the program 100 farmers engaged in rearing laying chickens were selected randomly. The overall mean family size included in this study from both districts was 4.92± 1.37. Higher family size (4.94 ± 1.43) was found in low than medium (4.91 ± 1.38) and high (4.91±1.35) altitude areas. However, there was no any statistically significant difference (p>0.05) mean number family size among the three altitude areas. The mean number of laying hens in medium altitude areas (4.29 ± 0.52) was higher than low (3.76±1.07 and high (4.28±0.63) altitude areas. There was statistically significant difference (p < 0.05) in mean number of laying hens among altitude areas. Higher number of eggs per hens was found in low altitude areas (27.22 ± 6.43) than medium (24.82 ± 1.37) and high (26.89 ± 1.07) altitude areas. The mean number of eqgs laid per hen in Simada district was significantly (p < 0.05)higher than Ebinat district. With respect to egg consumption by family members in under-two children, two to five, and lactating women were 57%, 47 % and 72% found consuming weekly egg consumptions > 4 eggs whereas 94 % of the pregnant women had <1 egg per week. In this study, it was found that district, altitude and interaction of district and altitude have significant effects on egg production performance and number of laying hens. Key words: Egg Production Performance/ consumption/ Simada / Ebinat /Ethiopia

PRODUCTION PERFORMANCE OF DUCK REARED UNDER THE RIVER BASIN AREAS OF PABNA DISTRICT OF BANGLADESH

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The study was conducted to find out the actual scenery and make a suggestion for duck rearing in the River Basin Areas. There were sixty (60) households studied. Total farmers were divided into two groups according to the system of rearing i.e. scavenging/control group (without supplementation and number of ducks ranges from 50 to 100) and semi-scavenging (75% scavenging + 25% supplementation and the number of ducks ranges from 101 to 300). The semi-scavenging group supplemented at the rate of 100 g mixed feed (Paddy + Wheat + Maize + Rice polish) /duck/day in adult age. Each group contained 30 farmers and the geographical location was the same. The farmers of these areas reared mainly Khaki Campbell and Zinding ducks and rare some mixed crossbreed (local name Duashal). All recorded data were analyzed using "SPSS" for the interpretation of the results. The farmers gave the floor space 1.8 sq. ft/duck of scavenging and 1.4 sq. ft /duck of semi-scavenging group which is comparatively lower to scavenging system. The daily live weight gain differed significantly (p<0.01) and maximum weight (g) gain (8.54 ± 0.34) was observed in semi-scavenging than scavenging system (7.23±0.23) as the scavenging group did not supplement any feed. There was no significant difference of age at sexual maturity (ASM) between the groups Zinding slightly earlier (15±0.5 weeks) and Khaki Campbell (16±0.5 but in weeks). Bodyweight at ASM differed significantly (p < 0.05) and maximum body weight (kg) observed in Zinding (2.4 ± 0.2) than Khaki Campbell (2.25 ± 0.12) . Duck day eqg production of semi-scavenging (48.46 ± 01.3) % system was significantly (p<0.001) higher than scavenging (32.54 ± 01.3) % system and observed pick production in Rainy season (May-June) each year. Mortality percentage varies from 3 to 4 were observed in whole the flocks. It may be concluded as Zinding in the semi-scavenging system of rearing that area is the best practice for the alleviation of poverty from the society in Bangladesh.

Keywords: Duck, scavenging, semi-scavenging, production, and supplement.

CAN VILLAGE POULTRY IMPROVEMENT EMPOWER WOMEN?

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Introduction

Gender analysis and gender sensitive instruments developed and used in development and research related to livestock and specifically to village poultry has evolved in the last three decades. Because poultry is the most widespread animal species in the world and because its potential for demand growth in the next decades is high it constitutes a very important animal husbandry to promote and improve. In addition, as poultry constitute one of the most common assets under the responsibility of women in the world, the motivations to use it as a leverage to increase rural women's visibility, economic benefit and empowerment is frequent.

Methodology

A dozen female key players who participated in projects and research related to village poultry in Asia (HPAI) and in Southern Africa (Newcastle disease) in the last three decades speak about women empowerment and village poultry work and comment on interventions carried out and results achieved individually and collectively. The paper also put in perspective, revises, compiles and analyses quantitative and qualitative data collected in different contexts through the prism of current knowledges and practices toward gender equality and women's empowerment.

Results

Women from different backgrounds and ages, in distinct period of their carrier and in diverse positions and roles in the several interventions under analysis experienced, exercised and valued different forms of power. Approaches shared, methodologies applied, and experiences discussed, both in relation to research and development processes, in the search for gender equality and women's empowerment highlight the importance of placing women at the centre of the process.

Conclusion

The diverse forms of women's empowerments experienced and achieved at different nodes of the interventions processes illustrate the multiplicity of ways by which gender sensitive interventions can transform individuals, processes as well as institutions.

ACTION RESEARCH TO IDENTIFY CONSTRAINTS AND OPPORTUNITIES FOR INCREASED NEW CASTLE DISEASE VACCINE ADOPTION AMONG WOMEN SMALL HOLDER POULTRY FARMERS IN KENYA, RWANDA AND UGANDA.

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In Rwanda, Kenya and Uganda, 75 to 90% of small-scale poultry farmers are women. Diseases are a major issue preventing them from optimizing production. Their flocks are frequently decimated by NCD despite availability of an effective vaccine. Packaging and reliable structures for vaccine delivery remain an obstacle to vaccine uptake and use. Women are disproportionately affected because of their reliance on poultry income. Our objectives were to: 1) identify barriers and opportunities for women's participation in NCD vaccine value chain (VVC) 2) formulate strategies that position women to effectively contribute to and benefit from poultry vaccines, 3) enhance women's participation in vaccine distribution, delivery and use, 4) provide data that impact programmatic and policy interventions. We used three methods for gender analysis and NCD-VVC analysis:1) The USAID Five Domains of Gender Analysis Framework administered through focus groups captured barriers, opportunities and strategies for improving women's participation in poultry ownership and VVC. 2)The Women Empowerment in Livestock Index (WELI) a standardized household quantitative survey tool captured the empowerment index of women farmers and vaccine usage. 3) Outcome Mapping systematically mapped and tracked critical stakeholders in the VVC focusing on changes in gender norms and cultural practices, organizational systems, institutional and governance policies that lead to increased vaccine adoption. We also introduced novel tools such as Vaxer Calendars, focus meals, jar voices, VacZines and PhotoVax to collect data and identify entry points for interventions. Preliminary results highlight limited vaccine availability and accessibility, as well as infrastructure challenges, lack of knowledge and information to quide poultry farmers on vaccinable diseases, women's limited decision-making agency in their households and communities, gender related technical, social, cultural, and economic barriers, lack of partnership/networking with public and private sectors as key reasons for low adoption of NCD vaccines. The project will pilot interventions that target women's individual and collective decision-making control within the households and communities, resulting in a recognized productive role in poultry management, strengthened skills and knowledge on vaccines, opportunities to influence governance structures leading to increased poultry vaccine adoption.

CHARACTERIZATION OF GROWTH PERFORMANCE, MEAT QUALITY, SERUM BIOCHEMICAL PROFILE, CELLULAR AND HUMORAL IMMUNE RESPONSE OF KADAKNATH CHICKEN UNDER MORINGA OLEIFERA FEED SUPPLEMENTATION

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Introduction: Backyard poultry farming in India has been rapidly growing in recent years. Consumer preference for chicken meat and eggs from the birds reared in semi-intensive or range system is increasing and also fetching higher prices. M.oleifera has the potential nutritional benefits for use in poultry feed as it is a good source of vitamins, minerals, and bioactive phytochemicals. The level of inclusion needs to be standardized in different phases of growth without effecting the health status and quality of meat.

Materials and methods: Dried leaf powder of M. oleifera (MOLP) was supplemented at 0, 5, 10 and 15% levels of basal diet from day old to 17 weeks old to Kadaknath chicken. Weekly feed intake, body weight, body weight gain and FCR were calculated on every 3week interval for each replicate. Sensory, physical and nutritive quality of meat from 17 weeks old chicken were studied. Serum biochemical profile of chicken were estimated during both 6 and 17 weeks of age. Humoral response to lasota vaccination and cellular immune response to PHA-P injection were studied at 8 weeks of age. Data was analysed statistically using SPSS 20.0 as per Duncan's multiple range test.

Results: Overall performance of 5% supplemented group was superior among the dietary treatment groups. Significant reduction in feed intake of supplemented groups compared to control group. Improvement ($p \le 0.05$) in the FCR of supplemented groups compared to control group birds. The was an added effect on sensory, physical and nutritive quality of meat without significant difference. Beneficial effect ($p \le 0.05$) of M. oleifera feeding on the oxidative stability of meat. 5 and 10% supplemented groups recorded improvement ($p \le 0.05$) in the serum lipid picture and functional profile of kidney. MOLP feeding significantly ($p \le 0.05$) increased humoral and cell mediated immune response.

Conclusion: M. oleifera can be included at 5% level during stater phase and can be increased up to 10 % level in grower phase. Supplementation of MOLP also reduces the feed cost besides having beneficial effect on meat quality, immunity and health status.

African Poultry Network

Selected short communications
EFFECT OF DIETARY PROTEIN AND ENERGY LEVEL OF INDIGENOUS GUINEA FOWL (NUMIDA MELEAGRIS) ON LAYING, BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS

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Feed is one of the main factors influencing the quality and number of hatching eggs of guinea fowl as in other poultry. Energy and protein concentrations are the major feed components that have impact on eqg laying. The purpose of this study was to assess the effects of variation in protein and energy intake on indigenous guinea fowl breeders. A total of 280 female local guinea fowls and 140 males were fed since day-old with 5 diets: control (ME: 2802; CP:17,13) low-energy (ME: 2701; CP:17,16), low-protein (ME: 2802; CP:15,67), high-energy (ME: 2906; CP:17,10) and high-protein (ME: 2799; CP:18,62). These guinea fowls were crossed at 23 rd week old with a sex ratio of 2 females for 1 male. The initial data such as feed intake and egg production were collected at the 28 th week. From the 32 nd to the 39 th week of age, a sample of 32 eggs per group was randomly taken, weighed then broken to assess their external and and internal quality. At the 22 nd and 60 th week of age, blood samples were collected from all groups to determine the blood count and to assay the biochemical parameters. The data were processed by Graph Pad Prism 5.00.288 software and the ANOVA test was used to analyse the results. The means were compared by the Tukey HSD test and the probability of p < 0.05 was considered as the significance threshold. The results showed that the guinea fowls fed the highenergy diet consumed $(88.43 \pm 0.28 \text{ g/j})$ less feed (p < 0.05) than those of control group $(91.88 \pm 0.61 \text{g/j})$. On the other hand, the birds of low-energy group ingested (110.30 \pm 0.37) more feed compared to control group (p < 0.05). The increase or decrease in protein content had no influence on feed intake (p > 0.05). High-protein and low-energy respectively increased (p < 0.05) egg weight (40.12g and 39.33g vs 38.46g), and decreased (p < 0.05) fertility (61.14% and 62.92% vs 72.49%). High-protein diet increased (p < 0.05) the laying rate (65.21% vs 50.35%), and low-energy diet decreased the laying rate (47.92% vs 50, 35%). Low-protein and high-energy diets respectively reduced (p<0.05) the laying rate (38.03% and 33.5% vs 50.35%), the eqg weight (35.38g) and 35.75g vs 38.46g). Hematocrit of low-protein and high-energy is significantly (p < 0.05) less than that of control group. The serum total protein and albumin level of low-energy and high-protein are high (p < 0.05) than those of control **Group.** This study showed that high-protein and low-energy diets improved laving rate, egg weight, Keywords: Guinea fowl, protein, energy, Togo.

COMPARATIVE PERFORMANCE AND CARCASS YIELD PREDICTION USING BODY LINEAR MEASUREMENTS IN A PURE AND RECIPROCAL CROSS BREEDING SYSTEM OF THE SASSO AND WASSACHE CHICKENS

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The study was aimed at improving the Wassache chicken and predicting carcass yield from body linear measurements in a pure and reciprocal crossbreeding experiments carried out to compare the performances of cross breds (Sasso x Wassache chicken (SW) and wassache x Sasso female (WS)) to pure breds (Sasso (SS) and Wassache (WW) breeds) simultaneously. Data on body weight and body linear measurements (BLM) was collected on 300 birds on the 12th week of experiment, 20 birds each across the genotypes were dissected to determine the carcass yield. Body weight and carcass weights were measured using a weighing scale, BLM were measured using a flexible measuring tape. The results were subjected to ANOVA and regression analysis using Minitab 19. Sasso chicken showed significant (p<0.05) supremacy compared to the wassache chicken, while the values for the reciprocal crosses were intermediate and significantly (p>0.05)different form the pure breeds in all parameters studied except for thigh weight, back length and carcass yield. There was no significant (p < 0.05) difference amongst the cross breeds in all the parameters. Body linear measurements predicted meat yield with the WW genotype showing the highest R2 value (63.22 %) and least in the SS genotype (33.22%). The prediction coefficients in the reciprocal crosses were improved for WS and SW (R2 = 55.8% and R2 = 51.43%). Cross breeding using SS improved the growth performance of the wassache chicken significantly (p<0.05). The mathematical models predicted carcass yield from body linear measurements. The use of Sasso sires against Wassache sires will give a greater advantage in breeding programs for improvements in body weight and BLM. Sasso sires are recommended for use in crosses involving WW hens for table birds in this region while the use of Wassache Sires on Sasso hens will enhance better carcass yield %.

INCIDENCE OF INFECTIOUS BURSAL DISEASE IN VILLAGE CHICKENS IN TWO DISTRICTS OF AMHARA REGION, NORTHWEST ETHIOPIA

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In Ethiopia, lack of coordinated implementation of importation policy and quarantine services have opened the door to the introduction of infectious bursal diseases that has not been reported previously. Therefore, a longitudinal study was conducted in village chickens owned by 775 households in two districts of Amhara Region, Ethiopia. Clinical, pathological and serological studies were conducted to confirm IBD as outbreaks in the study areas. During the study period, the incidence of IBD was found 38.39% in Bahir Dar and 17.40 % in Farta. The case fatality rates were 98.56% in Bahir Dar and 77.73% in Farta. There was significant difference (p < 0.05) in the incidence and case fatality rates of IBD among the study districts. Chicken in the households of Bahir Dar district was found 1.69 (OR=1.69) times more likely to be affected by IBD than those in Farta district. At postmortem examination, ecchymotic hemorrhages were observed on the thigh and breast muscles and at the proventriculus-gizzard junction. The bursa of Fabricius was hyperemic and enlarged and when opened the bursa was filled with necrotic yellowish debris. Histopathological evidences of infectious bursal disease were detected in the bursae and spleen. The prominent bursal lesions include haemorrhagic lesions in the interfollicular space, heterophilic infiltration in the follicles with necrotic centers, follicular atrophy, lymphoid cell depletion and the formation of cystic cavity in the medulla. The spleen of affected chickens was also enlarged. Similar necrotic process has been observed in the white pulp of the spleen. This is the first report of IBD in village chickens in Ethiopia. Thus, it is of paramount importance to design in depth survey of village poultry production systems and the spread of disease to poultry breeding centres and indigenous chickens, characterization of infectious bursal disease virus isolates from field cases and the IBDV vaccine strains used in Ethiopia, investigation on genetic resistance/susceptibility of local chickens breeds in relation to infectious burasl disease virus, assessment on economic impact of IBDV on village poultry production and evaluation of biosecurity measures implemented by commercial farms in Ethiopia are required in order to improve the productivity and welfare of village chickens and also to conserve the indigenous chicken genetic resources. Key words: Infectious bursal disease, incidence, indigenous chicken, villages

SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF INFECTIOUS BONCHITIS IN BROILER AND LAYER FLOCKS IN THE PERI-URBAN AREA OF DAKAR AND THIES (SENEGAL)

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A serological survey was carried out in 20 laying hen farms with more than 1,000 birds suspected of having respiratory infections in two regions with a high concentration of poultry in Senegal. A questionnaire was administered and blood taken from 20 birds samples were in the most affected building. The serological survey by indirect ELISA technique involved 400 sera for five (5) diseases: Newcastle disease (NCD), infectious bronchitis (IB), LPAI subtype H9 and mycoplasmosis with M. gallisepticum (MG) and M. synoviae (MS). serology Herd infection rates determined were in order of by decreasing importance 60% for MS, 50% each for LPAI subtype H9 and MG; 40% for BI and 35% for NCD. These prevalence values varied according to the areas surveyed, age, strains of layers and vaccination programmes applied. Coinfections were recorded with associations of 2 to 4 pathogens, in order of importance, MG-MS-H9 in 23.5% of cases, influenza-MG-MS-H9, MG-MS-BI, ND-BI in 11.8% of cases and 5.9% of associations of ND-Influenza H9, MG-H9, MG+MS+H9+ND, MS+BI+H9, MG+MS+BI+ND, MS+H9, ND+MS. Several risk factors such as irregular visits by the poultry advisor, multi-age sites, manual watering, lack of a second disinfection of the buildings after sanitation, poor quality bedding (wet in places), lack of foot baths, were associated with these respiratory infections.

Key words: Laying hens- respiratory infections - sero-epidemiology - Senegal.

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Selected short communications

RESIDUAL FEED INTAKE – A BETTER TOOL TO ASSESS FEED EFFICIENCY IN TURKEY

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Feed expenses account for over 70% of the total production cost. Selection and breeding of feed efficient bird is the ultimate aim of the researchers. In this study an attempt was made to evaluate the feed efficiency traits in growing turkey poults. Six wk old Beltsville small white turkey chicks (n=144) were randomly distributed in individual cage system and provided standard diets (NRC, 1994). The body weight gain (BWG), feed conversion ratio (FCR) and residual feed intake (RFI=TFI-{b0+b1MMW+b2BWG}) was calculated for the 6-12 wk period. Based on combine index (FCR & RFI data) poults were categorized into: high feed efficient (HFE) comprising top 10 % of all birds, medium feed efficient (MFE) group (middle 10%), and low feed efficient (LFE) group (bottom 10%). At 12th wk, six birds from each group were slaughtered; serum was separated for biochemical studies and 150 mg of liver & jejunum tissues each was collected for total RNA isolation and subsequent cDNA preparation. The qPCR was carried out for nutrient transporters & growth-related genes following standard protocol. Data was subjected to oneway ANOVA using SPSS-20 software. The feed efficient birds categorized based on RFI were almost similar to that of combined index with few closed out liars. The HFE group turkeys gained more weight (12.6 %), consumed less feed (15.1 %), and had better FCR (30.7%) & RFI (>200%) than the LFE group. BWG was positively correlated with feed intake (FI) but negatively correlated with FCR. RFI was positively correlated with both FI and FCR. Higher serum cholesterol and uric acid level was recorded in HFE than MFE & LFE group, however serum glucose & protein level was similar in all FE groups. The relative expression of CDX, SGLTI, EAAT & FABP genes in jejunum tissue of HFE group was lower in comparison to LFE group, but LFE and MFE groups had similar expression. The expression of IGF-II & GHRL gene in liver tissue was considerably increased in HFE group than LFE and MFE groups. It is concluded that the HFE turkeys gained more weight, consumed less feed & had better FCR than the LFE group. The RFI measurement was found to be highly correlated with FI & FCR, hence can be used as a tool to assess FE traits in turkeys. Differential expression of nutrient transporter & growthrelated genes in different feed efficient turkeys indicate possible polymorphic pattern of these genes in turkey.

Key Words: Residual feed intake, FCR, Feed efficiency, gene expression, turkey

EFFECT OF DIETARY SUPPLEMENTATION OF ORGANIC, INORGANIC AND NANO PARTICLES OF CHROMIUM ON GROWTH, CARCASS QUALITY TRAITS, DEVELOPMENT OF DIGESTIVE AND IMMUNE ORGANS OF TURKEY POULTS

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An experiment was performed to study the effect of dietary supplementation of organic, inorganic and nano chromium particles on growth, carcass guality traits, development of digestive and immune organs of turkey poults. One-hundred-andtwenty-day old straight run turkey poults were divided into four treatment groups comprising of three replicates and ten birds in each replicate. T1 birds were fed basal turkey diet, T2 were fed basal diet + inorganic chromium@ 0.5 mg/ kg diet, T3 were fed basal diet + organic chromium@ 0.5 mg/ kg diet, T4 were fed basal diet + nano chromium@ 0.5 mg/ kg diet. Results indicated that there was no significant difference in the average weekly body weight. feed intake and body weight gain of birds during the entire experimental period except at 6th week where birds in T3 (139.07gm) group had significantly higher (P<0.01) body weight gain than T1 (136.13gm), T2 (136.86 gm) and T4 (136.06 gm). There was no significant difference in FCR of birds during the entire experimental period except at 6th week where FCR was significantly better (P<0.05) in T3 (2.52) as compared to T4 (2.59) and comparatively better than T1 (2.54), T2 (2.57). Dressing percentage was significantly higher (P<0.05) in T3 (74.94) group as compared to T1 (73.47), T2 (73.39) and T4 (73.39) groups. However, no significant difference was observed in other slaughter traits viz. percent shrinkage and ready-to-cook yield. There was no significant difference observed in cut-up-parts among the different treatment groups except percent neck yield, which was significantly higher (P<0.05) in T3 (5.29) than T1 (4.26) and comparatively higher than T2 (4.45) and T4 (4.92). Significantly higher percentage of weight (P<0.05) of thymus was observed in T4 (0.3) than T1 (0.15) and comparatively higher than T2 (0.19) and T3 (0.2). Similarly, significantly higher percentage of weight (P<0.05) of bursa was observed in T4 (0.29) than T1 (0.16), T2 (0.19) and T3 (0.17). Thus, it may be concluded that dietary supplementation of organic, inorganic and nano chromium did not result in any adverse effect on growth and slaughter quality traits of birds throughout the experimental period. However, dietary supplementation of organic chromium significantly increased dressing percentage and percent neck yield. Further, dietary supplementation of nano chromium significantly increased weight of thymus and bursa of turkey poults at 8 weeks of age. Keywords: Chromium, Turkey Poults

THE PROTEIN PROFILE OF BREAST MUSCLE EXUDATE IN RESPONSE TO DIFFERENT DIETARY RATIOS OF LIMITING AMINO ACIDS IN TURKEYS SUBJECTED TO CLOSTRIDIUM PERFRINGENS CHALLENGE

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The aim of this study was to determine the effect of different Arg to Lys ratios in diets with low or high Met levels on the protein profile of breast muscle exudate in turkeys reared under optimal (no challenge) or challenge (Clostridium perfringens infection) conditions. The experiment was performed on female Hybrid Converter turkey poults (216 in total) that were randomly allocated to 12 pens with litter bedding based on their average initial body weight, and were reared over a 42day experimental period. The levels of supplemental Lys were consistent with the nutritional specifications in the Management Guidelines for Growing Commercial Turkeys at respective ages. The experiment had a completely randomized $3 \times 2 \times 2$ 2 factorial design with three levels of Arg (90%, 100% and 110%) relative to the content of dietary Met (30 or 45%) and C. perfringens challenge: without (-) or with (+). The birds referred to as "challenged" were infected with C. perfringens at 34, 36 and 37 days of age. An overnight culture of bacterial inoculum in a brain heart infusion (BHI) broth (Sigma Aldrich) containing approximately 108 CFU/mL of C. perfringens was administered to birds per os at a dose of 1 mL. At 42 days of age, 8 turkeys representing group average body weight from each treatment were sacrificed by cervical dislocation, and the right breast muscles (pectoralis major) were collected, placed separately in plastic bags, and transported to the laboratory. Meat pH was measured 48 h after slaughter (pH 48), and meat color was evaluated in the CIE L*a*b* system. The sarcoplasmic protein profile of breast muscle exudate was determined by electrophoresis (SDS-PAGE). The effects of three levels of Arg (90%, 100% and 110%) relative to the content of dietary Met (30% or 45%) and C. perfringens infection (-, +) were evaluated by three-way ANOVA ($3 \times 2 \times 2$ factorial design). It was found that the experimental factors had no significant effect on meat quality traits, except for dietary Arg content which affected color parameter a*. Protein profile analysis by SDS-PAGE revealed a predominance of glycolytic enzymes. The protein profile of breast muscle exudate was significantly influenced by all studied factors. Glycolytic enzymes perform many important functions in the cell (apart from glycolysis), and the results of this study indicate that they may be involved in cell metabolism, in particular the course of inflammatory processes, as well as DNA replication and status.

LATERAL TRANSMISSION OF HISTOMONAS MELEAGRIDIS IN TURKEY POULTS RAISED ON FLOOR PENS

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Histomoniasis (blackhead disease) is caused by the protozoa Histomonas meleagridis (HM) and has a great economic impact on turkey production. Under commercial conditions, HM gets laterally transmitted among birds and leads to high mortality in inflicted flocks. In research models, birds are individually inoculated by intracloacal administration of HM, which is both labor intensive (all birds need to be inoculated) and does not reflect field conditions as lateral transmission does not occur with birds raised in battery cages. This study tested an HM infection model assessing the lateral transmission of HM in turkey poults raised on floor pens. A total of 320, day (d)-old female turkey poults were individually wing-tagged and allocated to one of four treatment groups (4 floor pens/group and 20 poults/pen) based on the percentage of poults inoculated with HM: 1) 10% (HM10); 2) 20% (HM20); 3) 30% (HM30); and 4) 40% (HM40). On d 9, seeder poults received a 1 mL inoculum/bird containing □80,000 cells of HM (Buford strain) administered intracloacally. Poults had free access to a starter cornsovbean meal diet and water. Poults were individually weighed on d 0, 9, and 25 and feed intake recorded on per pen basis. Mortality was recorded twice daily, and performance data adjusted for the mortality. On d 25, all birds were euthanized by cervical dislocation and ceca and liver were evaluated for HM lesions. Data were analyzed using JMP (Pro16) and significance (P<0.05) between treatments were determined by LSD test. Mortality was 7.63%, 12.5%, 21.58%, and 20.59% in HM10, HM20, HM30, and HM40 groups, respectively. HM was successfully transmitted from inoculated to non-inoculated birds with infection rates of 62.5%, 57.5%, 92.43%, and 78.75% in HM10, HM20, HM30, and HM40 groups, respectively. Average daily feed intake was proportionally reduced from HM10 to HM40, which was associated with the increase in the number of poults inoculated per group. Average daily gain was significantly lower in HM30 and HM40 groups compared to HM10 and HM20 during the post-challenge period (d 10-25). Therefore, we herein report the successful lateral transmission of HM among turkey poults raised on floor pens. This research model closely resembles commercial field conditions and affords a much-needed opportunity for conducting relevant basic and applied research on histomoniasis in poultry.

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Turkeys

Posters

PROBIOTIC BACILLUS LICHENIFORMIS DSM28710 IN FATTENING TURKEY DIETS IMPROVES TECHNICAL PERFORMANCE

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The efficacy of a specific probiotic strain of Bacillus licheniformis (DSM 28710) was evaluated for use in fattening turkeys, with the goal of improving technical performance parameters. The trial was carried out at the Centro di Ricerche per La Zootecnia e L'ambiente (CERZOO, Italy), using 336 one-day old male BUT big 6 turkeys (average 62.5 g body weight at the start) for a trial duration of 84 days in total. Animals were divided at random over 18 pens per treatment (8 to 10 animals per pen) and vaccinated against Newcastle disease at day 21. There were two treatment groups: a control, fed a commercial basal diet (corn/soybean meal based) and a probiotic Bacillus licheniformis group, fed the control's diet supplemented with 0.5 kg B-Act®/mton of feed (1.6 x 1012 CFU Bacillus licheniformis DSM 28710/mton of feed). B-Act® is a single-strain probiotic formulation, produced and commercialised by Huvepharma®. Body weights and average daily feed intake (ADFI) were measured, to calculate average daily gain (ADG) and feed conversation ratio (FCR). All data was statistically analysed using SAS's General Linear Model procedure (GLM; SAS, 2002-2010, release 9.3). ANOVA (Analysis of Variance) provided the main statistical test, with student's "t" and Tukey tests used to compare the means of each group. The level of significance to indicate statistical differences stated in the ANOVA model was $P \le 0.05$. Body weights at the end of the trial differed significantly, with animals supplemented with B. licheniformis DSM 28710 achieving 408.1 grams more than animals in the control group (6487.4 g vs. 6079.3 g, P<0.05). Average daily gain (ADG) was significantly higher for the probiotic group as well, both in the period of day 56 to 84 as well as over the whole trail (D56-84: 116.9 g vs. 106 g, D0-84: 76.5 g vs. 71.6 g, P<0.05). At the same time, average daily feed intake did not increase significantly for either group (D0-84: 191.8 g vs. 182.5 g, P>0.05), resulting in a final numerically improved FCR for the B. licheniformis DSM 28710 group (D0-84: 2.51 vs. 2.56, P>0.05). Supplementing fattening turkeys with 0.5 kg B-Act®/mton of feed had a significant positive effect on final body weight and average daily gain, whilst FCR improved numerically. As such it can be concluded feed was used more efficiently when the animals were supplemented with the probiotic, positively impacting final technical performance.

THE IMMUNE RESPONSE OF TURKEYS TO EARLY ADMINISTRATION OF ANTIBIOTICS

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The experiment verified the hypothesis that the early administration of an antibiotic may weaken the immunity in the first days of life and, consequently, worsen the functioning of the immune system of growing turkeys. The experiment was carried out on 3080 one-day-old turkeys divided into 4 experimental groups. The Control group did not receive any coccidiostatic or antibiotic supplement. Group M received monensin at a dose of 90 mg/kg feed, group E received enrofloxacin at a dose of 10 mg/kg BW for the first 5 days of life, and group D received doxycycline at a dose of 50 mg/kg BW. On 7 and 56 days of life, blood was collected from 14 birds from each group, and during necropsy, the spleen was collected for the determination of selected immunological indicators (percentage of CD4+, CD8+, CD4+, CD8+ IgM+ cells, and level of IL-2, IL-6, IL-8, IL-12, TNFa). The results were statistically analyzed by ANOVA using the STATISTICA version 13 software. Early administration of enrofloxacin decreased the percentage of IqM+ (P=0.006) and the level of IL-2, IL-6, TNF-a (P<0.001, both) in the blood of 7 days old turkeys. This had no effect on the percentage of the tested blood and spleen T and B lymphocyte subpopulations in 56 day-old turkeys, but it resulted in a reduction in the levels of IL-12 (P<0.001) and TNF-a (P=0.002) in the blood. Early dosing of doxycycline caused an increase in the percentage of CD8+ and CD4+CD8+ subpopulations in the spleen in 56-day-old birds. Plasma levels of IL-6 (P<0.001, P = 0.007, respectively) and TNF-a (P<0.001, P=0.002, respectively) were reduced in both 7- and 56-day-old turkeys following the administration of doxycycline. The use of monensin caused an increase in the blood IL-8 level in 7day-old turkeys. Early administration of enrofloxacin and doxycycline had no effect on body weight, FCR, and mortality, while administration of monensin increased body weight (P=0.006) and decreased FCR (P<0.001) in 56-day-old birds. The obtained results indicate that early administration of enrofloxacin or doxycycline to turkeys may significantly affect both the percentage of different subpopulations of T and B lymphocytes and the expression of many cytokines regulating the immune response. This, in turn, may affect the level of protection built both after vaccination and after natural contact with numerous pathogens present in their environment.

This work was supported by the National Science Centre in Poland, Grant No. 2020/39/B/NZ9/00765

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Waterfowls

Selected short communications

STUDY OF BIOMARKERS OF FOIE GRAS IN DUCK: IDENTIFICATION BY METABOLOMICS

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Introduction

The main producer of "foie gras" is France. Foie gras is rich in lipids that are responsible for its organoleptic qualities. Too much fat loss during the cooking process is a major problem in the industry of foie gras. One of the main issues is to classify the foie gras in function of their technological yield (TY) before cooking them. The TY must be greater than 70% [1]. The TY is strongly correlated to the liver weight (LW) [2,3]. Thus, the study aims at identifying biomarkers specific to TY with non-invasive biomarkers in duck. 1H-NMR (nuclear magnetic resonance of the proton) analyses were performed on liver of male mule ducks at different time points during the overfeeding period to identify liver biomarkers of foie gras qualities. Material and Methods: The experimental designed is clearly described in [2]. Briefly 64 mule ducks were overfed during 6 to 12 days. Plasmas and livers were sampled every two days. The LW and TY were measured. 1H-NMR spectra of plasma were acquired on NMR 600 MHz Bruker Avance III HD. The spectra were transformed into a table of 720 buckets with WorkFlow4Metabolomics [3] and into a table of 80 metabolites with ASICS package of R [4]. Then PLS regressions were performed with SIMCA-P+ software to identify the biomarkers to LW and to TY, so as to focus on the biomarkers specific to TY. Results: The PLS models of LW and TY only contained one latent variable that enabled to separate the samples in function of LW or TY. We identified 14 biomarkers of LW and 13 biomarkers of TY. Nine biomarkers were common to LW and TY (alanine, allantoin, glucose, glyceric acid, glycogen, lactate, maltose, taurine and threonine), five were specific to LW (arginine, glucuronic acid, glycerophosphocholine, malic acid and trasn-4-hydroxyproline) and four were specific to TY (creatine, ethanolamine, glutamic acid and quanidinoacetic acid). Conclusions: It is the first study of biomarkers of foie gras quality with large approach. To complete this study, we also studied plasmatic biomarkers of foie gras guality.

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IDENTIFICATION OF PLASMATIC BIOMARKERS OF FOIE GRAS QUALITIES IN DUCK BY METABOLOMICS

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The main producer of "foie gras" (fatty liver) of ducks in the world is France. The liver weight (LW) and the technological yield (TY) when cooking are the main characteristics of liver quality. The TY must be superior to 70% [1]. One of the main issues for producers is to classify the foie gras with high or low technological quality before cooking them. Thus, in the current study, plasmatic biomarkers of LW and TY were investigated by metabolomics approach using proton nuclear magnetic resonance (1H-NMR). The experimental designed is clearly described in [2]. Briefly 65 mule ducks were overfed during 6 to 12 days. Then they were slaughtered, the LW and the TY were determined. Plasmas sampled during the bleeding 11 hr after the last meal. 1H-NMR spectra of plasma were acquired. The spectra were analysed by two methods: a usual bucket method with WorkFlow4Metabolomics [3] and a new metabolite method with ASICS package of R [4]. Then PLS models were performed with SIMCA-P+ software to identify the biomarkers of LW and TY with both methods. The validations of the models were estimated by 500 permutation tests. The X-variables with a VIP-value superior to 1 were considered as important and were kept for interpretation of the results. The PLS models of LW and TY only contained one latent variable that enabled to separate the samples in function of LW or TY. We identified 18 biomarkers of liver weight and 15 biomarkers of technological yield. As these two quality parameters were strongly correlated (-0.82), 13 biomarkers were common: the lactate was the most important biomarker and there were also amino acids. Contrary to the amino acids, the lactate increased with the liver weight and decreased with the technological yield. We also identified 5 biomarkers specific to LW (glucuronic acid, mannose, sorbitol, glutamic acid and methionine) that were negatively correlated to liver weight. It was of main interest to identify 2 biomarkers specific to the technological yield. Contrary to the isovaleric acid, the valine was negatively correlated to the technological yield. This study presents the first analysis of plasmatic biomarkers of duck foie gras gualities with a large approach.

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BIOTIC STRESSOR, AFLATOXINB1 EMERGE AS THE MOST SIGNIFICANT FACTOR BEYOND ABIOTIC STRESSORS: FLUCTUATING CLIMATE AND METEOROLOGICAL FACTORS IN INFLUENCING EGG PRODUCTION IN DUCKS

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The Ducks (Anas Platyrhynchos) as a prominent alternate backyard-Poultry is constantly gaining popularity in Indian Subcontinent. Towards this, ICAR-DPR Regional Station Bhubaneswar is propagating a strain of White Pekin ducks, matching farmer's expectation of dual-purpose ducks. Considering vulnerability of Pekin ducks to biotic factors: naturally-occurring feed AflatoxinB1(AFB1) and Ochratoxin (OTA), the Egg-production data of Pekin-layers over 5 Annual-egg production cycles (2013-21) were analyzed for their impacts, besides those of abiotic factors: ambient temperature (low/high) and Relative humidity (RH). The impacts of 4 seasons of year (summer, Rains, Autumn, Winter) and 4 natural Production-peaks (early, Mid, Late, declining Production-peaks) were also analyzed. Data of adult layers (N=250/Yr) across 21 to 72weeks of age (52Weeks) were included in the study. Standard health care and managemental practices were followed through the years, with provision of well-compounded isocaloric (2700ME Kcal/Kg) and isoproteinous (16.5% CP) diets. Statistical significance of various factors was determined using SAS's GLM Procedure. The mycotoxins (AFB1/OTA) were determined using a high-precision flurometer using Kits from Vicam® Inc, USA. The results revealed huge fluctuation in duck-day egg production throughout, the 5 generations (range: 57-159eggs/Year), for which both year effects and feed's natural AFB1-levels emerged as the significant most influencing factor (P<0.05), with most other factors as non-significant or secondary impact on egg production. Among 2 mycotoxins, the AFB1 ranging: 2.1 to 97ppb, across years clearly cast usual morbidity on ducks intermittently, whenever it was >50ppb levels. However, the impacts of numerically-fluctuating OTA levels, (3-76 ppb range), across seasons, remained statistically non-significant on production. Interestingly, the egg-weight's variation (40Wks) was non-significantly impacted across both biotic and abiotic factors, indicating limited impacts of AFB1 on eggsizes. The study concluded that for managing a sound egg production in a Backyard-production sector, the most important factor is minimizing natural buildup of AFB1 in duck-diets, employing permissible Nutritional supplementation, while ambient-temperatures, RH levels and seasonal effects besides different phases of lay, proved as non-significant or secondary factors influencing duck husbandry under coastal ecosystems.

FEEDING BEHAVIOR DURING GROWTH OF THREE BREEDING DUCK GENETIC TYPES

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The aim of this study was to describe in detail the longitudinal growth and feeding traits, that are key phenotypes for feed efficiency and force-feeding, in the three genetically different types of ducks farmed for fatty liver production in France (Muscovy, Pekin, and mule ducks). Forty-one Muscovy, 35 Pekin, and 40 mule ducks, all males, were reared with single-place electronic feeders to compare their growth performances and feeding traits. Data were analyzed from 28 to 49 days of age, after eliminating multiple-bird and unidentified visits (between 1 and 6% of data per genetic type). We studied the different traits at three time scales: for the entire trial (covering 4 weeks), by week and by day. The effects of the genetic type (for the three time scales), the time scale and their interaction (for week and day scales only) were tested using linear mixed models with SAS software. At the trial scale, the hybrid mule was characterized by a feeding behavior close to that of Pekin ducks, and a production performance similar to Muscovy ducks. Like Pekin ducks, mule ducks consumed two less per visit (26.8 g) than Muscovy ducks (P <0.0001), and their visits were six times shorter (73 s, P < 0.0001). Thus, their feeding rate was higher (22.6 g/min, P < 0.0001). The number of visits to the single-place electronic feeders was two times higher for Pekin and mule ducks (8.3 and 9.0 visits, respectively) than for Muscovy ducks (P < 0.0001). The body weight of mule ducks, although superior from the body weight of Muscovy ducks at the beginning of the test (1.69 kg vs. 1.42 kg, P = 0.0002), was found to be equivalent at 38 days of age (around 2.54 kg). At the week scale, feed intakes increased between week 5 and 7 (in average + 266 g). Except for Muscovy ducks, the visit duration was relatively stable over weeks and the number of visits decreased with age, leading to an increase in the length of the intervals between visits. At the day scale, all three genetic types of ducks fed mostly during the daytime, with more than 90% of the feed consumed between 5:00 and 23:00. Further development is required to limit multiple-bird visits to the single-place electronic feeder in particular with Muscovy ducks, and to enable data collection during the necessary feed restriction period before force-feeding for fatty liver production and during the mating period.

LIVE INSECT LARVAE AS ENVIRONMENTAL ENRICHMENT IN MUSCOVY DUCK: EFFECTS ON WELFARE AND BLOOD TRAITS

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Introduction. Interdisciplinary approach is necessary to evaluate animal welfare, since it comprehends animals' good health, comfort, and expression of their natural behaviors [1]. Aim of the present work is the evaluation of the effects of Hermetia illucens (HI) and Tenebrio molitor (TM) live larvae as environmental enrichment in Muscovy ducks on behavioral patterns and blood parameters. Material and methods. Three-day-old females Muscovy ducklings were allotted in 18 pens (6 replicate/treatment, 7 birds/pen) and assigned to 3 experimental treatments. The C group (control) was fed with commercial feed, while HI and TM groups where fed with commercial feed supplemented with HI and TM live larvae (provided as 5% of expected daily feed intake), respectively. Video recordings were made on 3 replicate/treatment every week during the trial and were performed in 3 periods during the day: the hour before insects' larvae provision (T1, 9.00-10.00am), the hour during the larvae provision (T2, 10.00-11.00am), and the hour after larvae provision (T3, 11.00-12.00am). At the end of the trial, blood samples were collected from 12 birds/treatments in EDTA tubes and in serum-separating tubes. The total red and white blood cell counts, serum protein, lipid, minerals, liver and renal function serum enzymes were evaluated. One-way ANCOVA was used to compare the observed behaviors in the experimental treatments using the week as a covariate, while one-way ANOVA was used to analyze the collected data for blood traits (P<0.05). Results. During T2 and T3 the birds of the C group showed higher time spent in stand position compared to the HI and TM groups (P<0.05). Moreover, during T3 the HI group showed lower time spent in walking activity compared to the C group (P<0.05). The overall blood traits were not affected by the experimental treatments (P>0.05) except for the H/L ratio that resulted lower in the insects fed groups compared to the control (P<0.05). Conclusion. The behavioral patterns observed were only slightly affected by the daily provision of live HI and TM larvae. However, the observed reduction of the H/L ratio results to be promising in terms of the improvement of animal welfare due to the dietary administration of live insect larvae.

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Waterfowls

Posters

DUCK PRODUCTION SYSTEM IN TAMIL NADU, SOUTH INDIA

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Duck production system in Tamil Nadu, South India is characterized by nomadic, extensive and seasonal. It is still held in the hands of small and marginal agricultural labourers as well as weaker section of the society. Duck has not yet been industrialized as that of chicken. In Tamil Nadu, 70 per cent of the duck population is concentrated in six districts namely, Kancheepuram, Thiruvallur, Villupuram, Cuddalore, Vellore and Thiruvannamalai. The duck farmers are rearing indigenous ducks such as Sanyasi and Keeri. Male Sanyasi duck has dull to dark brown throughout the body and presence of white patches, white bands in the neck; neck looks lustrous blackish green. Female Sanyasi duck has dull brown throughout the body with white patches; Neck is dull brown and with or without white bands. Keeri duck has blackish brown stripes all over body. Male Keeri duck is dull blackish green with partial white bands in the neck. Ash colour bill was observed. Female Keeri duck neck is dull to dark black in colour, back is predominantly black and bill is dull black in colour. The morphometric traits namely body, neck, bill and shank lengths were measured for 350 adult ducks. Body, neck, bill and shank lengths of male and female Sanyasi were 23.98, 23.05; 14.01,12.28; 6.72, 5.53 and 5.54, 5.49 cm respectively. Body, neck, bill and shank lengths of male and female Keeri were 24.72, 22.85; 13.27,12.33; 6.78, 5.73 and 5.67, 5.50 cm respectively. A data on duck production system was collected from 240 farmers covering 6 districts of the state of Tamil Nadu, South India. Duck farmers did not possess any permanent shelter and kept the ducks in the pen made of bamboo-paddy straw covered by tin-shed and soil. Most of the duck farmers fed with broken rice, rice bran between hatching and 4 weeks of age. After 4 weeks of age kitchen waste, paddy grains, insect, snails and weeds are the feed sources for ducks in addition to the feed received from foraging. The flock size is generally estimated in dozens. The common unit size is 25 to 30 dozen maintained by family members under free range system. These indigenous ducks have innate potential to produce eggs and meat at considerable quantity with lesser input. The annual egg production under the nomadic system of rearing averages 180-200 eggs. Non-availability of quality ducklings, balanced feed, vaccine and medicine, lack of diseases control, technical assistance are constraints perceived by the farmers.

IDENTIFICATION OF DUCK FAT LIVERS THAT HAVE BEEN FREEZE-THROWN

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The sale of fresh duck foie gras is mainly concentrated during the festive season, creating an imbalance between the demand of French consumers and the availability of the product. Some French market stakeholders are therefore tempted to freeze duck foie gras to sell it fresh, a practice prohibited by French and European regulations. Slaughterers would like to be able to gualify this type of products, to ensure that they comply with the regulations. In our study, 5 methods were tested: oxidation, fingerprinting by MALDI-TOF mass spectrometry, near infrared spectrometry (NIRS), Nuclear Magnetic Resonance (NMR), MRI and conductivity. Of these methods, two have shown very promising results: MALDI-TOF fingerprinting and NIRS. In the first phase of the project, on livers from the same batch and calibrated by weight, MALDI-TOF fingerprints were able to classify the livers well with internal validation and recognition capacity percentages between 93% and 100%. Similarly, the NIRS method showed that it was possible to develop calibrations with calibration model determination coefficients (R²c) of 0.82, and cross-validation coefficients (R²cv) of 0.8. In the second phase of the project, variability was incorporated in the origin of the livers in order to have a model specific to freezing/thawing and usable on all-origin livers. The MALDI-TOF method was able to recognise frozen fatty livers, but with many misclassifications, and too much variability to validate the first phase model. Regarding NIRS, the external validation revealed, as for MALDI-TOF, a level of classification error that was too high. However, external calibration/validation tests carried out on the second database, which is more variable, show encouraging results: R²p of 0.67 to 0.86 with 31 to 2% error, mostly false negative. A validation phase of these two methods on a larger pool of foie gras, coming from different technical itineraries seems necessary to have robust and reliable prediction equations.

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Keel bone damage

Selected short communications

PREVALENCE OF KEEL BONE DAMAGE IN RED JUNGLE FOWLS (GALLUS GALLUS)—A PILOT STUDY

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Keel bone damage (KBD) is a prevalent welfare issue in commercial egg production. Within-flock prevalences ranging from 30 to 97% has been reported [1,2]. KBD consists of two different conditions: Keel bone deviations and keel bone fractures (KBF). Deviations are linked to pressure on the keel, e.g., from perching [3]. The causative factors for KBF are not clear [4]; however, selection for efficient egg production has been suggested as a major contributing factor. An important step to shed light on the role of selective breeding as an underlying cause of KBF in modern laying hens is to evaluate the keel bones of the ancestor, the red jungle fowl. To the authors' knowledge, this has never previously been published. The aim of this novel study was therefore to describe the prevalence of KBD in a study group of red jungle hens and roosters housed in an aviary system. The study examined 29 red jungle fowls at 112 weeks of age, post-mortem; 12 hens and 17 roosters. Keel bones were evaluated by external palpation for deviations and fractures. Palpation was followed by autopsy. No fractures were detected in the 17 roosters; one had a very slight deviation. Of the 12 red jungle hens, one had a single fracture and 10 hens had a very slight deviation. In conclusion, the study found a high prevalence of slight keel bone deviations, and very low prevalence of KBF compared to published data from modern layer hybrids. To investigate the hypothesis that selective breeding might have contributed to an increase in keel bone pathology, large scale studies of jungle fowls and "historic" breeds are needed.

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THE IMPACT OF LIGHT INTENSITY ON KEEL BONE QUALITY AND TIBIA BONE STRENGTH OF PULLETS HOUSED IN PERCHERY SYSTEMS FROM 0 TO 16 WEEKS OF AGE

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Light intensity can impact bird activity, which can affect musculoskeletal development. The objective of this study was to determine the effect of varying light intensity (L) on keel bone health and tibia bone strength of Lohmann Brown Lite (Brown) and Lohmann LSL-Lite (White) pullets. A 3 x 2 factorial arrangement of L and strain (S) was performed in a randomized complete block design. Three L intensities (10, 30, or 50 lux, provided by white LED lights) and two S were used. Brown and White pullets (n=1,800 per S) were randomly assigned to floor pens (50 pullets per pen; 4 replications per L x S) from 0 to 16 weeks of age. Each floor pen contained a system of four parallel perches (0.3 m apart, 0.6 m high), ramp, two tube feeders, and a drinker line. All pullets were fed ad libitum on a commercial phase feeding program. Pullet body weight was collected on a pen basis at 0, 8, and 16 wk. At 16 wk, 10 pullets per pen (n=720) were palpated for keel bone deviations and fractures. An additional 9 pullets per pen (n=648) were euthanized and the keel bones and breast muscle were removed to assess keel bone damage and breast muscle weight. Right tibiae were collected, and bone strength was assessed using a 3-point-bending test. Mortality was recorded daily. The effect of L, S, and their interactions were analyzed using Proc Mixed (SAS 9.4) with Tukey's range test to separate means. Differences were significant when P < 0.05. There was no effect of L, and no interactions between L x S, for any of the measured parameters. Brown pullets were heavier than White pullets at 8 and 16 wk. There was no effect of S on keel bone damage from palpated and dissected keels. No fractures were found from dissected keel bones. Brown pullets had a higher breast muscle weight than White pullets (86.3 vs 77.0q; P<0.05), however relative to body weight, White pullets had a higher breast muscle mass (6.59 vs 5.97%, P<0.05). Brown pullets' tibiae were 1.31% heavier, 1.02% longer, and 1.18% wider than White pullets (P<0.05), however White pullets had a higher bone strength relative to bone size than Brown pullets (1,816 vs 1,277 kg/cm 2; P<0.05). Mortality was higher in White pullets compared to Brown pullets (4.00 vs 1.22%; P<0.05). Overall, the results indicate that L, between a range of 10-50 lux, does not affect pullet keel bone health or musculoskeletal development, however S may play a role in these parameters. Key words: Lohmann Brown-Lite, Lohmann LSL-Lite, bone breakage test, keel bone palpation

KEEL BONE DAMAGE EVALUATION IN LAYING HENS FED DIETS SUPPLEMENTED WITH DIFFERENT LEVELS OF DRIED OLIVE PULP.

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Introduction: The objective of this study was to investigate the effect of in-feed inclusion of dried olive pulp in laying hens' diet on the incidence of Keel Bone Damage (KBD) and to determine the inclusion rate that has the optimal effect. Materials and methods: The experiment was carried out in a commercial farm of laying hens. In total 300 Isa Brown layers 23 weeks of age with similar body weight were randomly accommodated in 30 enriched cages (10hens/cage) that were fully equipped and met the requirements of EU Directive 1999/74/EC. Hens were kept under 14h light-10h dark cycle (15 lux), they were beak trimmed and were fed with a basal layer diet containing approximately 17 % CP, 3200 kcal ME/kg, 4.4% Ca and 0.58% available P. Feed and water were offered ad libitum. After 2 weeks of adaptation, birds were randomly divided in 6 dietary groups CON, OP2, OP3, OP4, OP5 and OP6, with 50 hens/group, 5 replicates/group, 10 hens/replicate. CON group was fed the basal layer diet described while OP groups were fed the basal diet supplemented with Sparta INNOLIVE, a commercial feed supplement of dried olive paste, at the rates of 2%, 3%, 4%, 5% and 6% respectively. All rations were calculated on isonitrogenous and isocaloric basis. At the age of 39 weeks all hens were evaluated for the presence of KBD. Each hen was held gently by one person and another person palpated the keel bone by running fingers alongside and over it. The presence of KBD was recorded (deformation, fracture or both) and the incidence rate was calculated. For the assessment of keel bone deformation, the Li et al. scoring system was used1. The significance of the differences of KBD incidence rate among groups was assessed by Chi-square test. Results: The overall incidence of KBD was 45.3%; the lowest rate was recorded at OP3 (10%) and was significantly different among all groups (P<0.05). OP2 had the highest KBD incidence rate (68%) and was significantly different (P<0.05) than CON (46%), OP3 and OP6 (40%). The incidence of KBD in OP4 (54%) and OP5 (54%) was significantly different only in comparison with OP3 (P<0.05). The vast majority of deformations (91%) was rated as 1 and no significant difference was observed among groups (P>0.05). Fractures were detected at 1% of the hens. Conclusions: The in-feed inclusion of dried olive pulp at the rate of 3% reduces the incidence rate of KBD in laying hens.

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REARING ENVIRONMENT AFFECTS PECTORALIS MAJOR AND KEEL BONE DEVELOPMENT IN LAYING HEN PULLETS

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As global egg production shifts away from conventional cage housing, the use of complex open aviaries for laying hens is increasing. Aviaries are associated with an increased risk of injury, particularly to the keel bone – the anatomical anchor for the breast muscles that facilitate flight. Thus, there is an urgent need to identify strategies for improving the hen's ability to adapt to complex three-dimensional housing. It is known that exposure to aviary housing during rearing improves the hen's ability to navigate aviary systems in adulthood. However, it is unknown how various designs of commercial rearing aviaries differ in their effects on the pullets' musculoskeletal development. We hypothesized that pullets given the most opportunity to perform wing loading behaviours would develop proportionally larger breast muscles and larger keels than birds reared in more spatially restrictive and barren environments.

Two flocks of Lohmann LSL-lite (W) and Lohmann Brown-lite (B) pullets were each reared in one of three styles of rearing aviaries differing in environmental complexity and opportunities for wing loading behaviours: Style 1 (S1; 345 birds/flock) offering the least, Style 2 (S2; 432 birds/flock) being moderate, and Style 3 (S3; 600 birds/flock) offering the most. A control group was reared in restrictive conventional cages (CC; 120 birds/flock). At 6 (171 birds), 11 (170 birds), and 16 (165 birds) weeks of age, n=10-15 birds/age/strain/style were dissected and analyzed. The pectoralis major breast muscle was weighed, and the keel bone was extracted, photographed, and traced using ImageJ software to quantify lateral surface area. Statistical analyses were conducted using SAS 9.4 applying a generalized linear mixed model on values corrected for body weight. We found that birds raised in rearing aviaries had proportionally heavier pectoralis major muscles than birds raised in CC (p < 0.001) at all three ages, though there were no differences between aviary styles. Keel area was proportionally larger in birds from S1 and S2 compared to CC (p<0.03) with S3 being intermediate. Strain also affected both traits. W pullets had proportionally heavier pectoralis major (p<0.0001) and larger keel area (p<0.0001) compared to B.

These results suggest that rearing aviaries promote improved development of key musculoskeletal traits in laying hen pullets, though there may not be appreciable differences in these benefits between rearing aviary designs.

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Welfare of layers

Selected short communications

DEVELOPMENT OF AN EASILY APPLICABLE MONITORING TOOL FOR EVALUATION OF FEATHER PECKING DAMAGE ON COMMERCIAL LAYING HENS

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Damaging feather pecking is a well-known behavior in laving hens, which has a negative impact on wellbeing and hen-performance. With a recent ban on the practice of beak trimming by European legislation and specification-demands, the number of non-beak trimmed (NBT) flocks increased in Flanders. In order to monitor the progress of pecking behavior in a flock, feather condition scores (FCS) are conducted. Therefore, different body areas of a large number of birds need to be examined. This practice is time consuming and stressful for the birds, which makes it unsuitable for use in commercial laying houses. A pecking checklist, developed by the Experimental Poultry Centre (EPC), offers an alternative monitorina tool which is less disruptive and easier to carrv out. The checklist was performed on 23 commercial flocks of NBT laying hens between 50 and 79 weeks of age. In each flock, FCS of 100 hens were inspected, according to the method of Tauson (Tauson et al., 1984). This implies that a score is given for the body areas 'Neck', 'Wing', 'Back', 'Tail' and 'Cloaca region', ranging from 1 (largely bald) to 4 (no feather damage). In addition, 15 hens were scored according to the checklist. Here, a score was given to the areas 'Head/Neck' and 'Back/Cloaca', ranging from 0 (largely bald) to 4 (no feather damage). All FCS's and checklist scores were averaged out for each flock. A Pearson correlation matrix was computed to assess the linear relationship between the scores of different measuring methods and body areas. All of the variables were positively correlated, and the strongest correlations were found between 'Checklist: Head/Neck' and 'Tauson: Neck' [r(21) = 0.901, p < 0.001]; 'Checklist: Back/Cloaca' and Tauson: Back' [r(21) = 0.894, p < 0.001]; 'Checklist: [r(21) = 0.825, Back/Cloaca' and 'Tauson: Cloaca' < 0.001]. р These strong correlations suggests that the pecking checklist is a valid way of measuring feather damage in commercial flocks of laying hens, when compared to the method of Tauson. This way, the checklist could play an interesting part in the development of a continuous monitoring system on pecking behavior, that can be applied in commercial laying houses.

HOW TO DEAL WITH INTACT-BEAK LAYING HENS IN PRACTICE?

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Feather pecking is an important welfare and economic problem in laying hens. Because of national legislation or market demands, more and more hens in Europe are non-beak trimmed (NBT). It has been shown that the consequences of injurious pecking in NBT hens can be much larger compared with beak trimmed hens (BT) resulting in a serious decrease of animal welfare, health and flock performance. Field studies show different outcomes of NBT flocks and conclusions are often contradictory. The Experimental Poultry Centre (EPC) followed 3 flocks with NBT hens in a controlled set-up. During the first 2 flocks (7680 and 10600 Isa Brown hens resp.) BT and NBT hens were compared while in the third flock all hens were NBT (9140 Isa Brown vs 9140 Dekalb White). All hens within a flock had the same birth date, came from the same breeder flocks, were reared in the same rearing house and placed in identical aviaries. All groups received pecking blocks and roughage as distraction. Production data were recorded, a flock behavior checklist was performed and plumage condition was assessed monthly. Differences between the groups were ascertained with MANOVA's. The aim of this study was to evaluate the evolution and consequences of injurious pecking in semicommercial conditions. The first experiences with NBT hens showed significant higher cum mortality (25% vs 9%), lower laying % per hen present (php) (-6%) and very poor plumage condition at 77 weeks of age. Both groups in the second flock performed above breed standards until 85 weeks of age. No differences were seen in cumulative mortality and laying percentage. However, NBT hens showed higher feed intake (+2,6g php) and higher feed conversion ratio. In addition, plumage condition started to deteriorate at 60 weeks of age and more pecking behavior was observed in the NBT hens. In the third flock large differences were seen between the hybrids. Brown hens had a significant higher cumulative mortality (25,25% vs 5,08%), lower cum laying % php (82,20 vs 88,60), high cumulative daily feed intake php (131,92 g vs 115,25g) and poor plumage condition.

In conclusion, in 2 out of 3 flocks brown NBT hens showed poor technical results, animal welfare and health. In addition, the NBT hens in the flock with good technical results also showed a worse plumage condition and behavior compared to the BT hens. White hybrid NBT hens show better technical results and plumage scores compared to brown hybrid NBT hens in identical conditions.

IS TOE PECKING A SELF-INFLICTED BEHAVIOR?

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Toe pecking (TP) is a serious behavior problem affecting laying hens with severe impacts on animal welfare and productivity. Unlike feather pecking, there are few experimental studies on TP. The aims of this study were to relate toe lesions with the behavior of TP under two settings: a slightly stressful situation and control to describe its spatial and temporal occurrence. Eight adjacent pens in an experimental barn with an aviary and 225 LSL hens each were equipped with cameras. When the hens were 32 weeks of age (WOA), the pens were filmed continuously for three days. All occurrences of TP (self and directed at other hens, i.e. allopecking) and pecking at the comb were recorded using the software Solomon. At 32 WOA, in a period excluded from video recording, 25 hens per pen were collected in a stratified manner (50 individuals in total) and their toes scored according to a developed visual analogue scale. Afterwards, two treatments were applied. Half of the pens skipped the last feeding one hour before lights-off. After seven weeks of treatment, video recordings and scoring of toes (50 hens/100 feet per pen) was repeated. The scores of both feet were added for each hen. Data were analyzed with generalized mixed models. Pen was taken as a random variable and, for the number of pecking events, a Poisson distribution was chosen. For inter-observer reliability of scoring, the rptR package was used (R-project, LMM REML with 10 bootstraps). Hens spent more time pecking their own toes than allopecking or pecking at the comb of other hens (F2,14 = 61.9, P<0.0001). The least pecking was observed during midday, with no pecking observed in the litter while most events happened in front of the nests and on the top tier of the aviary (F3,21=6.47, P=0.003). Allopecking (toes and comb) was only reduced by treatment in the afternoon when feeding was skipped (interaction treatment x time of day: F1,34=12.11, P=0.001, N=44) but there was no effect of treatment at any time for self-directed pecking (time: F1,35=10.72, P=0.002, treatment and interaction: NS, N=44). The assessment of toe damage had a high inter-rater reliability of R = 0.99 (0.985, 0.994). Comparing pens, comb and toe injuries were correlated (rS=0.54, P = 0.007, N=23) but pecking at their own toes and toe lesions were not (rs=0.29, P=0.18, N=23). In conclusion, skipping the last feeding seemed to have decreased TP in conflict with our prediction. There was no evidence that TP was a self-inflicted behavior.

EARLY LIFE ENVIRONMENT AND GENETIC STRAIN ARE KEY FOR THE DEVELOPMENT OF LOCOMOTION SKILLS IN LAYING HENS

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Laying hens housed in aviaries need locomotory skills to move between different vertical levels. Failure can have negative consequences for hen welfare (injury or emaciation) and production (floor-eggs) alike. We investigated the effect of early life environment and genetic strain on the development of these skills. We raised 4 flocks of Lohman Selected Leghorn lite (LSL) and Lohman Brown lite (LB) in 4 systems with varying spatial complexity: Conventional pullet cages (CC, least complex), a rearing aviary of low complexity (A1), intermediate complexity (A2), and high complexity (A3). A total of 459 15-week-old pullets were tested in 5 tasks to reach a reward on raised platforms. Tasks 1 and 3 required 1 jump (60 and 120 cm respectively), task 2 could be completed in 2 jumps (2x 60 cm), and task 4 in 3 (3x 60 cm), task 5 required a downward jump (60 cm). Quality was scored for each jump, and success for each task. Quality was analysed as a proportion of the total possible score per task and divided by 100 to fit a generalized linear mixed effect model with a binomial distribution. Housing, strain, and task were fixed effects, flock and bird ID random effects. Likelihood of success (odds ratio; OR) was based on two generalised linear models (upward and downward jumps) with housing and strain as fixed effects. Jump quality was affected by a task and housing interaction (χ^2 =30.35, p=0.002) and strain (χ^2 =37.78, p<0.0001). A3 pullets performed better than A1 in task 1, A2 in task 5, and CC in tasks 1 and 3. A2 pullets did better than CC pullets in tasks 1 and 3, and A1 did better than CC in tasks 3 and 1. Jump quality was higher for LSL than LB. LSL were more likely to succeed in both, upward (OR= 3.2, 95%-Confidence Interval (CI)= 2.4, 4.4) and downward movements (OR= 1.9, CI= 1.1, 3.3). For upward jumps, aviary reared birds were more likely to succeed than CC (ORA1-CC= 9, CI= 4.5, 20.7, ORA2-CC= 9.9, CI= 4.9, 22.5, ORA3-CC= 14.1, CI= 7.2, 32.1). Moving downwards, A3 pullets were 2.2 times more likely to succeed than CC (CI = 1.1, 4.6) and 5.1 times more than A2 birds (CI= 2.2, 13.1), while A2 birds were less likely to succeed than A1 birds (OR = 0.4, CI = 0.1, 0.96). The results show consistent strain differences with LSL performing better and succeeding more than LB, and an improvement of locomotion skills with increased complexity during early life. We aim to improve hen welfare by understanding the factors involved in the development of locomotion skills.

26th World's Poultry Congress, abstracts selected in 2022

Welfare of broilers

Selected short communications

EVALUATION OF ADAPTABILITY RESPONSE, THROUGH A BEHAVIOURAL OBSERVATION, OF FOUR DIFFERENT CHICKEN GENOTYPES REARED IN A FREE-RANGE SYSTEM

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Alternative poultry rearing systems such as organic and free range should be developed following the "One Welfare" concept, a link between animal and human welfare. Thus, the choice of chicken genotypes should take into account their adaptabiliy to environmental conditions strictly linked in turn to animal welfare. The aim of this study was to assess the adaptability through a behavioural observation, of four different Slow Growing (SG) chicken genotypes: RedjA (A), Lhomann Dual (LD) Necked Neck (NN) and a Crossbreed (CB, Robusta Maculata x Sasso) free range reared. At hatching 400 chickens were randomly housed into 8 pens (2 pens per genotype; 50 animals each, 25 females and 25 males) and given outdoor access from 35 days of age, (0.10 m2/bird indoor and 4 m2/bird outdoor). The behavioural pattern of each pen was video-recorded from 42 d of age during 5 weeks, 2 times week and 2 hours per recording (9:00 to 11:00 am). Static, active, eating, comfort and social behaviours were then scanned every 30 minutes to record the percentage of animals expressing each specific behaviour. Data were analyzed by ANOVA with genotype, day, and their interactions as fixed effects and pen as a random effect. Static behaviours were more frequently observed in A chickens followed by NN chickens compared to LD and CB genotypes (55.4% 46.3% vs. 34.8% and 35.4% of chickens; P<0.001), which depended on differences in chickens resting (13.5% and 11.9% vs. 8.5% and 9.9%; P<0.05) and roosting (41.8% and 34.4% vs. 26.3% and 25.5%, P<0.001). Conversely, LD and CB chickens showed more active behaviours compared to A and NN genotypes (33.9% and 32.0% vs 16.3% and 23.9%; P<0.001), which is determined by the number of birds walking (21.8% and 24.8% vs. 10.0% and 20.9% P<0.001). On the contrary, the number of birds hiding was lower in A, NN and CB chickens compared to LD (2.3%, 0.6% and 1.9% vs. 8.7%; P<0.001). Concerning the eating behavior a higher number of A and NN chickens were found eating grass as compared to CB and LD (15.7% and 18.9% vs. 14.8% and 10.3%; P=0.001). A lower number of A and NN birds showed comfort behaviours respect to CB and LD genotypes (7.0% and 5.1% vs. 7.9% and 11.3%; P<0.001), which was due to the lower percentage of birds scratching and dust bathing (P<0.001). In conclusion, the A genotype showed the less adaptive response, while LD chickens likely fitted better to free range systems based on their higher overall outdoor activity and a more complete ethogram.

A LOWER STOCKING DENSITY STIMULATES SPECIES-SPECIFIC BEHAVIOR AND IMPROVES WELFARE OF BOTH FAST- AND SLOWER-GROWING BROILERS

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There is a trend towards broiler production systems with higher welfare requirements, often including slower-growing broilers and a reduced stocking density. Although we know that fast-growing broiler welfare improves when stocking density reduces, it is unknown whether slower-growing broilers respond in the same way to stocking density in terms of welfare. Thus, the aim of this study was to compare the behavior and welfare of fast- (Ross 308) and slower-growing broilers (Ranger Classic) housed at different stocking densities (24, 30, 36 and 42 kg/m2). Each experimental group was replicated 4 times, resulting in a total of 32 experimental pens. Behavior was observed at pen level using instantaneous scan sampling at 4 target weights (0.4, 1.1, 1.7 and 2.1kg), where 2 locations of approximately 3m2 were observed per pen. In addition, the novel object and human approach test were performed once per pen at the same target weights to assess fearfulness. Gait score, footpad dermatitis, hock burn, cleanliness and injuries were assessed using the Welfare Quality® protocol at 2 target weights (2.0 and 2.3kg), with 30 birds assessed per pen (15 male/15 female). No interaction effects between breed and stocking density were found for any of the variables, indicating that breeds responded in the same way to the different stocking densities with regard to their behavior and welfare. Slower-growing broilers showed less ingestion, but more active and foraging behavior, and further had better scores for all welfare indicators compared to fast-growing broilers. Broilers housed at lower stocking densities showed less ingestion, but more foraging and comfort behavior, and further had better scores for all welfare indicators compared to those housed at higher stocking densities. No clear differences between breeds or stocking densities were found for behavioral responses to the fear tests, except for a higher percentage of slower-growing broilers approaching the human compared to fast-growing broilers. In conclusion, regardless of breed, broilers housed at lower stocking densities showed more species-specific behaviors and had improved welfare compared to those housed at higher stocking densities. Further, slower-growing broilers showed more speciesspecific behaviors and had improved welfare compared to fast-growing broilers. These findings demonstrated that a lower stocking density is beneficial for behavior and welfare of both fast- and slower-growing broilers.

EFFECT OF GENOTYPE AND STOCKING DENSITY ON BEHAVIOR AND HEALTH OF BROILER CHICKENS

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The poultry industry wishes to improve the welfare of broilers reared in claustration while controlling production costs. Improving housing conditions is expected by consumers and citizens, and a decrease in growth rate is also being considered. The objective of this study was to evaluate the behavior and health status for six genotypes with various growth rates (from 42 to 61 g/d), and reared at conventional or reduced density (39 or 30 kg/m²) for a total of 12 experimental treatments. Chickens were reared in 48 pens of 18 m² (4 pens per treatments) with natural light and had unlimited access to enrichments (pecking blocks, straw/alfalfa bales). Birds were slaughtered between 1.8 and 2.2 kg, at D32 (Ross 308), D39 (Redbro, Rustic Gold, Ranger Classic, JA787), or D46 (JA757). Animal behavior was observed by scan sampling, 6 times a day during 9 to 13 d throughout the rearing period. Gait, pododermatitis and hock burns were scored on 160 birds per treatment the day before slaughter. Behaviors were analyzed by ANOVA taking into account the genotype and density as explanatory variables and their interaction, while health indicators were analyzed using a Chi-square test. Compared to Ross 308, the two slower growing strains (JA757 and JA787) had twice as many animals standing (30 vs. 14%), moving (9 vs. 5%) or exploring the environment (14-15 vs. 9%) over the entire rearing period. Ross 308 perched less (1.6 vs. 3%) and pecked less at enrichments (0.2 vs. 0.3-0.5%) than other genotypes. At reduced density, the presence along enrichments increased (3.3 vs. 2.9%) and the proportion of inactive birds decreased (62.4 vs. 63.9 %). Gait score was also improved for the strains slaughtered at D32 and D39, comparatively to Ross 308. Finally, at reduced density and for the slowest growing genotypes, we observed less pododermatitis (4 vs. 21%) and hock burns (18 vs. 37%). These results confirm that reduced growth rates coupled with lower stocking densities are two relevant options to improve animal welfare for chickens reared in claustration. In particular, new genetic strains could represent good compromises between the preservation of economic criteria (i.e. animal performance and meat quality; see Méda et al., WPC 2022) and improved animal welfare. This study should therefore help the poultry industry in the transition of its conventional production model.

THE EFFECT OF LIGHT WAVELENGTH TREATMENTS ON BROILER BEHAVIOUR

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LED bulbs for poultry lighting are of great interest, as they can produce specific wavelengths. Changing light colour may lead to changes in bird' behaviour. To assess the effects of different wavelengths treatments (L) on behaviour, broilers were raised from 1-35d under 3 L (blue (BL, 455 nm), green (GL, 510 nm) or white (WL) LED bulbs) in 2 blocked trials (N=14,256 birds). In addition to L, sex (SE) and strain ((ST) Ross YPM-708 and EPM-708) were assessed. Broilers were housed in 9 rooms (3 rooms/L), each subdivided into 12 pens. Lighting program started at 23L:1D at d1 and reached 16L:8D by d6 (dawn and dusk transitions of 15min). Wk 1 light intensity was 9.6 clux (trial 1) or 14.3 clux (trial 2), followed by 14.3 clux for the grow-out periods. Bird behaviour was recorded 24h with infrared cameras at 11-12 and 33-34d. Scan sampling was conducted at 20 min intervals. Behaviours recorded were feeding, drinking, inactive/resting, walking, standing, running, preening, leg/wing stretching, dustbathing and foraging. Data were logtransformed for normal distribution and statistically analyzed (Proc Mixed, SAS 9.4). All behaviour data is given in percent of time (24 hr). At 11-12d, broilers raised under BL preened more than birds raised under GL (P=0.05). Females spent more time drinking (P=0.04) and dustbathing (P=0.002), and males foraged more (P=0.05). At 33-34d, broilers raised under WL walked more (P<0.0001), while broilers raised under BL spent more time on inactive/resting behaviour (P=0.03). YPMx708 broilers preened (P=0.05) and leg/wing stretched (P=0.0002) more than EPMx708 birds. Interactions were noted between LxST, where YPMx708 raised under BL or GL foraged more than EPMx708 birds raised under GL. An interaction was found between STxSE, where YPMx708 males and females foraged more than EPMx708 females. Interactions were also found between LxSTxSE, where EPMx708 females raised under GL spent more time feeding and EPMx708 females raised under WL spent more time performing drinking behaviour. EPMx708 males raised under WL and EPMx708 females raised under GL performed running behaviour for more time. YPMx708 males raised under BL, YPMx708 females and EPMx708 males raised under WL spent more time performing dustbathing behaviour. The results indicate that exposure to light wavelength treatments impacted mobility and resting behaviour of broilers, with WL generally increasing broiler activity and BL increasing resting behaviour.
USE OF BLACK SOLDIER FLY LARVAE TO IMPROVE THE WELFARE AND BEHAVIOR OF BROILERS

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Fast-growing broilers, often faced with limited environmental stimulation, spend most of their time inactive and are therefore susceptible to leg problems. Enrichment of their living environment can help reducing these problems by encouraging the expression of their natural behaviors and increasing their activity. Thus, the use of attractive enrichments, such as insect larvae, seems promising but little technical evidence is available in the literature. In order to identify realistic methods of their use in production conditions, a trial was conducted to study the effect of providing black soldier fly larvae (live, LL or dried, DL) as a supplement to a complete feed, on the behavior, activity, health and performance of Ross 308 broilers. 1200 as-hatched chicks (sex ratio 50:50) were randomly allocated to 24 pens (50 birds/pens) and fed ad libitum with the same diets from D0 to D36. From the growth phase (D12), 6 treatments were tested (4 pens/treatment): a negative control (C) with no enrichment; a positive control using whole wheat as enrichment and four treatments with LL or DL distributed once or twice a day (LL1 and DL 1 vs. LL 2 and DL 2, respectively). Animal performance and footpad dermatitis were recorded at each feed transition (D12, D22 and D36). At D36, the general condition of the animals (wounds, dirtiness, feathering) was also assessed. Broilers activity was measured by scan sampling at regular time intervals (during enrichment distribution and 2h before/after) on 9 occasions during the trial (D10, D13-J17, D21, D31 and D35). The larvae supply did not have any negative effects on the performance and health status of the animals. Feed consumption was reduced when DL were fed, improving feed conversion ratio by more than 0.13 points compared to C. Furthermore, intake of LL or DL increased animals' activity, but only at the moment of distribution. This effect was greater when larvae were distributed twice a day, but did not vary according to the type of larvae. A higher palatability for LL was also observed, compared to DL or wheat. Further analyses are still required, especially to investigate the effect of larvae on specific behaviors (exploration, comfort). Larvae distribution directly into the litter rather than on a tray, although not ideal from a sanitary point of view, would perhaps prolong the effect of larvae on chicken's activity, and would stimulate more foraging and litter-scratching behaviors.

THE ORGANIC SELENIUM HYDROXY-SELENOMETHIONINE IMPROVES BROILER ANTIOXIDANT CAPACITY UNDER HEAT STRESS CONDITIONS

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This study investigated whether hydroxy-selenomethionine (OH-SeMet) improves broiler oxidative stress response to a greater extent than sodium selenite (SS) or seleno-yeast (SY) under environmental stress. Day-old male Cobb 500 broilers (12 cages/diet, 9 broilers/cage) were fed a selenium (Se)-deficient diet (0.047 mg Se/kg) supplemented with SS, SY or OH-SeMet at 0.3 mg Se/kg for 42 days. Animals were raised at a high stocking density (18 broiler/ m^2) and during the summer period inducing heat stress conditions (average 1-42 days temperature $33.4^{\circ}C \pm 0.7$, average relative humidity 70.9%). OH-SeMet significantly (P < 0.05) improved the mortality corrected feed conversion ratio (FCR), SS:1.74; SY:1.72; OH-SeMet:1.71g/g; and Se concentration in pectoral muscle, SS:0.12; SY:0.17; OH-SeMet:0.29 mg/kg as compared with SS (P < 0.05). Relative selenoprotein W (SELENOW) quantities were also significantly (P < 0.05) increased by OH-SeMet compared to SS as observed in pectoral muscle (SS:100; SY:128; OH-SeMet:141) and jejunum (SS:100; SY:112; OH-SeMet:324). OH-SeMet increased total antioxidant capacity compared to SS (SS:14.7; SY:16.9; OH-SeMet:18.3µmol/g), and it reduced malondialdehyde in the muscle more than SY (SS:0.21; SY:0.28; OH-SeMet:0.13 µmol/g) (P <0.05). Circulating antiinflammatory cytokine IL-10 was higher (P < 0.05) for OH-SeMet compared to SS and SY, indicating a better ability of OH-SeMet to promote an anti-inflammatory response (IL-10: SS:8.7; SY:9.2; OH-SeMet:12.4 pg/ml). Intestinal morphology measures indicated that OH-SeMet resulted in higher (P < 0.05) villus height to crypt depth ratio than SS in the duodenum and ileum (SS:3.53; SY:3.93; OH-SeMet: 4.14 and SS: 3.87; SY: 4.80; OH-SeMet: 4.76, respectively). OH-SeMet as a pure organic form of selenium, has the potential to improve Se status, antioxidant capacity and anti-inflammatory response under oxidative stress situations like heat stress.

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Welfare

Posters

SUNLIGHT AFFECTS RANGING BEHAVIOUR OF COMMERCIAL FREE-RANGE HENS

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Free-range commercial layer farming is popular with consumers as it is perceived to improve hen welfare due to the more 'natural' outdoor access. However, observed proportions of hens using the range can be low which fails to meet consumer expectations of a free-range system. Range use may be affected by many variables including surrounding climatic conditions during access periods. In Australia and other countries, summer brings intense sunlight with high UV radiation and this might impact hen ranging behaviour. To determine if range use was correlated with sunlight variables and other climatic factors, a study was carried out on a commercial free-range laying hen farm in Tasmania, Australia across the summer/autumn period (December-March). A high-resolution video camera was set-up for daily video recording of hens ranging outside within a portion of one shed of 20,000 hens starting at 28 weeks of age. The solar radiation spectrum [total solar radiation (TSR) (100 nm- 1 mm)/ photosynthetically active radiation (PAR) (400-700 nm)/ ultraviolet radiation (UVAB) (280-400 nm)] and weather data (temperature and relative humidity) were recorded through a weather station located on the farm. Across a total of 102 days of video recordings, image snapshots were taken at 30 min intervals from pop-hole opening until sunset. The number of hens within direct sunlight (as opposed to shaded areas from range shelters or the shed) were counted using Image-J software. Using JMP® 14.0, three multiple linear regression analyses were conducted separately (to account for collinearity in sunlight measures) using each sunlight variable in consort with the climatic factors as related to ranging hen numbers. In the models, all variables (sunlight, temperature, humidity) were significantly associated with the number of hens outside in direct sunlight (PAR had the highest adjusted $r^2 =$ 0.34, F (3, 1171) = 204, P < 0.0001). The most contributory variable in the models was the sunlight parameter accounting for 48%, 59%, and 61% of the variation for UVAB, TSR, and PAR respectively. The relative humidity was positively associated with the hens' access, while sunlight and air temperature had a negative relationship. This study contributes to an understanding of how sunlight affects range access with confirmation across additional commercial farms required.

EFFECTS OF THE EARLY-LIFE AND ADULT ENVIRONMENT ON LAYING HENS' SPATIAL COGNITION

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Rearing and production environments for laying hens often differ in their degree of complexity, which can affect individuals' characteristics and welfare. We investigated the effects of the rearing environment complexity on spatial cognition, and how the adult environment can modify the effects of the rearing environment. The objective was to obtain a deeper understanding of the effects of environmental complexity different life stages laving at on hen coanition. We tested 64 laying hens in a detour task to assess their spatial cognitive abilities. These hens were cage or aviary- reared, and then housed in standard or enriched furnished cages during the laying period (4 groups, n=16 per group). The degree of complexity of the adult environment was higher in enriched furnished cages than in standard furnished cages, with dustbathing platforms and curtains. For the test, a hen was placed in a detour compartment with solid side walls, an open rear end and a grid front. It was facing a stimulus compartment containing two familiar hens and food as a motivation to make the detour. Each hen was tested twice (at 62 & 64 weeks) and latencies to perform the detour were recorded, with a cutoff of 10 minutes. The results were analyzed using survival analyses, and pairwise comparisons between each treatment group were performed using log-rank test. The results of the analyses showed a significant difference between hens reared in cages and housed in standard furnished cages and hens reared in aviaries and housed in enriched furnished cages for both ages (62w: p=0.028; 64w: p=0.032). For each rearing condition, hens housed in enriched furnished cages during lay tended to perform better than those housed in standard furnished cages. The hens were also faster to perform the detour during the second test (62w: mean=476s vs 64w: mean=397s), and more individuals accomplished the task before the cutoff (62w: n=26 vs 64w: n=32). Thus, the early-life environment and its complexity has long lasting effects on spatial cognition and the ability to solve a detour task. Rearing hens in an aviary produces individuals with better spatial skills, probably due to the possibility for the hens to navigate in three dimensions and in a larger space. However, the adult environment can partly compensate for this if it offers a higher degree of complexity than the rearing environment.

EFFECTS OF REARING AVIARY STYLE AND GENETIC STRAIN ON THE LOCOMOTION OF LAYER PULLETS

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For laying hens to succeed in aviaries, they must be reared in a structurally complex environment. However, there are many styles of rearing aviaries that offer varying levels of structural complexity, particularly during the brooding period. This study compared the locomotory behaviours of brown- and whitefeathered strains of pullets in 3 basic styles of commercial rearing aviaries: Style 1 (S1) was the least complex, with chicks placed in brooding compartments outfitted with low perches. Style 2 (S2) was a more complex brooding compartment with different perch heights and an additional platform. Style 3 (S3) was open concept, multi-level, and spanned the length of the barn. In all styles, producers opened the system after a few weeks, providing pullets access to a litter floor, as well as additional platforms, perches, and multiple tiers. Fifteen commercial pullet flocks (5 flocks per style; 7 white and 8 brown) were visited 3 times: at 26.6 ± 6.51 (before systems were opened; V1), 68.7 ± 6.07 (V2), and 112.0± 4.46 (V3) days of age. Cameras were mounted throughout the barns to view within the system and over the litter. Videos were recorded 3 times throughout the light cycle, and 30 mins of each video were analyzed. From each video, 60 focal birds selected from predetermined locations were followed for 30s. All locomotory behaviour – walking, running, flying, wing-assisted running (WAR), wing flapping, jumping, group running & walking – was recorded. Data were analyzed using Proc Glimmix in SAS 9.4 with time of day, visit, style, and strain feather colour as fixed effects, visit as a repeated measure, and flock as a random effect. Percentage of time locomoting peaked at V2 after the brooding compartments were opened, and decreased significantly at V3 (p=0.0057). Pullets in S3 spent more time locomoting than pullets in S1 (p=0.0023) and S2 (p=0.0057). The effects of aviary style on locomotion were most apparent before the brooding compartments were opened. The percentage of time pullets in S3 spent running, WAR, as well as the rate of group runs was the highest at V1 than any other combination (run p=0.0114; WAR p=0.0110; group run p<0.0001). White-feathered pullets in S3 also performed the highest rate of wing flaps, aerial transitions, and total vertical transitions (wing flaps p=0.0012; aerial transitions p=0.0129; vertical transitions p=0.0201). The results from this study indicate that aviary style and genetic strain affect the locomotion of pullets.

COMPLEX REARING AVIARY DESIGN PROMOTES DIFFERENT LOAD BEARING ACTIVITIES IN LAYING HEN PULLETS

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Compared to layer pullets raised in cages, pullets raised in aviaries have stronger bones due to increased opportunity for exercise. Dynamic load bearing exercise engenders osteogenic levels of mechanical strain on the bone, promoting bone formation and enhancing strength. The type of load bearing exercise will impact different bones in the avian skeleton. E.g., wing-assisted activities likely affect the keel bone, which is particularly prone to fractures. Commercial rearing aviaries vary greatly in their structural complexity and offer vastly different experiences for pullets. Especially the brooding phase (BP) differs when chicks are confined within brooding compartments before given access to litter and multiple tiers in the freerun phase (FP). We hypothesised that the prevalence of dynamic load bearing activity (dlb; walking, running, flying, jumping, stepping up/down) as well as winginvolved load bearing activity (wlb; wing-assisted locomotion and wing-flapping) differ between aviary styles as well as between brown (B) and white (W) layer strains. We raised 3 flocks of B Lohmann-lite and W LSL-lite pullets in 3 commercial rearing aviary styles. Aviary style 1 (S1; n= 345) offers the least complexity, style 2 (S2; n = 432) intermediate and 3 (S3; n = 600) the most, with main differences being the space and complexity of the brooding compartments where chicks spend the first 6 weeks. Live behaviour observations were done at 3 ages during the BP and FP each. Statistical analysis was done in R by applying generalised mixed effect models. Both dlb and wlb were affected by style and strain. During the BP, S3 performed more dlb than S2 and S1 (p<0.001) and W more than B (p<0.001). There was a trend for a higher odds ratio (OR) of wlb in S2 and S3 (p<0.1), with no effect of strain. In the FP, there was an interaction of style and strain (p<0.05)with S2W and S3W showing more dlb than any other group. There was an interaction of style and strain (p<0.05) on wlb with S2W showing the highest OR and S1W the lowest. These results confirm that birds perform more dlb in more complex aviaries and W perform more dlb than B. W perform more wlb than B, with S2 showing the most wild behaviour. In conclusion, rearing aviary design can promote different dynamic and wing involved load bearing activities, which will have varying affects on bone strength and subsequent fracture risk. We aim to improve laying hen welfare by growing pullets fit for complex free-run housing.

EFFECTS OF A PHYTOGENIC FEED ADDITIVE ON ZOOTECHNICAL PERFORMANCE AND LITTER MOISTURE CONTENT DURING CYCLIC THERMAL STRESS

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Heat stress (HS) is a crucial influence on the performance, health and well-being of poultry. The present study investigates the potential effect of a phytogenic feed additive during cyclic thermal stress in broiler production and the effects on zootechnical performance parameter and litter moisture content. A feeding trial was conducted with 200 male broiler chickens (Cobb 500) during the period from day 1 to day 42. Two treatment groups with 10 replications (n = 100 in each treatment) were randomly allocated. The first group was a negative control (NC), the second group received the NC diet supplemented with a phytogenic additive (200 g/t Anta®Phyt MO, APMO). The additive was given throughout the duration of the experiment. APMO consists of a combination of natural plants and their extracts with hops as the primary ingredient. The birds were fed with starter (day 1 - 14), grower (day 15 - 28) and finisher (day 29 - 42). Diets and drinking water were offered ad libitum. The experimental birds were exposed to cyclic HS. In the 3rd to 6th week of age, the temperature was between 30 and 36°C during the day. In addition, the animals were challenged by previously used bedding material. Body weight (BW) and feed intake were recorded weekly. Litter samples were collected from each of the pens towards the end of the feeding trial at day 42. All data were subjected to one-way analysis of variance with the dietary treatments as the grouping factor, applying Tukey's B-test. Supplementation of APMO increased BW (42d; NC: 2.48 kg, APMO: 2.53 kg, p = 0.072). Feed conversion ratio in the APMO supplemented groups was better as compared to the NC group at the time of harvest (NC: 1.62, APMO: 1.56, p =0.002). Mortality was not affected. Litter moisture content decreased when APMO was supplemented to the diet as compared with the NC group (NC: 25.64 %, APMO: 21.36 %, P = 0.002). The data indicate that the negative effects due to exposure to cyclic HS, such as lower body weight and higher feed conversion rate, can be reduced by the supplementation of APMO. Overall, it was concluded that supplementation of Anta®Phyt MO can be used as a tool to alleviate chronic HS and sustain performance and gut health stability in commercially reared broiler chickens during heat stress conditions.

PROVIDING RESTING ENRICHMENTS NEXT TO ACTIVE ENRICHMENTS SEEMS TO SEPARATE ACTIVE AND RESTING BEHAVIOUR MORE AND TO IMPROVE CONTACT DERMATITIS IN SLOWER-GROWING BROILERS

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In the Netherlands, bales are often provided as environmental enrichment in production systems with slower-growing broiler chickens. Bales may have different functions, as they can stimulate explorative behaviour and may offer an elevated resting place as long as they are intact. However, broilers may have more benefits from specific resting enrichment in addition to bales, so that active and resting behaviour can be better separated and possibly the behavioural needs of the broilers are better met. We performed an experiment on a commercial broiler farm with three houses during three production cycles and with three treatment groups, each in one house: Control (C; lucerne bales only), Platform (PI; solid platforms with ramps in addition to lucerne bales) and Perch (Pe; round metal perches in addition to lucerne bales). Treatments were alternated between the houses in each cycle. The platform area was 5% of the floor area and capacity of perches and platforms was for a similar number of birds. Each house had roof windows (>3%) of floor area); stocking density was 30 kg/m2 and the breed was Hubbard JA757 (as hatched). Behaviour was observed on locations with and without enrichment in addition to enrichment use and response to a novel object/human approach test at 2, 4 and 6 weeks of age. Contact dermatitis, gait score, cleanliness and injuries were measured at 6 weeks of age (just before depopulation). Platforms were more occupied at all ages as compared to perches. With respect to behaviour, in the Pl and Pe treatment chickens showed more locomotion on locations without enrichment and more inactive behaviour on locations with enrichment, whereas the C treatment showed the opposite. Foraging and explorative behaviour was highest on locations without enrichment for PI, but on locations with enrichment for C. Further, standing was more observed in Pe and C and comfort behaviour was more observed in Pl and C. Although generally scores for welfare indicators were low (good) for all treatments, Pe and Pl had less footpad dermatitis as compared to C. These findings suggest that providing additional resting enrichment next to bales may have added value as this seems to create functional areas in the house for resting and active behaviour. Platforms were preferred over the perches and well used by the chickens, not only to rest on but also to rest under, and would therefore be the more suitable as resting enrichment.

TRANSPORTATION STRESS- IMPACT ON BEHAVIOR AND WELFARE IN MEAT TYPE CHICKEN UNDER INDIAN SCENARIO

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In recent years, welfare issues in rearing birds were increasing at an alarming rate globally. During the transport of birds to the slaughterhouse leading to stress is a major cause of concern of animal welfare and also it is associated with economic losses. Especially in developing countries like India having tropical environment, poor road and transport facilities this issue is still not researched much. In order to find the standard transportation time and its impact on the welfare of broilers, the following experiment was conducted. The experiment was executed with 480 marketable commercial broiler chickens were transported for 2, 4, and 8h. A control group was kept without transport in crates. Transport significantly affected welfare as well as behavior parameters in broilers. Transport duration (8h) significantly (P<0.001) worsened gait score, tonic immobility, and Run-away time (279.20sec) of birds. Increased transport duration magnified physical injuries among birds. Birds transported for a longer duration took more runaway time to move out of the runaway corridor indicating physical injuries and gait impairment. Birds transported for a short duration took less runaway time. Transport stress resulted in a significant (P<0.001) reduction in body weight especially 8h transit groups exhibited greater (8.21%) loss. From the above results, it is concluded that a transport period of more than 4h under Indian conditions is not recommended as it's causing a significant level of stress in the birds leading to stress, production loss, and hampering welfare of broilers.

EFFECT OF CHANGING LIGHT SPECTRA ON WELFARE AND PRODUCTION IN LAYING HENS

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Unlocking the genetic potential of prolonging the production cycle in laying hens can only be profitable when a flock shows optimal production, good welfare and health. Farmers are looking for strategies and tools to meet this ambition. Over the last few years, the importance of light in managing laying hens has been reconsidered. The evolution in lighting technology makes it possible to adapt light day spectra and illuminance during the or the production cvcle. At the Experimental Poultry Centre, a trial was conducted with 8 groups of 960 brown laying hens. Four groups had a standard LED light program with a fixed spectrum during the day. The other four groups received a dynamic light spectrum during the day. All the hens received the same management, feed and distraction materials. Production and welfare parameters were evaluated from 20 to 60 weeks of age. Illuminance (in lux) was measured at week 20, 40 and 60. Feather scoring and keel bone evaluation were conducted every 4 weeks. Adaptations of both light programs were based on the daily assessment of group behavior. At 60 weeks of age, there were no significant differences between the two treatments in mortality, laying percentage, daily feed consumption, feed conversion, percentage of second choice eggs and the amount of produced eggs per hen. Feather and keel bone evaluation showed no significant effect of changing daily light spectra. Although the trial could not demonstrate an effect on selected parameters, we can still conclude an important difference. The groups who received a dynamic light schedule could be kept at an average of 20 lux at week 60 while the groups with a fixed program were kept at an average of 14.5 lux.

INCREASED DIETARY FIBER, PROTEIN AND TRYPTOPHAN LEVELS REDUCES FEATHER PECKING BEHAVIOR IN PEN-HOUSED INTACT-BEAKED LAYING HENS

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Severe feather pecking (SFP) in laying hens has been described as a redirected foraging behavior that is often displayed in stressful situations (Rodenburg et al., 2013), and its predisposition has been associated with a reduced serotonin metabolism (Birkl, 2020). Intact beaks have been associated with more severe consequences of feather pecking in animal welfare. Crude fiber increases feeding time, satiety and may stimulate serotonin production by enterochromaffin cells in the gut (de Haas et al., 2018). Previous research highlighted an association between SFP and dietary protein and amino acid levels (Rodenburg et al., 2013), especially tryptophan, an important precursor of serotonin (Birkl, 2020). Our study aimed at investigating the effects of diets with either low or high levels of crude fiber (44 vs 55 g/kg), protein (164 vs 184 g/kg), and tryptophan (1.7 vs 2.7 g/kg) on laying hen behavior and feathering condition. Seventy-two intact-beaked 25weeks-old brown laying hens were allocated into 2 groups of 36 birds and placed in 12 pens of 3 birds each, according to their SFP behavior and feathering condition. For 4 weeks, half of the hens got a low fiber, protein, and tryptophan diet (LFPT) and the other half a high concentrated diet (HFPT). Behavioral frequencies and feathering condition were recorded for all pens on a weekly basis via scan sampling, and egg production was monitored daily. Plasmatic serotonin and corticosterone were analyzed at the beginning and at the end of the trial. Layers that received the HFPT diet showed decreased frequency of SFP behavior (P<0.05) and an increased frequency of feeding (P<0.1). Exploratory behavior was also significantly reduced (P<0.01), as expected due to increased satiety and feeding time. Frequency of visits to the drinker, aggressive behavior, standing and preening did not differ between the groups. Unexpectedly, feather condition did not differ between groups, probably because of the short duration of the trial. Egglaying rate was not changed, but the egg weight was increased with the HFPT diet (P<0.01). Serotonin and corticosterone levels were similar amongst groups (P>0.05) In conclusion, a diet promoting satiety and neurotransmitter modulation was efficient in promoting animal welfare by decreasing feather pecking behavior while improving egg weight in intact-beaked laving hens.

DIVERSITY AT IT'S FINEST – CHALLENGES AND CHANCES IN FUTURE POULTRY PRODUCTION

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Constant changes in poultry production in the EU create major challenges but also chances for producers, consumers and animals. One alternative strategy is crossbreeding local breeds with high-performing production lines. However, welfare related behavioral traits such as fear and exploration are largely unexplored in those local breeds. As a prerequisite for the use of local breeds along the food chain, this research gap needs to be filled. Therefore, fear of objects and humans of the local breeds Bielefelder and Malines was tested in Novel Object Test (NOT) and Avoidance Distance Test (ADT) on an animal-specific level. Thus, Bielefelder and Malines were observed in an 2m2 Open Field. Animals were sat into one corner of the arena facing the opposite corner containing an unknown object/a person and tracked for ten minutes using EthoVison®XT (Noldus information technology). For analysis, the arena was subdivided into 3 zones; the start zone (SZ), the object/human zone (OZ/HZ) and the intermediate zone (IZ) in-between. Cumulative duration (CD) in every zone was calculated. With the assumption that a high CD in the SZ and low CD in OZ/HZ show avoidance of the object/human and thus a fear reaction and, in contrast, high CD in the OZ/HZ show approach and thus exploration, the animals were divided into groups, using the k-means clusteranalysis. In NOT, the behavioral groups "fearful", "balanced" and "explorative" could be distinguished in both breeds. In total, 40 fearful animals with the highest CD in SZ (cluster centerSZ = 592.8), 12 balanced animals with highest CD in IZ (cluster centerIZ = 419.88) and 2 explorative animals with highest CD in OZ (cluster centerOZ = 417.74) could be identified. In ADT, only the groups "fearful" (n = 45; cluster centerSZ = 584.31) and "balanced" (n = 9; cluster centerIZ = 396.82) could be identified as only 3 animals entered the HZ with low CD. These results show animal-specific diversity in behavioral traits of both breeds but also chances for selective future breeding, e.g. using animals which show balanced ratios between the heritable behaviors fear and exploration. Furthermore, studies with larger numbers of animals and more breeds of local origin or high performance would be interesting, based on individual and on-farm testing. These data would allow more detailed conclusions e.g. about requirements of the different behavioral groups for their preferred husbandry systems.

THE EFFECTS OF SPECIALISED BEAK BLUNTING FEEDERS AND STRAIN ON THE BEHAVIOUR OF LAYER PULLETS AT 10 AND 16 WEEKS OF AGE

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Beak treatment is one of the most effective methods of reducing the damage inflicted from severe feather pecking behaviour. However, the demand to stop the practice of beak treatment and use viable alternatives is increasing. Natural beak blunting by the inclusion of abrasive materials in the feeder design has been suggested, but the impacts of these materials on pullets are not well understood. The objective of this study was to examine the effects of specialised feeders that promote beak blunting on the behaviour of Lohmann Brown-Lite (LB) and Lohmann LSL-Lite (LW) pullets. Newly hatched chicks (n=900 per strain) were sorted into 3 beak treatments: sham untreated control (C), infrared beak treated (IR), or provided with a specialised beak blunting feeder (SF), which had an abrasive inner pan. Pullets were housed in 18 floor pens (n=100 per pen) from 0 to 16 weeks of age. Behaviour was recorded for 24 continuous hours in all pens at 10 and 16 weeks of age using ceiling-mounted infrared video cameras. Data were analysed using PROC MIXED (SAS 9.4) with Tukey's test to separate means. Differences were significant when $P \le 0.05$. At 10 weeks, interactions were noted for percent of time spent at the drinker and object pecking. LW C pullets spent more time at the drinker than LB C pullets with no differences between IR and SF pullets of both strains. LW C pullets also spent more time object pecking than LB C, LB IR, and SF pullets of both strains. Strain influenced time spent walking, resting, preening, and gentle feather pecking at 10 weeks with LW pullets spending more time performing these behaviours than LB. Both beak treatment and strain influenced behaviour at 16 weeks of age. IR pullets spent more time wing stretching (a comfort behaviour) compared to C and SF pullets. C pullets spent more time dustbathing compared to SF pullets. For pecking-related behaviours, IR pullets spent more time gentle feather pecking compared to SF and more time object pecking compared to both C and SF. LB pullets spent more time at the drinker and gentle feather pecking but less time resting and preening than LW. Overall, the results demonstrate that behaviour was minorly impacted by the specialised feeders, particularly beak-related behaviours such as feeding, drinking, object pecking, and environmental pecking. However, as more countries move towards banning beak treatment, research investigating the applicability of these feeders to pullet and laying hen production is needed.

EFFECT OF LOCAL BREEDS USE ON INDUSTRIAL POULTRY PRODUCTION: PERFORMANCE AND WELFARE

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Genetic selection in poultry farming has enabled to obtain High Performance Genotypes (HPG), which can reach the weight of 3 kg in just 40 days [1], but also presents frequent health pathologies (ascites, sudden death syndrome (SDS)) and welfare issues (reduced kinetic activity, foot-pad dermatitis (FPD)) [2]. In this scenario, the possibility of exploiting the intrinsic quality of resistance of Local Breeds (LBs) in а semi-intensive system, should be investigated. The aim of the trial was to evaluate performance and welfare of five different genotypes: an HPG, two LBs and their crossbred. 240 male birds, 21 days old, of five different genotypes (Ross 308® (C), Robusta Maculata (LB1), Bionda Piemontese (LB2) and their crossbreeds with Sasso® (F1s)), were randomly allotted in an intensive farming system (controlled environment, 33 kg/m2 of animal density and commercial diet). Birds live weight were recorded weekly, mortality daily while FPD and behavioural records 1 week before slaughter. Slaughtering was performed when birds reached the 45% of the expected adult weight (EAW). Birds reached the EAW at different ages, with different LW, in particular Ross reached it at 42 days with 2.7 kg, while LBs and F1s at 84 days of age, with 1.8 kg and 2.6 kg LW, respectively (P < 0.005). Mortality has settled at 3% and was significantly higher in C (65% due to SDS and 35% to ascites), compared to LBs (1%) and F1s birds (1%) (P<0.05). FPD was totally absent in LBs and F1s birds, contrary to Ross (P<0.005). Accordingly, C birds performed more resting and stretching, whereas LBs and F1s birds performed more social and locomotor behaviours (P<0.005). Results clearly showed an improvement of welfare in F1s and LBs compared to C birds, moreover crossbred birds (F1s) had significantly higher LW compared to LBs birds. This study highlights the opportunity offered by the use of crossbreeding to enhance poultry welfare and at the same time to valorize and develop LBs and then maintaining poultry biodiversity. This last, in fact suffer from the economic competition of HPG. Further studies will be necessary to develop a new farming system adequate to the use of crossbreed and to maintain LBs with appropriate conservation plans.

^[1] Aviagen, Ross 308: Broiler Performance Objectives. Aviagen Inc., Huntsville, AL, pp. 1–15, 2019

^[2] R. J. Julian, Production and growth-related disorders and other metabolic diseases of poultry - A review, Vet. J., vol. 169, no. 3, pp. 350–369, 2005.

CONTINUOUS MONITORING OF PREDICTIVE HEALTH AND WELFARE INDICATORS IN LAYING HENS TO FACILITATE DECISION-MAKING IN COMMERCIAL FARM MANAGEMENT.

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Traditionally, laying hen farmers monitor health, welfare and productivity of their flock based on birds' feed and water intake, several climate factors, the productive output of the flock and observations on behavioural performance. Due to the growing number of birds per layer farm and the decreased availability of personnel with sufficient knowledge on poultry (de Olde et al., 2020; Economic Research Service, 2020), it becomes increasingly difficult to safeguard and control animal health and welfare. Concurrently, there is a global trend towards more sustainable livestock farming with amongst others profitable and efficient animal production with a low ecological footprint. To keep up with these developments, laying hen farmers can benefit from state-of-the art sensor technology, serving as artificial nose, ears and eyes that gather 24/7 data on flock health, welfare and productivity. This project aims to improve laying hen welfare by early detection of stressors based on continuous assessment of reliable, predictive animal-based indicators. A qualitative survey will gain insight into the perception of knowledgeable, experienced laying hen farmers, poultry veterinarians and other poultry experts on sensor use and automation for on-farm health and welfare assessment. The predictive value of egg quality in welfare assessment will be identified, using data from recent innovative sensors that continuously measures individual egg characteristics on 10 commercial flocks, combined with sensorenabled data on feed and water intake, climate parameters and egg numbers. The predictive value of odour will be identified by gualitative and guantitative analysis of volatile organic compounds present in manure and air on commercial layer (rearing) farms during (induced) stress. An experiment will determine the potential of odour sensors, acoustic sensors and cameras to detect stress-induced changes in hen performance. Patterns in odour, vocalisation and activity/movement will be discovered with 'machine learning' models in a commercial setting during one full flock cycle. Based on developed algorithms, relationships between sensor output and changes in hen welfare, health and productivity will be detected. A predictive monitoring platform will be developed for the poultry farmer, supporting farm management decisions and validating the effect of data-driven decisions on laying hen health, welfare and productivity. Survey results will be available at time of the Congress.

IMAGE-BASED EVALUATION OF THE PLUMAGE CONDITION IN LAYING HENS VIA AN AUTOMATED CAMERA SYSTEM

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Plumage condition (PC) is a well investigated welfare-related indicator in laying hens. Different manual scoring systems are well-established to monitor the PC, however manual systems are labour-intensive, subject to an observer bias and its results may not represent the whole flock. Therefore, the objective of this study was to develop and test an automated, image-based system to detect feather damage in laying hen flocks. The study was performed using a near infrared camera (600-1,100nm) in combination with a 3-D-imaging software (CS) under 3 different experimental setups with brown feathered hens: (1) 15 hens from the experimental farm were manually scored and photographed (CS) weekly between 27 and 61 weeks of age. (2) The CS was installed for 20 min in 13 laying hen flocks (1.55 m above the scratching area) to obtain 600 automatically taken pictures form birds. PC in all 13 flocks was also manually assessed using a randomized sample of 40 birds per flock. (3) In two additional flocks, the CS was mounted on tracks under the barns' ceiling and pictures of the birds were taken during movement of the camera (1pic/2sec; speed of the camera: up to 4m/min). For all experimental setups a system with 4 scores was used to evaluate the PC at the body regions back, wings and tail. The interrater reliability (k, PABAK) of the applied scoring scheme was 0.69 (back), 0.98 (tail) and 1.00 (wing), respectively. The CS detected and contoured the birds and their anomalies regarding PC (damages, blank areas) so that percentage of anomalies could be calculated. The results showed that the overall correlation between manual and camera-based scoring was 0.679 (Spearman-Rho). Thus, an increase in feather damage could be detected with both, the manual scoring system and the CS, however the extent of alterations (quantitative) did not match with the results of the manual assessment, sufficiently. Image analysis showed, that the CS misjudged the surface of the hens (contouring) due to overlapping as well as boundary and contrast issues leading to miscalculated percentages of alterations. The current CS is capable of detecting hens and plumage alterations however, further studies are needed in order to train the algorithm for an increased viability regarding detection of the birds (contouring) and thus, to calculate representative percentage of plumage alterations per each bird.

EFFECT OF CAGE SPACE ALLOWANCE ON EGG PRODUCTION, EGG QUALITY, IMMUNE RESPONSES AND ANTI-OXIDANT VARIABLES IN WL LAYERS

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Optimum cage space allowance (CSA) is one of the critical components of the bird management. Lower cage space is known to reduce health status and production potential of birds. The standards for space allowances for egg laying hens are generally derived from a variety of studies conducted elsewhere in temperate countries with different climatic condition. Limited studies were carried out to find out the effect of CSA for laying chicken in the tropical countries, where the birds are typically reared in open sided poultry house. Therefore, an experiment was conducted to study the effect of three different CSA on egg production (EP) and anti-oxidant and immune responses in WL layers. A total of 900 layers were housed in California colony cages in an open sided poultry house at three different CSA (422, 563, 844 cm2/bird). Each of the CSA was studied with 20 replicates. Records on daily EP, feed intake (FI) and feed efficiency (FE, FI/egg mass) and egg quality traits were recorded at the end of each 28-d interval. Lipid peroxidation (LP) and activities glutathione peroxidase, glutathione reductase and super oxide dismutase in blood, and the antibody titres against ND vaccine, and cell mediated immune response (CMI) against PHA- P were measured at 80 weeks of age. The variation in CSA did not influence (P>0.05) the EP, egg mass, weight gain and shell thickness. The FI reduced and FE improved (P<0.05) with reduction in CSA. The egg density and egg shell percentage increased with increase in CSA. The LP was higher (P<0.05) and the activities of anti-oxidant enzymes were lower at 422 cm2/bird as compared to the higher space allowances. No effect of CSA on ND titre was observed, while the CMI response was higher (P<0.05) at 844 cm2/bird than the other two CSA. It could be concluded that, though the FE was better at 422 cm2/b, the trends of shell quality traits and antioxidant variables indicated 546 cm2/bird as requirement for WL layers in open sided poultry house under tropical regions.

DESCRIPTION OF FEATHER DAMAGE IN FLEMISH LAYING HENS WITH UNTRIMMED BEAKS.

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Beak trimming is considered negative for animal welfare. Therefore, in some EU Member States it is prohibited by law, but also some retailers demand to keep hens with untrimmed beaks. This requires a very close follow-up by the farmer in order to detect harmful pecking behaviour very early. Flemish layer farmers have limited experience with untrimmed beaks, as a result of which outbreaks of pecking and consequently higher mortality rates linked to lower production occur. It was found that flocks with pecking had up to 5.1% less egg production (Jones et al., 2019). This study describes characteristics of the plumage of Flemish layer hen flocks with untrimmed beaks. Seventeen conventional layer farms were visited between 50 & 75 weeks of age and subjected to a standardised protocol in 2020-2021. The hens were housed in aviaries, with or without free range. An in-depth interview was conducted and a house visit was made. In the house, a pecking checklist was carried out at 3 locations and 100 hens were subjected to feather scoring following the Tauson method (Tauson et al., 1984). The scoring system used is a 4-1 scale to score 5 body zones. Score 4 stands for a perfectly feathered body zone and score 1 for a bare body zone. A mean score of \leq 2 indicates severe damage of the integument. A score between 3 & 2 indicates mild to moderate feather damage (Tauson et al., 2006). The mean age was 65 weeks (min 51 and max 75), with a mean mortality of 10% (min 5% and max 17%). All hens together (N=1700) showed mean scores (SD) of Neck 2,44 (0,5); Back 1,78 (0,78); Cloaca 1,73 (0,76); Tail 2,25 (0,44); Wing 2,66 (0,46). Free ranged hens (N=600) showed scores of Neck 2,58 (0,39); Back 1,80 (0,70); Cloaca 1,71 (0,72); Tail 2,30 (0,48); Wing 2,62 (0,27). Hens without free range access showed scores of Neck 2,36 (0,54); Back 1,76 (0,72); Cloaca 1,74 (0,78); Tail 2,23 (0,41); Wing 2,68 (0,53). These results show that back and cloaca areas are most subject to pecking. We conclude that all body zones in untrimmed beaks are moderately to severely affected at an average age of 65 weeks. No major differences are seen between hens with or without free range. Hens with free range showed less damaged neck and tail. These results are part of a larger project that is still ongoing. Further insight is gained into the preliminary causes of pecking and how this can be prevented. Also, a tool for poultry farmers that helps detect pecking at an early stage is currently being tested.

LACTOBACILLUS RHAMNOSUS IMPROVES STRESS-INDUCED FEATHER DAMAGE AND INCREASES IMMUNOSUPPRESSIVE REGULATORY T CELLS

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The link between the gut microbiome, immune system and behaviour is well established. As depression and anxiety in humans are associated with a dysregulated microbiome and the immune system, microbial- and neuroimmunemediated changes in the development of behavioural problems is receiving increasing attention, in both humans and animals. Addressing prevalent, damaging behaviours, like severe feather pecking, by targeting the microbiome is, therefore, an exciting, new field of research. To this end, we assessed the effect of an oral Lactobacillus rhamnosus supplement on stress-induced feather damage, fearfulness and T cell populations in adult laying hens. Eighty-six hens (33 weeks of age/woa) received L. rhamnosus in water (Lacto, 6 pens) or a drinking water placebo (Placebo, 6 pens). Three pens from each group received a 3-week chronic social stress regimen (woa 33-35) to induce severe feather pecking. Tonic immobility was used to assess fearfulness at 36 woa, and feather damage was scored from 0 (no or slight wear, nearly intact feathering) to 2 (\geq 1 featherless area \geq \$2 Canadian coin) at 37 woa. The proportions of T helper cells, cytotoxic T cells and regulatory T cells in the spleen and cecal tonsils were measured via flow cytometry at 38 woa. Generalized linear mixed models were used to assess treatment (Lacto, stressors) effects on feather cover, fearfulness, and T cells populations. Social stress aggravated plumage damage (P < 0.05), as 74% of stressed birds had clear feather loss (score > 2) compared to only 50% of nonstressed birds. Overall, Lacto treatment favored less feather damage under stress as only 51% of Lacto birds exhibited feather loss (score > 2) relative to 74% of Placebo birds (P = 0.074). While Lacto also improved the feather cover in nonstressed birds, it did not impact fearfulness. Furthermore, the probiotic treatment increased the immunosuppressive regulatory T cell proportion in both the cecal tonsils (P < 0.001) and the spleen (P < 0.001) compared to Placebo. The T helper and cytotoxic T cell proportions were not affected by Lacto, stress, or their interaction (P > 0.05). Taken together, the data suggest that plumage damage due to severe feather pecking may be, in part, an immune system-related behaviour. Importantly, we demonstrate the beneficial effect of L. rhamnosus on the avian immune response which can be harnessed as a therapeutic to deter plumage damage in millions of laying hens, thereby improving their welfare.

KEEL BONE FRACTURES ARE NOT ASSOCIATED WITH WING FEATHER LOSS IN LAYING HENS

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Laying hens have a high wing-load (weight/wing area) limiting flight. This may be further exacerbated by the loss of flight feathers, a common occurrence in up to 95% of backyard and commercial birds. Consequences of feather loss may include decreased wing use, reduced access to elevated resources, pectoral muscle loss, and adverse impacts on the keel bone which anchors the pectoral muscles. Notably, keel bone fractures, a welfare concern, are reported in up to 98% of laying hens. To assess the impact of flight feather loss, 120 white- (34 weeks of age/woa) and brown-feathered (39 woa) laying hens received a wing feather treatment: full clip or half clip (55.4% and 32.5% reduction in wing areas, respectively) or no clip. We compared the birds' ability to utilize an elevated feeder (70 cm) to a ground feeder via RFID technology. Ultrasound was used to measure changes in pectoral muscle thickness and X-rays to assess keel bone fracture prevalence before clipping (week 0) and six weeks (week 6) after clipping. Data were analyzed via a general linear mixed model. Prior to clipping, white-feathered hens spent significantly more time feeding at the elevated feeders than brownfeathered hens (53.4% vs. 24.0 %; p = 0.0218). Full clipping of white-feathered hens resulted in a 39.2% decrease in time spent on elevated feeders by week 6 (from 53.4% to 25%; p<0.0001). The time spent on elevated feeders did not change in half-clipped white-feathered hens and brown-feathered hens (half- or full-clipped). Six weeks after wing feather loss, pectoralis muscle thickness decreased by $\sim 5\%$ in white-feathered birds (half clipped; p=0.0165 and full clipped; p=0.0129). This may reflect muscle atrophy due to wing disuse. Interestingly, the muscle thickness in brown-feathered hens was unaffected by wing feather loss. Finally, the keel bone fracture rate was unchanged at 48% in all birds at baseline and at week 6. We conclude that wing clipping status and pectoralis muscle thickness are not associated with keel bone fractures in either white- or brown-feathered birds. Our data show that white-feathered birds are more sensitive to feather loss, exhibiting loss of pectoralis muscle thickness and impaired access to elevated feeders following wing clipping. Consequently, the data suggests that future feather loss prevention strategies should focus on bird strains that are more susceptible to muscle loss associated with wing feather damage to maximize the impact of these measures.

NEW INSIGHT IN CAUSES OF FEATHER PECKING: DISTURBED CIRCADIAN CLOCK TRIGGERS FEATHER PECKING AND RELATED BEHAVIOURS IN LAYING HENS.

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High locomotor activity has been assumed to be related to feather pecking. Experimental results, however, did not provide consistent results to support this hypothesis. Experimental studies showed very low correlations between both criteria and bidirectional selection for locomotor activity did not influence feather pecking. Potential changes of the circadian rhythm have not been considered so far. Recent transcriptomic studies in lines selected for high (H), low (L) feather pecking revealed differentially expressed PER2 and PER3 genes, which are known as core genes of the circadian clock. There is increasing evidence that disturbance of the circadian rhythm leads to behavioural and physiological disorders in humans and animals. This led to the hypothesis that disturbance of the circadian rhythm may be involved in the development of feather pecking. To test this hypothesis, activity records of H and L birds and an unselected control C have been reanalysed. Data of 682 individual 18 weeks old pullets of three consecutive hatches were used. H, L and C birds were kept in intermingled flocks. Locomotor activity was measured during the 13 hours light phase for 7 days continuously using RFID technology. Generalized linear mixed model with hatch, line, time of day and interaction line x time of day were used as fixed effects. Hourly means per time of day among lines were tested for significance using Tukey's t-Test. There was a significant line x time of day interaction. The effect of line was not significant. There was a tendency of lower activity in H birds. All lines showed a bimodal cicadian activity pattern. Differences among lines in the circadian rhythm were particularly expressed in the evening activity peak which was significantly lower in H than in L birds with C taking an intermediate position. The results support the hypothesis that feather pecking is related to the disturbance of the circadian clock system.

26th World's Poultry Congress, abstracts selected in 2022

Poultry welfare in low-input outdoor production systems: the PPILOW project

Selected short communications

PERCEPTIONS AND EXPECTATIONS OF PRACTITIONERS AND CITIZENS TOWARDS WELFARE IN POULTRY LOW INPUT OUTDOOR AND ORGANIC PRODUCTION SYSTEMS

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To promote animal welfare, it is important to understand the views of practitioners and citizens to poultry welfare in different farming systems. The aim of this study was to examine the perceptions and expectations of practitioners and citizens towards poultry welfare in low input outdoor and organic production systems. In total 15 online focus group discussions and a policy focus group were carried out in Autumn 2020 in France, Belgium, Finland, United Kingdom, Italy and Romania. The participants were low-input outdoor and organic laying hen and chicken farmers, processors and retailers of poultry meat and eggs, consumers, and policy actors. Welfare issues and themes examined by the focus group meetings were identified based on a literature search and key informant expert interviews carried prior the focus groups. All discussions were recorded, analysed and summarized first in local language and then in English, to facilitate cross-country analysis. Consumers, farmers and veterinarians were seen as key actors when animal welfare is considered. Consumers themselves considered space, investment required, disease control, over-use of medicines and predation as major issues relating to animal welfare in low-input outdoor and organic poultry production systems. The discussions suggested that many consumers have limited knowledge about different poultry production systems and quality assurance schemes. Locally produced, better-quality food and value for money were among the main factors that people considered when purchasing organic or low-input outdoor-produced eggs and chicken. The concept of low-input farming was understood poorly by the participants. Supply side actors considered the management of environmental parameters and a well-maintained range with appropriate vegetation or agroforestry as challenges in poultry production. Achieving the full use of range was also indicated as a challenge. Poultry meat producers considered the cost of production and weather challenges as the main issues affecting production whereas thermal comfort and disease were mentioned by egg producers as the key issues affecting production. Feeding organic poultry was an issue affecting both chicken and egg production. In conclusion, the analysis suggested several animal welfare issues that were common to producers in indifferent countries. The PPILOW project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°816172.

OPINIONS OF ORGANIC AND FREE-RANGE BROILER FARMERS ON ANIMAL WELFARE AND THE EBENE® APP FOR ANIMAL WELFARE SELF-ASSESSMENTS

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The EBENE® app was designed by ITAVI to let poultry farmers conduct welfare assessments of their own animals. It contains specific assessments for organic and free-range farms. The app focusses on animal-based welfare indicators and includes automated feedback and anonymous benchmarking. A longitudinal study on free-range and organic broiler chicken farms in Belgium and France has started in order to determine the long-term effect of the use of the app on animal welfare. At the start of the study, a survey was conducted among the participants (n=14)to assess their views on animal welfare and expectations of the EBENE® app. This survey will be repeated at the end of the study to document possible changes in their opinions. Answers were given on a scale of 1 (disagree completely/not important at all) to 7 (agree completely/very important). The farmers were asked to rate the importance of 15 welfare indicators related to the app. The highest scores were given for water availability (x=6.7, sd=0.6), food availability (x=6.6, sd=0.7) and enough space (\bar{x} =6.4, sd=0.8), while the lowest scores were given for possibilities to use enrichment (\bar{x} =5.2, sd=1.8), on-farm killing method (\bar{x} =5.4, sd=1.7), and reaction to humans (x=5.7, sd=1.2). Thus, it seems that resourcebased welfare aspects received high scores, whereas aspects related to positive behaviour and animal handling received lower scores. This indicates that perhaps more information should be provided to farmers to increase awareness of the importance of behaviour and animal handling for welfare. Farmers were also asked to rate their own performance on these same indicators. A comparison of the two scores showed that they gave a lower average score for their own performance than for the importance of the welfare aspect for 11 of 15 aspects. The difference between the two scores was largest for absence of wounds/lesions ($x_1-2=0.6$, sd=0.9), absence of footpad dermatitis (x1-2=0.4, sd=1.1) and absence of lameness (x1-2=0.4, sd=0.9). Thus, it is likely that these are the welfare aspects for which the farmers think improvement is most desirable. Farmers rate the expected usefulness of an app for welfare assessments with a 4.5 out of 7 (sd=1.6). This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°816172. The study complies with the EU regulations and ethics requirements and all participants signed an informed consent form.

CONVERSION EFFICIENCY OF THE MAIN BIOACTIVE COMPOUNDS (PUFA, TOCOLS, CAROTENOIDS) IN DIFFERENT COMMERCIAL SLOW GROWING CHICKEN GENOTYPES EXTENSIVELY REARED

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Organic rearing system of poultry requires an outdoor run, which enhances the exploratory behavior of animals. Genetic strain modulates the foraging activity of chickens: chickens with high growth rate have a low locomotory activity and are not adapted to organic system whereas adapted strains explore the outdoor area and eat grass, worm and insects and generally have more bioactive compounds in their meat. However, the conversion efficiency of some bioactive compounds coming from feed and grass by different strains has never been estimated. The aim of this paper is to assess the storage efficiency of n-3 and n-6 PUFA, tocols and carotenes on meat of seven commercial slow-growing genotypes. One hundred chicks/strain of both sexes were used: Ranger Classic (RC), Ranger Gold (RG), Rowan Ranger (RR), RedJA (RJ), Nacked Neck (NN), CY Gen 5 × JA87 (CY), and M22 \times JA87 (M). Chickens were reared in pens (2 pens/strain) with an indoor (0.10 m2/bird) and outdoor (4 m2/bird) area according to organic EU Regulation. The animals fed ad libitum the same diet (starter 1–21 d, grower 22 to slaughter 81 d). The chickens were weighed weekly; feed consumption and forage intake were also estimated. At 81 days of age, 15 chickens/pen were selected and slaughtered. The breast, thigh and drumstick muscles were excised from the carcasses, sampled and stored at -20°C for analytic evaluations. Bioactive compounds (fatty acids, tocols and carotenoids) of feed, grass and meat were analyzed. The conversion efficiency of compounds was estimated as ratio between the amount stored in the body muscles and their intake (feed and grass). The results showed that strains had a different foraging behaviour which affected the intake of bioactive compounds (mainly n-3 PUFA, carotenoids and tocols). In RR, NN and RJ the n-3 intake, which are often scarce in standard poultry feed, was provided for more than 50% by grass, while in the other genotypes was less than 20%. Accordingly, chickens eating more pasture, which, in organic system, could be considered a free and cheap source of bioactive compounds, showed the best nutritional profile of meat (less fat, more n-3 long chain PUFA and tocols). However, the conversion efficiency of the genotypes to store what provided by feed and grass into muscles was not connected with grass intake: strains with higher grass intake (RR, NN, and RJ) contemporary had lower storage rate.

MOTIVATION FOR FORAGING BEHAVIOR AND FEED PLACE PREFERENCE IN FREE-RANGE BROILER CHICKENS

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Free-range broiler chickens usually show an uneven utilization of the outdoor range, but reasons of this variability remain largely unknown. In this work, we tested whether individual differences in the exploration of the outdoor range is related to different motivation for foraging activity between individuals. We therefore compared chickens with different ranging levels (low- and high-ranging chickens, LRC and HRC, respectively) using a conditioned place preference paradigm to test whether and how individuals differ in contrafreeloading (when individuals work for food instead of acquiring it freely) and during an association/extinction of a learned food place preference. During the contrafreeloading study, chickens (nLRC = 13, nHRC = 16) were conditioned to one chamber presenting a foraging substrate and mealworms, while in the other chamber mealworms were freely available on the floor. For the feed place preference study, individuals (nLRC = 8, nHRC = 11) were conditioned to one chamber, always presenting a freely accessible feed reward (mealworms), while the other chamber was always empty. During testing trials, for both studies, animals had access to both empty chambers, and the time spent in each chamber was quantified. During contrafreeloading tests, HRC showed a marked preference for chamber with both the foraging substrate and mealworms (p = 0.02), whereas LRC did not show any preference (p = 0.52). For the first testing day during the feed place preference, both HRC and LRC spent significantly more time in the conditioned chamber (F1, 17 = 13.70, p = 0.002), where they could previously find mealworms. During the extinction trials, HRC were also more immobile than LRC $(3.00 \pm 1.94 \text{ and } 1.25 \pm 1.75 \text{ for HRC and LRC, respectively, U = 20, p = }$ 0.048). Our results suggest that individual chickens may value foraging opportunities differently, LRC being less prone to contrafreeload and being more resistant to the extinction of a learned feed place preference, partially explaining their motivation to remain near the barn where free feed is available constantly and predictably.

The project PPILOW has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N°816172.

REDUCING THE RISK OF FEATHER PECKING THROUGH LIGHT DURING INCUBATION AND LIVE INSECT FEEDING DURING THE REARING PERIOD OF ORGANIC LAYING HENS

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Feather pecking is the pecking at- and pulling out of feathers of other hens. Feather pecking remains one of the major welfare issues in laying hens. To minimize feather pecking in commercial flocks of laying hens, adaptations in management are needed. One potential route to influence behavioural development of the birds is the incubation period. During natural incubation, eggs are frequently exposed to light – especially towards the end of the incubation period. Light exposure may stimulate brain lateralization in the chick and allow early onset of hormonal rhythms. As feather pecking in laying hens is linked to the ability to cope with fear and stress, light during incubation may a promising route to reduce the risk of feather pecking. A recent pilot study showed that exposure to green light during incubation reduced severe feather pecking in pullets. A second promising avenue to reduce feather pecking is environmental enrichment. It is known that feather pecking is closely related to normal foraging behaviour and that feather pecks may be redirected foraging pecks. By keeping birds in a more stimulating environment during the rearing period (0-17 weeks of age), they may be less likely to develop feather pecking behaviour. An interesting source of enrichment may be the provision of insect larvae, as this is also a natural food source for poultry that they find attractive to eat. The larvae can be offered in a way that requires the hens to work for a food reward, resulting in a reduced motivation to direct pecking behaviour to flockmates. Data analysis is currently ongoing to investigate the effects of green light during incubation (12L:12D) and insect feeding during the rearing period on the development of feather pecking. Preliminary results from the first batch indicate that light during incubation resulted in chicks that were more reluctant at 6 days of age. Insect feeding seemed to result in birds that were less fearful of objects and humans at a later age. No major differences in feather pecking were found, but more tail feather damage was found in the 'light, no larvae' group. Results are also shared with commercial pullet rearers and laying hen farmers to see if some of these factors can also be tested in commercial flocks.

The PPILOW project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement N°816172. <u>www.ppilow.eu</u>

ON THE ROAD TO DUAL PURPOSE CHICKENS FOR EUROPE -EXPERIENCES FROM PULLET REARING IN DENMARK AND GERMANY

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The culling of hatched male layer chicks has been subject to widespread disapproval and led to its ban in Germany and France in 2022. One option to end this practice is a shift towards dual purpose breeds with a balanced performance in egg and meat production. As part of the EU project PPILOW, males and females of three dual purpose genotypes (GT) were compared in Denmark (DK) and Germany (DE) in their productivity, behavior and welfare. Here, we present the characteristics of the pullets in the rearing. Two dual purpose (A, C) and one rustic (B) GTs were selected for evaluation in this experiment: A leans towards a higher meat production, B is a rustic breed with balanced performance, and C leans towards higher egg production. In each country, a group of layers was kept as a control (D). In DK 250 chicks and in DE 80 chicks per GT were reared under organic standard and same rearing conditions for each group. They were fed a starter, a grower, a pre-lay diet based on 99-100% organic components. Data collection included feed consumption, live weight, welfare and behavior observation to study genotype-specific differences. Mortality up to week 4 ranged from 0% B in D to 6.9% A in DK. Live weights in week 4,10,18 differed significantly across all GTs and affirms breeding focus of each. Both in DK and in DE, A showed highest while D showed lowest growth. The average live weight at 18 weeks of age ranged from 1.4-1.7kg of D to 2.3-2.5kg of A in DE and DK, respectively. Fresh matter feed consumption per bird and day did not differ significantly in the starter phase (DE 26-35g; DK 15-19g) nor in the grower phase (DE 59-84g; DK 45-61g). There were significant genotype-specific differences in feed consumption in the pre-layer phase for DE (83-114q), but not in DK (81-91q). Due to technical difficulties, feed data for A in DK is excluded, explaining a lower range in feed consumption compared to DE. Studied welfare indicators show no abnormalities across GTs. Based on behavior observations in week 7,11,16 we can summarize that chicks showed high interaction with enrichment. Only minor aggressive behavior was observed. Pullet rearing is key to a successful laying period, thus promising results are expected.

Acknowledgement: We thank our partners SYSAAF, ITAB, Novogen and Hendrix Genetics for supplying the birds and valuable support. This project has received funding from EU's Horizon 2020 research & innovation program under grant agmt. N°816172.

26th World's Poultry Congress, abstracts selected in 2022

Alternatives to antibiotics

Selected short communications

COMPARATIVE EFFICACY OF POSTBIOTIC, PROBIOTIC, AND ANTIBIOTIC AGAINST NECROTIC ENTERITIS IN BROILER CHICKENS

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Necrotic enteritis (NE) caused by Clostridium perfringens (C. perfringens) is a significant bacterial disease of chickens. Here, we compared the efficacy of two antibiotic alternatives such as a postbiotic [dry(a) and aqueous(b) nonviable Lactobacillus species fermentation] and a probiotic [dry(c)] and aqueous(d) Bacillus subtilis and B. lischeniformis mixture] with an antibiotic [amoxicillin(e) in water] to control NE in broiler chickens. Four hundred, day-old chicks were divided into 8 equal groups (Gs), n=50 each (5 replicates; 10 each). G1 (a), G2 (b+e), G3 (a+b), G4 (c), G5 (d+e), G6 (c+d) treated and G7 (non-treated), were orally inoculated with a toxigenic C. perfringens type A on days 19-21 of age, predisposed with mixed Eimeria oocysts species for induction of NE. However, G8 was kept without treatment or challenge. The severity of NE signs was greatly decreased in G3 and the mortality rates were 22%, 10%, 16%, 22%, 12%, 20%, and 36% in Gs 1, 2, 3, 4, 5, 6, and 7, respectively. The highest significant ($P \le 0.05$) average final body weights were recorded in G8 (1904 g) and G3 (1753 g) compared to G7 (1603 g). In addition, the best significant ($P \le 0.05$) feed conversion ratio was in G3 (1.51) then G2 and G8 (1.61) compared to other Gs. Moreover, the European production efficiency factor was significantly ($P \le 0.05$) improved in G3 (279.33) followed by G2 (266.67), but decreased in G7 (177.33), compared to G8 (339.33). Normal intestinal histopathology appeared in G2, G5, and G6. Chickens of G3, G4 and G7 showed very mild, moderate, and severe villi degeneration, respectively. In addition, chickens of G2, G3, G5, and G6 had normal hepatocytes compared with severe vascular damage in G7. The lowest significant ($P \le 0.05$) C. perfringens and total coliform counts were observed in chickens of G2 followed by G5. Besides, G3, G6, G1, and G4 showed significant ($P \le 0.05$) decrease in bacterial counts, when compared with G7. The highest significant ($P \le 0.05$) haemagglutination inhibition antibody titers for Newcastle vaccination were in chickens of G1 and G3 as 6.4 log2 in comparison with other Gs. Significant ($P \le 0.05$) improvement of liver function tests was detected in G3 in relation to G7 and the other treated Gs. In conclusion, combined feed and water postbiotic treatment is promising in ameliorating the severity of NE and improvement of some immunological and biochemical parameters in broiler chickens compared to the used probiotic and antibiotic.

INCREASE OF ANTIMICROBIAL POTENTIAL OF ACID LACTIC BACTERIA OVER SALMONELLA HEIDELBERG THROUGH QUORUM SENSING

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Brazil is still the world's largest chicken meat exporter1, wich causes great pressure over the poultry chain production and its microbiological challenges. As an alternative to antibiotic use in large scale animal production, brazilian industry has been investing in probiotic products and its associations2. Mainly represented by acid lactic bactéria (ALB), probiotics are microrgnisms that uses the quorum sensing (QS) mechanism, trough recognition, production and secretion of oligopeptides3, inducing microbial behaviour in an unitary manner, inhibiting pathogens as Salmonella spp. The aim of this work was to evaluate ALB's increase of antimicrobial action against Heidelberg, mediated trough QS. Of clocal swabs from 40 birds (Ross 308 AP, 45 days-old), were isolated and identificated trough molecular biology, 5 ALBs of 4 genera: Enterococcus, Lactobacillus, Pediococcus and Lactococcus (20 samples). Yet, these pure ALBs were submited to the plate inhibition test, Spot on the Lawn, enabling measurement of inhibition towards Salmonella Heidelberg. From the sensibilization of the 20 ALBs with S. Heidelberg, 20 sterile filtered inducers were produced. The 20 filtrates were inoculated separately in the 20 pure cultures of ALB, and the set was submitted to the Spot on the Lawn test, resulting in the inhibition halo formed after OS_{\neg} was induced.

Data were compared between inhibition halos formed before and after the filtrates were added, applying Wilcoxon test, comparing medians (1° and 3° quartiles). Significant diferences were considered when p < 0,05. The results point out for Enterococcus and Pediococcus genera as the probiotics with better action, both being statistically induced by all 20 sterile inducers, increasing S. Heidelberg's inhibition. In addition, the mean stakes of halo increases were compared under both genera, being 26,65% and 102,5% for Enterococcus and Pediococcus, respectively. In conclusion, the ALB genera with optimal response to QS is Pediococcus due to better response when added to all inducers, in addition to higher potencialization, compared to Enterococcus.

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IMPACTS OF PREBIOTIC PREPARATIONS ON GROWTH OF SUSCEPTIBLE AND RESISTANT ESCHERICHIA COLI

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Globally, the use of antibiotics in agriculture is widespread. Their use is not simply as a treatment for infection, but often as an unnecessary prophylactic and growth promoter. This overuse has contributed in part to the development of antimicrobial resistance (AMR) in bacteria such as Escherichia coli (E. coli). The role of nutritional supplementation enabling a move towards antibiotic free diets is an area of interest. Of the functional ingredients currently in use for microbial control, prebiotic yeast preparations are widely used in animal nutrition and have been shown to aid the transition away from antibiotics. Previous studies have demonstrated that yeast mannan rich fraction (MRF) has the capability of enhancing antibiotic sensitivity of resistant strains (Smith et al., 2020). This study aims to examine antibiotic sensitivity with regards to the growth rate of resistant E. coli in the presence of different types of prebiotic preparation.

Experiments were carried out in triplicate using a Bio-tech Synergy HT microplate reader (Mason). E. coli were treated in a microplate with 0.01% w/v of 5 different prebiotics (MRF, yeast prebiotic preparations 1 and 2 (YPP1, YPP2), mannose and inulin) and an increasing concentration of tetracycline ranging from 0mg/ml -1mg/ml. Microplates were then incubated at 37oC for a period of 18 hours, with medium shaking applied every hour. Optical density (OD) measurements were taken hourly and compared to the reference control of E. coli alone. Statistical evaluation of growth curve data was assessed using GrowthRate (GR) software. For each set of replicate cultures, GR reports the mean growth rate. DesignExpert Software (Version 13) was then used to perform one-way analysis of variance (ANOVA) and produce 3D surface graphs of predicted growth rate values. In this study prebiotic type was found to have a significant impact on the growth rate of tetracycline susceptible (p<0.0001) and resistant (p<0.0001) E. coli. 3D Surface graphs of predicted growth rates support this analysis. Growth rate of susceptible and resistant E coli were lower with MRF when compared to YPP1, YPP2, mannose and inulin. The study indicated that yeast MRF had greater potential to provide an agonistic effect on the efficacy of antibiotics against both susceptible and resistant E. coli when compared with other prebiotics. These findings could aid in the transition to antibiotic free diets and in to combatting the further development of AMR.

THE USE OF AN OLIVE POMACE EXTRACT FROM OLEA EUROPAEA AS AN EFFECTIVE NATURAL SOLUTION TO THE REPLACEMENT OF ANTIBIOTICS IN BROILER CHICKEN DIETS

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The current need to find alternatives to the use of antibiotics in poultry feeds is opening opportunities for natural extracts with functional properties. The present study aims to evaluate the effect on performance of 2 solutions based on natural extracts compared to a commercial mixture of antibiotics in broiler diets. A total of 2400 1 d-old Ross AP 308 broiler chickens were housed in floor pens (16 pens/treatment, 8 of males and 8 of females, with 50 birds/pen). Standard commercial diets were used, comprising three feeding phases: pre-starter (1-7 d), starter (8-21 d) and grower (22-38/22-41 d, females and males, respectively). Birds were randomly assigned to 1 of 3 treatments based on the solution added on top of the basal diet: i) antibiotic mixture (CON); ii) a standardized olive pomace extract from Olea europaea at 1,25 kg/Tn (OPE); iii) a mixture of essential oils containing thymol and carvacrol as main functional molecules at 0.9 kg/Tn (EOS). Body weight (BW), average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) were recorded. Data were analyzed using a mixed-effects model including treatment, gender and its interaction as fixed effects (SAS software, v.9.4). CON group presented a higher (P < 0.01) ADG and FI from d0-d21 compared to OPE and EOS groups, resulting in a higher (P < 0.01) BW at d21. However, during the grower phase OPE displayed the highest ADG followed by CON and finally EOS (P < 0.01). Furthermore, global ADG and final BW were lower (P < 0.01) in EOS group compared to CON and OPE. Nevertheless, a significant (P = 0.03) treatment by gender interaction was observed in final BW, where BW of males at d41 tended (P = 0.09) to be higher in OPE than CON. FCR presented different treatment effects depending on the feeding phase. In the pre-starter phase FCR was higher (P < 0.01) in CON compared to OPE and EOS; while in the starter phase was the contrary. During the grower phase, EOS group presented a significant higher (P < 0.01) FCR than CON and OPE; with a trend of OPE group to have a lower FCR than CON group (P = 0.07). Globally, FCR during the whole period was significantly higher (P < 0.01) in EOS group, and no differences were observed between CON and OPE. In summary, animals receiving OPE in the diet performed similarly than CON, suggesting the use of olive extracts as a promising natural tool to reduce the use of antibiotics in poultry production.

EFFECT OF THE ADDITION OF HUMIC SUBSTANCES IN BROILERS CHALLENGED WITH A LIPOPOLYSACCHARIDE ON THE PRODUCTIVITY AND ANTIMICROBIAL RESISTANCE OF COMMENSAL ESCHERICHIA COLI

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Humic substances (HS) promote chicken growth, but it is unknown whether they can suppress antimicrobial resistance (AMR) factors in birds exposed to infectious challenges. The objective of the study was to assess the productive response, antioxidant activity of meat, and the presence of Escherichia (E.) coli carrying AMR factors in the excreta of broilers supplemented with HS extracted from wormicompost and increasing levels of digestible lysine (DLys) after being challenged with capsular lipopolysaccharide (LPS) from E. coli. Forty-eight male Ross 308 broilers housed in cages from 6 to 24 days of age were were randomly assigned to one of six treatments based on a factorial combination of two growth promoter programs: 1 = BMD and nicarbazin (APC) and 2 = 0.30% HS, and three dietary levels of DLys (1.1, 1.2, and 1.3%). At 11, 13, and 15 of the study, all birds were intraperitoneally injected with LPS. At the end of the study, excreta samples were taken to evaluate the presence of AMR in E. coli isolates using the Kirby Bauer technique. Data collected was subjected to ANOVA. The interaction of treatments had an effect on feed intake, feed conversion ratio and the water loss by compression in breast meat (P 0.05). The addition of HS improved the weight gain, the weight and yield of carcass and the tibia weight and ash content (P 0.05) compared to APC. The DLys influenced the breast and carcass weight, carcass yield, and tibia weight and ash content (P < 0.05). On the AMR tests, the treatment interactions and the DLys effects were not statistically different. The proportion of E. coli isolates in excreta of broilers fed APC and HS were 76.2 and 87.5%, respectively. A high proportion of resistant E, coli isolates in most of the tested antibiotics were observed: ampicillin (64%), carbenicillin (60%), cephalothin (69%), cefotaxime (36%), ciproflacin (36%), chloramphenicol (44%), gentamicin (44%), norfloxacin (40%), sulfamethoxasol/trimethropin (51%), regardless of the treatment. The E. coli isolates from broilers fed HS showed higher (P 0.05) resistance to cephatoxin (25 vs 75%) and norfloxacin (28 vs 72%). It is concluded that broilers added with HS showed better performance compared to those fed APC, but also had higher proportion of E. coli isolates resistant to cephatoxin and norfloxacin. Overall, the proportion of resistant E. coli to different antibiotics ranged from 36 to 69%.

USING A POTENTIAL PROBIOTIC STRAIN BACILLUS SUBTILIS PS-216 FOR PATHOGEN CONTROL AND WEIGHT INCREASE IN POULTRY

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Antibiotic resistance and the spread of pathogenic bacteria in livestock and food are a serious and increasing problem worldwide. Poultry meat is a frequent vehicle for the spread of pathogenic bacteria, causing economic losses in the industry and healthcare. Stricter regulations on antibiotic use, health and economic concerns have opened the door for more intensive research into alternative solutions to this problem, such as the use of probiotics. In this study, we focused on the use of Bacillus subtilis PS-216 as a potential probiotic in poultry for pathogen reduction and weight increase. Bacillus subtilis PS-216 spores survive harsh gastric conditions, making it suitable for use in the gastrointestinal tract and increases the shelf life of this probiotic. In co-cultivation in vitro experiments we have confirmed a strong inhibition (from 80 to 99,9%) of Campylobacter jejuni, Escherichia coli, Staphylococcus aureus, Salmonella Infantis, S. Enteritidis, and Listeria monocytogenes by B. subtilis PS-216. In addition, we investigated the efficacy of B. subtilis PS-216 for the control of the gastrointestinal pathogen C. jejuni in vivo in broiler chickens. Different settings were used to test the activity of the probiotic. First, PS-216 was added as a spore solution (2.5x106 CFU spores/mL) to the water supply before broilers were infected with C. jejuni (preventive measure), second, broilers were infected with C. jejuni and B. subtilis was administered 7 days after infection (therapeutic measure), and thirdly broilers were infected with C. jejuni and B. subtilis was administered immediately from day 1 until sacrifice (21 d) to prevent or reduce C. jejuni colonization. It was confirmed that B. subtilis PS-216 significantly reduced C. jejuni in the cecum content of broilers when treated for the entire period (21 days) for $1,3 \log CFU/g$ cecum. Interestingly, the addition of spores to water resulted in a significant weight gain in broilers in all B. subtilis treated groups (average 130 g increase). We conclude that B. subtilis PS-216 has the potential to be used as a probiotic in poultry for the reduction of foodborne pathogens and weight gain in broilers.
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BONILLA S. ID:1796, ID:1983

BONNEFONT C. ID:1673, ID:1716

BONNEFOUS C. ID:2256

BONNET A. ID:1673, ID:1716

BONOS E. ID:1573, ID:1634, ID:1635

BONTEMPO V. ID:1688

BORCHARDT G. ID:1901

BORDEAU T. ID:1925, ID:1943

BORDIGNON F. ID:1798, ID:2085, ID:2086 BORDINI M. ID:2079, ID:2136 BORDONARO S. ID:1750 BORGES L. ID:2088 BORTOLUZZI C. ID:1602, ID:2065, ID:2227 BOŠNJAK-NEUMÜLLER J. ID:2220 BOSTVIRONNOIS C. ID:1628, ID:1834, ID:1901 BOTI M. ID:1776, ID:2199, ID:2200 BOTTJE W. ID:1760 BOUCHEZ O. ID:1713, ID:1771 BOURASSA D. ID:2006 BOURGEOIS A. ID:1638 BOURIN M. ID:1920, ID:1924, ID:1925 BOUVAREL I. ID:1933, ID:1943, ID:2115 BOUVET R. ID:1535 BOUWHUIS M. ID:2167 BRAND Z. ID:2215 **BRAUN U. ID:2160** BRAVO-LANDA E. ID:2070 BREGEON M. ID:1676 BREVAULT N. ID:1587 BRIENS M. ID:1967, ID:1969 **BRINK M. ID:1862** BRISTER R. ID:1760 BROOKS L. ID:1859 BROUARD B. ID:1932

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- CRISCIONE A. ID:1750
- CROUBELS S. ID:1574
- CROWE T. ID:2064
- CURRIE D. ID:2167
- D'AURIA G. ID:2068
- DA SILVA A. ID:2253
- DABBOU S. ID:2156
- DAL BOSCO A. ID:1846, ID:1900
- DALLOUL R. ID:2216, ID:2268
- DAMME K. ID:1675, ID:1719
- DARDIR S. ID:1840
- DAVID I. ID:1561
- DAVIES R. ID:1990
- DAVIN R. ID:1763, ID:2062
- DAVOLI R. ID:2079
- DAYAN J. ID:2023, ID:2160
- DAYONNET A. ID:2177
- DE BAERE K. ID:1879, ID:1880
- DE BRUIN M. ID:2102
- DE CARVALHO MATOS C. ID:2125
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- DE OLIVEIRA N. ID:2238
- DE PAULA DORIGAM J. ID:2271
- DE PAULO L. ID:2238
- DE RAUGLAUDRE T. ID:2278

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DEV K. ID:2036

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DEWEER C. ID:1630 DEWEZ M. ID:2113 DEWO T. ID:2045, ID:2047 DEY S. ID:1930 DHARA A. ID:1861, ID:1930, ID:2059 DI BIANCO S. ID:1758 DI NUNZIO M. ID:2136 DIERKS C. ID:1675, ID:1719 DÍEZ D. ID:1942 DILÉ B. ID:1758 DING J. ID:1806, ID:2044 DINH A. ID:2192 DISHON L. ID:2098 DISTL O. ID:1775 DJIOMO TANKEO S. ID:2046 DJITIE KOUATCHO F. ID:2046 DOHMS J. ID:1815 DOKOU S. ID:1905 DONER S. ID:1702 DONKOR J. ID:1807 ĐORĐEVIĆ M. ID:2093 DORET-AUBERTOT M. ID:2196 DORIGAM J. ID:1564 DOS SANTOS V. ID:2061, ID:2063 DOTTA E. ID:2127 DOUBLET B. ID:2017 DOUTART E. ID:1932 **DOWNS K. ID:1495** DRAŻBO A. ID:1554, ID:2145 DRÉANO E. ID:1934 DRIDI S. ID:1760, ID:1982 DROBNYAK A. ID:1881

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FIORILLA E. ID:1947, ID:2086, ID:2137 FIRMAN J. ID:2191 FLORES-SALES R. ID:2070 FLOREZ-CARDENAS J. ID:1754 FOLEGATTI E. ID:1809 FONTAINE S. ID:1919, ID:1944, ID:2054, ID:2278 FONTANA ABS DA CRUZ R. ID:1976 FONTINHAS NETO G. ID:2171 FORGA A. ID:2285 FORSYTHE P. ID:2262 FORTE C. ID:2157 FOTOU E. ID:1776, ID:1963, ID:2119, ID:2199, ID:2200 FOTOU K. ID:1573, ID:1634, ID:1635 FOTSCHKI B. ID:2091 FOUBERT I. ID:2017 FRANZO G. ID:1860 FREDRIX M. ID:1489 FREHEN-VAN CALMTHOUT E. ID:1536 FREITAS C. ID:1669 FRENCH N. ID:2064 FRIES-CRAFT K. ID:1842 FRISCH M. ID:2014 FUCHS D. ID:2262 FULLER L. ID:2268 FURUSE M. ID:2130 GABARROU J. ID:2087, ID:2242 GABRIEL S. ID:1627 GAGLIANO M. ID:2137 GAI F. ID:2156, ID:2157

GAIGNON P. ID:1786 GALLARDO R. ID:1895, ID:2253 GANPULE S. ID:2024 GARANT R. ID:2264 GARCÉS-NARRO C. ID:2140 GARCÍA GAVIRIA L. ID:1954 GARCIA RUIZ A. ID:2100, ID:2102 GARCÍA RUIZ A. ID:2146, ID:2148, ID:2150 GARCIA-CORONEL J. ID:1688 GARCIA-LLORENS J. ID:2070 GARIGLIO M. ID:2156, ID:2157 GASCO L. ID:2127, ID:2156, ID:2157 GAUTRON J. ID:1809 GAYCHIN S. ID:1958 **GEBHARDT-HENRICH S. ID:1959** GEERINCKX M. ID:1968, ID:1971, ID:1974 GEISLER S. ID:2262 GELAUDE P. ID:1981 GEMO G. ID:1688 GEORGEAULT S. ID:1566 GERBER P. ID:1984, ID:2129 GERMAIN K. ID:1964 GERSTENKORN H. ID:2052 GESTIN O. ID:1664 GHARIB NASERI K. ID:1865 GHARIB-NASERI K. ID:1864 GHOLIZADEH M. ID:2172 GIANNENAS I. ID:1635, ID:1905 GIERUS M. ID:2288 GIGAUD V. ID:1809, ID:2005

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Cinarel			
Bronze			
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Delacon. performing nature	iff		CREATE TRUST
Kersia.		Z ZINPRO	
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