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ORAL PRESENTATIONS

Monday, October 13, 2025

Tuesday, October 14, 2025

Wednesday, October 15, 2025

Thursday, October 16, 2025

POSTER PRESENTATIONS

SYMPSOSIUM PRESENTATIONS

ORAL PRESENTATIONS

Student Competition: Metabolism and Nutrition: General Nutrition

1 Interactive effect of breed, sex, and dietary nutrient density on nutrient intake and digestibility in straight-run broiler chickens.

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The aim of this study was to investigate the interactive effect of breed, sex and dietary nutrient density (metabolisable energy; ME, and digestible lysine; dLys on nutrient digestibility in broiler chickens. The feeding study consisted of three nutrient densities and two breeds designed as a 3 × 2 factorial arrangement. The three different diets were formulated to either Ross 308, Cobb 500 or experimental specifications with 100 kcal/kg ME reduction in the starter and 75 kcal/kg ME reduction in the grower phases compared to Ross 308. Each of the six treatments was replicated eight times, with 45 birds per replicate. Diets were prepared in four phases of starter (0-10 days), grower (10-24 days), finisher (24-35 days) and withdrawal (35-42 days). Digesta from the distal ileum was collected from 3 males and 3 females from each pen at day 35 of the experiment. Feed intake and bird body weight were recorded at the end of each feeding phase and used to calculate ME (kcal/bird/day) and dLys (g/bird/day) intake and ME (Mcal/kg BW) and dLys (g/kg BW) conversions. Digestibility data were analysed using 3-way ANOVA of GLM procedure in JMP®13.0.0 to assess the main effects of breed, diet specs and gender and their interaction. Nutrient intake and conversion data were analyzed using 2-way ANOVA of GLM procedure to assess the main effects of breed and diet specs and their interaction. Significant differences between treatments or main effects were separated by least square differences test. There was no interactive effect of breed, nutrient density and sex on any of the measured digestibility coefficients. However, Cobb specs resulted in lower gross energy and fat digestibility ($P < 0.001$). Sex also affected protein, gross energy and fat digestibility ($P < 0.001$), with male birds recording higher digestibility values. Cobb birds had significantly higher intakes of both ME and dLys (0-42 days), however, they had higher ME and dLys conversion compared to Ross ($P < 0.001$). The experimental specifications resulted in both higher dLys intake and conversion ratio compared to Ross and Cobb specifications ($P < 0.001$). Diets based on Cobb specifications exhibited lower gross energy and fat digestibility. Cobb birds grew faster, gained more weight and had higher feed consumption leading to higher intake of both ME and dLys, however, the conversion ratio of these nutrients to live body weight was higher in Cobb birds. Female birds had lower protein, gross energy and fat digestibility when compared to their male counterparts.

Keywords: broiler chicken, digestible lysine, metabolizable energy, nutrient digestibility, breed

2 The effect of lard and chicken fat ratio on production performance and nutrient utilization in meat ducks

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Currently, lard and chicken fat have been utilized as dietary lipid sources in poultry farming due to their cost-effectiveness as well as lard has a lower price than chicken fat. Studies have demonstrated that dietary unsaturated fatty acids (UFAs) can promote the digestion and absorption of saturated fatty acids (SFAs). Specifically, lard is rich in stearic acid (a SFA), while chicken fat is abundant in linoleic acid (a PUFA). Whether an optimized ratio of these two fats can further enhance their utilization efficiency remains unreported. Therefore, the present study aimed to investigate the effects of varying ratios of lard and chicken fat (as lipid sources) on the growth performance and nutrient utilization in Cherry Valley meat ducks fed high-fat diets.A total of 480 male Cherry Valley ducks (14 days old, average body weight 625±25 g) were randomly allocated to six treatment groups, with eight replicates per group and 10 ducks per replicate. The experimental design was as follows: Group T1 received a basal diet supplemented with pure lard, while Groups T2–T6 received basal diets in which 20%, 40%, 60%, 80%, and 100% of the lard was replaced with an equal amount of chicken fat, respectively. All diets contained 7.5% lipid supplementation. The experiment lasted 28 days, from day 14 to day 42 of age. Results showed that: 1) No significant differences were observed in 42-day body weight (BW), 14–42-day body weight gain (BWG), or average daily gain (ADG) among all treatment groups ($p > 0.05$). Numerically, ducks in Group T3 (60% lard:40% chicken fat) exhibited the highest BW, BWG, and ADG values. Meanwhile, the feed-to-gain ratio (F/G) in Group T3 was significantly lower than that in Group T1 (100% lard, $p < 0.05$) and numerically lower than those in the other four groups. A similar trend was observed for the European Efficiency Factor (EEF). 2) The utilization rates of dietary dry matter (DM) and total phosphorus (TP) were significantly higher in Group T3 than in the other five groups ($p < 0.05$). Dietary energy utilization and apparent metabolizable energy (AME, kcal/kg) were also significantly increased in Group T3 compared with Groups T1 (100% lard), T4 (40% lard:60% chicken fat), T5 (20% lard:80% chicken fat), and T6 (100% chicken fat; $p < 0.05$).In conclusion, a blend of 60% lard and 40% chicken fat enhanced nutrient utilization, improved growth performance, and reduced the F/G in meat ducks, outperforming diets containing 100% lard, 100% chicken fat, or other tested ratios.

Keywords: meat duck, lard, chicken fat, production performance, nutrient utilization

3 Study on the effects of *Prevotella* and protein succinylation modification on liver fat metabolism

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The composition dynamics of the intestinal microbiota and the metabolites it generates have a regulatory effect on the metabolic function of the host (Indiani *et al*., 2018). As an important member of the intestinal microbiota, the

genus *Prevotella* has multiple effects on the metabolism. However, the influence of *Prevotella* on liver lipid metabolism and its regulatory mechanism remain unclear. The main part for fat synthesis in poultry is the liver. Liver is one of the important regulators of lipid steady-state. When lipid synthesis exceeds lipid breakdown or output, it causes lipid accumulation within liver cells, eventually leading to liver steatosis (Yuan *et al*., 2022). Fatty liver hemorrhage syndrome (FLHS) leads to a sharp decline in egg production in laying hens and acute death of laying hens, seriously threatening laying hen production (Yao *et al*., 2022). With the changes in people's dietary structure and lifestyle, the prevalence of Nonalcoholic fatty liver disease (NAFLD) is also constantly rising, threatening people's lives and health (Visscher *et al*., 2001). Amber acylation is a kind of highly conservative modification after translation. Succinylation has a strong influence on protein stability, enzyme activity and transcriptional regulation (Zhang *et al*., 2010). Protein succinylation is widely present in the liver. More and more evidence indicates that succinylation is closely related to liver metabolism. This article first by feeding high fat forage to establish mouse model of nonalcoholic fatty liver disease, to explore the effects of *Prevotella copri* (*P. copri*) and its metabolite succinic acid (sodium succinate, SS) on lipid metabolism in the liver. Furthermore, we explored the effect and mechanism of succinylation modification on liver fat metabolism in laying hens. The main results were as follows: (1) *P. copri* and SS improved mice liver lipid deposition, reducing liver tissue inflammation. (2) *P. copri* and SS reduced lipid droplet deposition in hepatocytes and inhibits the secretion of inflammatory factors by activating SUCNR1. (3) SS reduced liver M1 macrophages accounted for relieve inflammation of the liver tissue. (4) The level of pansuccinylation in liver tissues of laying hens in the fatty liver group was higher than that in the control group. (5) HADHA that catalyzes mitochondrial β -oxidation of long-chain fatty acids can be modified by succinylation, and the desuccinylase SIRT5 may bind to HADHA to regulate its succinylation level. (6) After over-expression of Sirt5, the level of pan-succinylation decreased, and the level of HADHA succinylation also decreased. After knocking down Sirt5, the succinylation level of HADHA increased.

Keywords: Intestinal flora, Prevotella, Succinic acid, Liver fat metabolism, Protein succinylation

4 Effect of cyclic heat stress on biochemical parameters and some biomarkers of oxidative stress in broiler chickens fed with diets containing palm kernel meal

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This study aims to evaluate the effectiveness of using palm kernel meal to combat the harmful effects of heat stress in broiler chickens. A total of 288 21-day-old AA chickens were used. The birds, which had similar initial weights and health statuses, were randomly divided into two temperature groups (23°C and 33°C for nine hours per day), each of which had two diet subgroups (P0 and P1), with six replicates of twelve birds each. Diet P0 contains 0% palm kernel meal (PKM), and diet P1 contains 10% PKM. Prior to heat exposure on day 21 and on days 28, 35, and 42, immediately following the heat challenge, two birds from each repetition and group were randomly selected, and their rectal temperatures were recorded using a digital thermometer. Serum corticosterone content was measured using commercial kits purchased from the Jiancheng Bioengineering Institute. All statistical analyses were performed using GraphPad Prism 8.0.2 software, and a two-way analysis of variance was performed on the results. The results showed significant variation in zootechnical parameters (FI, BW, and BWG) depending on the week. Regarding cloacal temperature, there was a significant difference between subjects exposed to heat and subjects in the thermal neutral zone ($p < 0.05$). Compared to the relative organ weight results, the subjects exposed to heat stress had significantly higher thigh muscle weight ($p < 0.05$) than the subjects in the thermal neutrality zone. Conversely, the weight of the intestines, liver, and kidneys was significantly higher ($p < 0.05$) in subjects in the thermal neutrality zone than in subjects exposed to thermal stress. Biochemical results revealed that costicosterone content was higher in subjects exposed to heat than in their counterparts. Additionally, there was a numerical difference in these same subjects with regard to cholesterol and aspartate aminotransferase (ASAT) content. Finally, there is no significant difference in creatine kinase activity between subjects in the two temperature groups. The biomarker results show no significant difference between groups with regard to CAT and SOD. However, the values for subjects in the P1 group exposed to heat were numerically higher than those for the other subjects. Regarding malondialdehyde values, we observed a decrease in subjects exposed to heat stress compared to those exposed to thermal neutrality. According to our results, PKM alleviates the effects of heat stress in these subjects. Further studies can be conducted to examine the H/L ratio, blood pH, and dosage of these biomarkers in certain organs.

Keywords: biomarkers of oxidative stress, biochemical parameters, broiler, cyclic heat stress, palm kernel meal

5 Effect of ambient temperature on dietary energy partitioning in broilers determined using computer-controlled closed-circuit respiratory calorimetry chambers

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This experiment aimed to investigate the effects of ambient temperatures on growth performance and energy partitioning in Arbor Acres growing broilers using computer-controlled closed-circuit respiratory calorimetry chambers. Sixty male AA broilers with similar body weight (**BW**; 1004±11 g) were randomly assigned to four temperature treatments (23°C, 25°C, 27°C, 29°C) and were fed the same corn-soybean diet. Five respirometry chambers per temperature (three broilers each chamber) were used to assess the influence of ambient temperature on growth performance and dietary energy utilization in broilers. Summary statistics for the monitored chamber environment data (temperature, humidity, O_2 or CO_2 concentration), performance (BW, average daily gain [**ADG**]) of broilers, and

energy partition (O_2 consumption, CO_2 production, apparent metabolizable energy [**AME**], net energy [**NE**], heat increment [**HI**], retained energy [**RE**]) were calculated using the MEANS procedure of SAS 9.4. Significant differences were identified using the Tukey honest significant difference test. Linear models of O_2 consumption or CO_2 production on average daily feed intake [**ADFI**] and $BW^{0.70}$ were developed using the REG procedure of SAS 9.4. The measured ambient temperature for the four treatments during test period were 23.14°C, 25.11°C, 27.00°C and 28.85°C, respectively ($P<0.01$). No significant differences were observed among the treatments for humidity, O_2 concentration, or CO_2 concentration in the chambers. Additionally, no differences were observed among the treatments in initial or final BW, ADG, ADFI or feed conversion ratio for broilers aged 25 to 28 days. However, the growth curve of broilers in the 25°C group was positioned below those in the 27°C and 29°C groups, with the 23°C group showing the lowest growth curve among all treatments. The daily O_2 consumption or CO_2 production per kg of metabolic BW were greater at 23°C and 25°C compared to 29°C ($P<0.05$). The HI and HI/AME of the experimental diet were greater for broilers at 23°C and 25°C than those at 29°C ($P<0.05$). In contrast, the NE, RE, NE/AME and RE/AME of the experimental diet were greater for broilers at 29°C than at 23°C ($P<0.05$). Compared to the linear regression of ADG on AME, CP, and metabolizable CP (**MCP**) intake, those involving NE or RE, along with CP, and MCP intake, showed higher R^2 values, indicating stronger correlation with broiler growth. The findings demonstrate that ambient temperature affects energy partitioning and metabolic efficiency in growing broilers aged 25 to 28 days, with temperatures between 27°C and 29°C benefiting the improvement of energy utilization.

Keywords: broiler, net energy, respiratory calorimetry, temperature, energy partitioning

6 Comparison of digestive physiology and starch digestive dynamics in broiler chickens fed with diverse dietary starch sources

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The objective of this study was to investigate the digestive physiological differences and in vivo starch digestion kinetics in broilers fed diets with different starch sources. One hundred and eighty 22-day-old male Arbor Acres broilers were divided into six blocks based on initial body weight. Within each block, thirty broilers were randomly assigned to one of five dietary treatments. Physiological parameters (pH, ion concentration, and digestive enzyme activity) of digestive fluids were compared between broilers fed diets with corn, wheat, barley, sorghum, or broken rice as the starch source, respectively. In another separate trial, one hundred and eighty 22-day-old male Arbor Acres broilers were used to measure mean retention time **(MRT)** of digesta, and extent of starch digestion across the five diets. This enabled the calculation of starch digestion rate **(*k*)** using the equation: $D_t=D_{max} \times (1-e^{-kt})$. Experimental data were analysed as one-way ANOVA and multiple comparisons were drawn by Tukey-Honest significant difference via JMP Pro 16 (SAS, Institute Inc, Cary, NC, USA). The dietary starch sources influenced the pH of ileal fluid, certain ion concentrations, and activities of specific digestive enzymes in broilers ($P<0.05$). The five starch sources showed similar patterns of starch digestibility variation along the digestive tract of broiler, but significant differences were observed in starch digestibility at the same gastrointestinal sites ($P<0.001$). The MRT of digesta in the total gastrointestinal tract was significantly shorter in broilers fed broken rice diets compared to the other four treatment groups ($P<0.001$). No significant difference was observed in potential digestible starch between broilers fed the sorghum diet and those fed wheat or barley diets, whereas the values were significantly lower than those fed the corn diet or the broken rice diet ($P<0.05$).The starch digestion rate ($k=0.184\ \text{min}^{-1}$) in broilers fed broken rice diets was significantly faster than those fed corn diet ($k=0.115\ \text{min}^{-1}$), wheat ($k=0.072\ \text{min}^{-1}$), barley ($k=0.107\ \text{min}^{-1}$), and sorghum ($k=0.057\ \text{min}^{-1}$) diets ($P<0.001$). These results demonstrate that although dietary starch sources significantly altered digestive physiological parameters in broilers, starch digestibility in distal ileum exceeded 90% across all the treatment groups. The observed variations in starch digestion rates highlight distinct digestive characteristics of different cereal starches in the broiler intestinal tract. These findings provide a biological benchmark for developing in vitro starch digestion kinetic assessment methods.

Keywords: starch, digestive physiology, mean retention time, digestion rate, broiler

7 Fermented palm kernel meal modulates gut barrier and inflammatory gene expression via short-chain fatty acid production in broilers

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This study aimed to evaluate the effects of dietary inclusion of fermented palm kernel meal **(FPKM),** produced using *Limosilactobacillus fermentum* BN21, on growth performance, cecal short-chain fatty acid **(SCFA)** profiles, jejunal histomorphology, and the expression of genes related to intestinal barrier function and inflammation in broiler chickens. A total of 320 day-old male broiler chicks were randomly assigned to five dietary treatments: 0% (control), 2.5%, 5%, 7.5%, and 10% FPKM. Each treatment consisted of 8 replicates with 8 birds per replicate. Diets were administered during the grower phase (days 11–21) and finisher phase (days 22–35). Data were analyzed using one-way analysis of variance **(ANOVA)**, followed by Tukey's post hoc test, and linear and quadratic polynomial contrasts to assess response trends ($P < 0.05$). Inclusion of FPKM up to 7.5% did not negatively affect growth performance; however, 10% inclusion significantly reduced weight gain and increased feed conversion ratio ($P < 0.01$). Cecal concentrations of acetate, propionate, and total SCFAs increased linearly in response to FPKM inclusion ($P < 0.001$). The villus height to crypt depth **(VH:CD)** ratio was significantly higher in the 2.5% and 5% FPKM groups ($P < 0.05$), although villus height and crypt depth were not affected. The expression of *claudin-1* **(CLDN-1)** was significantly downregulated, while *zonula occludens-1* **(ZO-1)** was linearly upregulated ($P < 0.01$). Additionally, FPKM diets reduced the expression of interleukin-18 (**IL-18**) and increased the expression of interleukin-10 **(IL-10)** and interleukin-13 **(IL-13)** ($P < 0.001$). These findings suggest that moderate dietary inclusion (2.5–5%) of *L. fermentum*-fermented palm kernel meal can enhance gut health by improving SCFA production and modulating gene expression related to intestinal integrity and inflammation, without compromising broiler growth performance.

Keywords: broiler, palm kernel meal, Limosilactobacillus fermentum, gut health, gene expression

Student Competition: Microbiology and Physiology

8 Adventures in poultry physiology: Neuropeptides, hormones and other signaling molecules in the responses to stress, pathogens and in gastro-intestinal functioning

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The “classic” stress response consists of the hypothalamus of the stressed bird secreting corticotropin releasing hormone (CRH) and, thereby, stimulating releasing adrenocorticotropic hormone (ACTH). In turn, ACTH stimulates the production and release of glucocorticoid hormones including corticosterone (CORT) from adrenocortical cells. The situation is considerably more complex. For example, both Met-enkephalin release and Pro-met-enkephalin (PENK) gene expression in the adrenal glands is also enhanced by a series of stresses. There are also stress effects on other hypothalamo-pituitary axes including increasing plasma concentrations of thyroxine (T 4) and triiodothyronine (T 3) while depressing those of growth hormone (GH) and insulin-like growth factor-1 (IGF-1). In addition, there are stress-induced increases in the release of epinephrine (EP) and norepinephrine (NE) from chromaffin cells. Administration of Met-enkephalin in vivo completely ablates or at least attenuates stress-induced shifts in CORT, T 4, T 3, EP, and NE in young female chickens. Neuropeptides influence pituitary functioning. Chicken somatotrophs were identified by their ability to respond to GH releasing hormone with increased intracellular calcium and immunocytochemistry. Some, but not all, of these cells also responded to thyrotropin releasing hormone, ghrelin, pituitary adenylate cyclase- activating polypeptide and leptin. Neuropeptides are critical to gastro-intestinal functioning. There was both synthesis and release of both Met-enkephalin and ghrelin from the duodenum, proventriculus and crop in young chicks with both influenced by cholinergic and opioid antagonists in vitro. This is a novel observation. The crop also acts as a site for storage of feed during the night together with fermentation. In addition, Salmonella not only colonize the crop and influence the crop in a manner prevented by antibodies to toll-like receptor 4 and other inflammatory agents. Both IGF-1 and somatostatin (SRIF) were released from the duodenum and proventriculus but not from crop in young chicks. Cell division in chick duodenal explants in vitro was stimulated either IGF-1 or Met-enkephalin and with mutual potentiation.

Keywords: Met-enkephalin, ghrelin, insulin-like growth factor-1, growth hormone, adrenal

9 Not Presented

10 Co-adaptation of the symbiotic microbiome and host genome across altitudes in chickens

Jiaming Jin*[1], Chaoliang Wen[1], Junying Li[1], Chunning Mai[1], Jingwei Yuan[2], Ping Wang[1], Da Peng[3], Yiqiang Zhao[1], Congjiao Sun[1], Xueying Ma[3], Jing Feng[3], Ning Yang[1] [1]*China Agricultural University, Beijing, China;* [2]*Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China;* [3]*Institute of Animal Husbandry and Veterinary Medicine, Xizang Academy of Agricultural and Animal Husbandry Science, Lhasa, China*

High-altitude environments pose significant challenges for animal survival. The physiological and genetic adaptations of plateau species to these extreme conditions have been extensively studied. However, the responses and contributions of the symbiotic microbiota to host adaptation remain unclear. This study conducted an integrated analysis of the cecal and lung microbiomes in Tibetan chickens and other breeds raised in high-altitude Lhasa and low-altitude Beijing. The cecal microbiota was enriched in metabolic pathways, while the lung microbiota was primarily involved in environmental information processing. Lhasa chickens exhibited higher cecal microbial diversity but lower lung diversity. Consistent with the varying altitudes, the microbial communities in the ceca and lungs could be classified into distinct enterotypes and pulmotypes, respectively. Lung microbiota exhibited a more rapid environmental adaptation response, as 88 microbial genera were identified as potential signatures of high-altitude adaptation compared with only 7 in the ceca. Additionally, cecal *Acetobacteroides* was jointly regulated by the environmental conditions and host genetics, with higher abundance in Lhasa chickens. F_{ST} analysis and mbQTL mapping identified *NAT8L* as a key gene subject to natural selection influencing *Acetobacteroides* colonization. Moreover, genotype-associated differences in metabolite levels suggest a potential link between *NAT8L* and *Acetobacteroides*, possibly through shared involvement in alanine, aspartate, and glutamate metabolism. These findings reveal host-microbe interactions that potentially enhances energy efficiency and provides new perspectives on host-microbe co-adaptation in high-altitude environments.

Keywords: high-altitude adaptation, cecal microbiota, pulmonary microbiota, genetic regulation

11 Genotyping of infectious bronchitis virus and evaluation of commercial live vaccine efficacy against a Japan-like strain in Taiwan

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Infectious bronchitis (IB) is a highly contagious viral disease in chickens caused by the infectious bronchitis virus (IBV), which can lead to respiratory and renal lesions or egg production disorders in infected chickens, resulting in severe economic losses to the global poultry industry. In Taiwan, indigenous IBV strains are classified into Taiwan group I (TW-I) and Taiwan group II (TW-II) based on S1 gene. A Japan-like IBV strain, CK/PT/P108-19, was isolated from an IB case at the Animal Disease Diagnostic Center (ADDC) of National Pingtung University of Science and Technology (NPUST) in 2019. To investigate the current epidemiological status of IBV in Taiwan and evaluate the protective efficacy of currently used commercial live IB vaccines against this strain, this study was divided into two experiments. Experiment I was a genotypic survey of IBV strains in Taiwan. A total of 53 IBV-positive samples collected between 2020 and 2024 from the ADDC of NPUST were analyzed based on the S1 gene. In experiment II, three combinations of commonly used vaccines were administered via ocular and nasal routes on day 1 and day 14. At 3 weeks of age, chickens were challenged with CK/PT/P108-19. At 4 weeks of age, protective efficacy was evaluated based on tracheal ciliostasis scores, tracheal mucosal thickness, and statistical comparisons among groups, including the Kruskal-Wallis test for ciliary activity and one-way ANOVA for tracheal mucosal thickness. Vaccine protection rates were calculated based on tracheal ciliostasis scores. Results from experiment I indicated that the TW-I genotype was the most prevalent (45%) among the tested samples. Although Japan-like strains accounted for only 13%, their continued presence in clinical cases suggests a potential risk of endemic circulation. Experiment II revealed that the tested commercial live vaccine combinations failed to provide adequate protection against the Japan-like strain, with protection rates ranging from 19.33% to 31.25%. Taken together, the results indicate that Japan-like IBV strains continue to circulate in poultry farms in Taiwan. Current commercial live vaccines used in Taiwan do not provide adequate protection against the Japan-like strain, highlighting the need to introduce homologous or develop strain-specific vaccines targeting Japan-like IBV as a key strategy for effective disease control.

Keywords: infectious bronchitis virus, Japan-like IBV, genotyping, vaccine efficacy, Taiwan

12 CSE/H2S regulates glucose metabolism via AMPKα S-Sulfhydration in skeletal muscle of broilers

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Hydrogen sulfide (H2S) is an important endogenous gaseous signaling molecule, metabolically produced through the catalysis of key enzymes such as cystathionine γ-lyase (CSE) using cysteine as a substrate. Notably, chicken skeletal muscle exhibits lower insulin sensitivity compared to mammalian muscle. While mammals maintain stable blood glucose levels around 5 mM, broilers exhibit higher levels, ranging from 8 to 14 mM. Our previous study demonstrated that broiler skeletal muscle is a key target tissue for H2S-mediated metabolic regulation of glucose metabolism. Given that the chicken pectoralis major muscle consists primarily of fast-twitch glycolytic fibers, this study aimed to investigate the role of the CSE/H2S pathway in regulating glucose metabolism specifically in this muscle. This study employed CSE interference, AMPK site-directed mutagenesis, and S-sulfhydration proteomics to elucidate how CSE regulates endogenous H2S production, muscle fiber growth, and glucose metabolism in fast-twitch fibers. The results demonstrated that: (1) CSE is the primary source of H2S in broiler fast-twitch muscle; (2) The CSE/H2S system coordinately modulates myotube growth, glycolysis, and mitochondrial function; (3) S-Sulfhydration is critically involved in glycolytic regulation; (4) Cys302 on AMPKα2 is an essential S-sulfhydration site for regulating glucose uptake. Collectively, these results demonstrate that endogenous H2S, catalyzed by CSE, regulates metabolic processes via S-sulfhydration modification. This study provides a theoretical basis for developing technologies to regulate broiler muscle growth. Given that fast-twitch muscle fibers are more prone to atrophy than slow-twitch fibers, we identify the CSE/H2S signaling pathway and AMPK S-sulfhydration as promising novel targets for modulating skeletal muscle glucose metabolism.

Keywords: Hydrogen sulfide, Cystathionine γ-lyase, Glucose metabolism, AMPK, Broiler

13 In ovo injection time and dose of black soldier flies maggot oil (Hermetia illucens) on hatching and post-hatching physiological parameters of broiler chicks.

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Oils are energy sources used in animal nutrition to improve animal performance and health. In order to know the influence of maggot oil from black soldier flies (BSF) on the hatching and post-hatching parameters of chicks, in ovo injection of this oil was carried out at different incubation times in the yolk sac of the embryo. A total of 1400 Arbor Acres broiler eggs were used for incubation. All the eggs were incubated in the same incubator and the incubation standards were strictly followed. On the tenth day of incubation, 1260 fertilized eggs were selected after candling and then divided into three groups of 420 eggs. Each group was divided into seven lots such as CON-; CON+; L0.1; L0.2; L0.3; L0.4 and L0.5 for in ovo feeding. The eggs were injected on the 11th, 14th and 17th day of incubation. The CON- batches were not pierced or injected. The CON+ batches were only drilled but not injected. On the other hand, the L0.1; L0.2; L0.3; L0.4 and L0.5 were pierced and then received the injection respectively 0.1ml; 0.2ml; 0.3ml; 0.4ml and 0.5ml of maggot oil per egg. After hatching, the chicks from the sub-groups of each group were housed separately in cages and then fed the same diet for six weeks to evaluate post-hatching performance. Data analysis was performed using two-way ANOVA with GraphPad Prism 8.02, with $P < 0.05$ as the threshold for significance. Following the results obtained, the hatch rates and post-hatching performances of lots L0.1 and L0.2 were improved compared to the other lots for in ovo injection on the 14th and 17th days of incubation ($P < 0.05$). However, no improvement in hatch and growth performances was noted for the injected lots compared to the control lots for in ovo injection on the 11th day. But, the blood LDL cholesterol levels of the injected batches in each group were decreased compared to those of the control batches ($P < 0.05$). So BSF maggot oil can be successfully used for in ovo injection either on the 14th or 17th day of incubation to improve hatchability and post-hatch performance of broiler chickens.

Keywords: in ovo injection, black soldier fly maggot oil, hatching rate, broiler health, post-hatch performance

14 Gut microbiota-derived propionic acid mitigates age-related albumen quality deterioration by modulating magnum functions

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Albumen quality deterioration represents a prevalent issue in late-laying hens and may be influenced by gut microbiota via the gut–oviduct axis. This study aimed to elucidate the regulatory role of gut microbiota and its metabolites on oviduct function and albumen quality in laying hens. To address this objective, albumen quality, oviduct function, and gut microbiota were compared between peak (30 wk, n=100) and late-phase (70 wk, n=100) Hy-Line Brown hens. To evaluate causality, fecal microbiota transplantation (FMT) was performed from peak-phase donors to late-phase hens (n=50/group), followed by assessment of albumen quality, oviduct histology, and gut microbiota. Untargeted metabolomics and oviduct gene expression analyses were conducted to identify functional microbial metabolites and their effects on mucosal functions. In vivo sodium propionate supplementation further validated the role of the candidate metabolite. Statistical comparisons were performed using unpaired two-tailed Student's t-test or Mann-Whitney U test, with Spearman's correlation and random forest analysis for key feature selection. Results indicated that late-phase hens exhibited significant albumen thinning ($P < 0.0001$), with concurrent deterioration of oviduct magnum structure ($P < 0.05$). Gut microbiota composition shifted, with 31 differential genera; 28 were correlated with albumen quality and oviduct traits ($P < 0.05$). FMT improved albumen height ($P < 0.05$), mucosal fold height ($P < 0.01$), tubular gland width ($P < 0.05$), and mucin levels ($P < 0.05$). Among five genera altered by FMT, only *Anaerotruncus* and *Incertae Sedis* overlapped with the 31 taxa altered in late-phase hens. Both were reduced with age, restored by FMT, and positively associated with oviduct traits ($P < 0.05$). Propionic acid, identified as a key metabolite by random forest, was correlated with *Anaerotruncus* abundance ($P < 0.05$). Furthermore, FMT enhanced mucosal barrier integrity (Claudin-1, *MUC2*, *HEP21* upregulated, $P < 0.05$) and reduced inflammatory cytokines (*IL-1β*, *IFN-γ*, $P < 0.05$). Sodium propionate supplementation replicated FMT effects, improving albumen height ($P < 0.001$), oviduct morphology ($P < 0.05$), and mucosal barrier gene expression ($P < 0.05$). In conclusion, gut microbiota and the microbial metabolite propionic acid play an important role in improving oviduct function and albumen quality in laying hens. These findings offer novel insights into the gut–oviduct axis as a regulatory pathway for mitigating age-related albumen thinning in late-laying hens.

Keywords: Laying hens, Albumen thinning, Gut microbiota, Oviduct magnum, Propionic acid

15 Effects of *in ovo* polyphenols from brown seaweed (*Fucus spiralis*) and green tea (*Camellia sinensis*) on the ileal and cecal microbiome of broiler chickens

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With the phase-out of in-feed antibiotics, managing gut health and the gut microbiome of broiler chickens has become a priority of the poultry industry. Nutritional provision during the late embryonic stage could facilitate positive and lasting modulation of the gut microbiome. This study followed a 5×2 factorial design (*in ovo* supplementation × dietary challenge with oxidized oil) to evaluate the effects of seaweed (*Fucus spiralis*) and green tea (*Camellia sinensis*) polyphenols on the ileal and cecal microbiome of broiler chickens using 16S rRNA amplicon sequencing. On embryonic day 17.5, viable embryos received one of five *in ovo* treatments via a 50 µL amniotic injection: (1) standard control (no injection), (2) antibiotic control (no injection, post-hatch in-feed bacitracin, BMD® 110G), (3) diluent control, (4) seaweed polyphenols (2.5 mg/egg), or (5) green tea polyphenols (2.5 mg/egg). Post-hatch, chicks from each *in ovo* group were assigned to either a standard diet with unoxidized oil or a diet supplemented with oxidized oil as a model for stress. Compared to the standard control, a single dose of seaweed polyphenols administered *in ovo* significantly suppressed *Clostridium sensu stricto 1*, a genus associated with necrotic enteritis, in the ileum on day 28 post-hatch ($p < 0.05$), without altering dominant microbial taxa, as indicated by unchanged alpha diversity indices and phylum-level relative abundances ($p > 0.05$). This effect was comparable to what was achieved by 28-day dietary supplementation with in-feed antibiotics ($p > 0.05$). In contrast, *in ovo* green tea polyphenols had no significant effect on the ileal microbiome based on alpha diversity, beta diversity, or differential abundance analyses ($p > 0.05$). In the ceca, only in-feed antibiotics significantly altered the microbial composition compared to the standard control group, while neither polyphenol had a significant impact on the cecal microbiome. The dietary challenge with oxidized oil had minimal impact on both the ileal and cecal microbiome. Hatchability and overall growth performance metrics, including weight gain, feed intake, and feed conversion ratio, were not impacted by treatments ($p > 0.05$). Our findings suggest that a single dose of seaweed polyphenols supplemented *in ovo* is a safe, cost-effective, and sustainable alternative to the prolonged use of in-feed antibiotics for suppressing necrotic enteritis-associated microbes. This highlights early life as a critical window for nutritional provision to manage poultry gut health.

Keywords: In ovo, Polyphenols, Broiler chickens, Gut microbiome, Antibiotic alternatives

16 Effects of *in ovo* docosahexaenoic acid supplementation on early post-hatch neural and intestinal gene expression in broiler chickens

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Docosahexaenoic acid (DHA) is essential for the development and function of the nervous and digestive systems in poultry. *In ovo* supplementation of DHA during embryonic development may offer a promising strategy to enhance early post-hatch health and performance. However, the effects of DHA on neural and intestinal gene expression in broiler chickens remain underexplored. This study investigated the effects of *in ovo* DHA supplementation on neural and immune gene expression in the brain and ileum during the early post-hatch period. A total of 480 fertilized Ross 308 broiler eggs were injected with DHA solution into the yolk sac on embryonic day 11 and assigned to three groups: non-injected control (CON), 50µl DHA (DHA50), and 100µl DHA (DHA100). Chicks were reared on a standard diet for 21 days, after which brain and ileum tissues (n=5/group) were collected. Total RNA was extracted, and cDNA synthesized for qPCR. Expressions of the target genes were normalized using the housekeeping gene *GAPDH*. Data were checked for normality, log-transformed, and analyzed using one-way ANOVA followed by Tukey's HSD in JMP® Pro 17. Spearman's correlation test was performed to assess relationships among variables. Significance was set at $P \leq 0.05$. The study demonstrated a significant difference among groups for *NPY2R* ($P<0.0001$), along with trends for *NPY* ($P=0.0568$) and *PYY* ($P=0.0947$) in the brain, and significantly lower *CD3* expression in DHA100 compared to CON and DHA50 ($P=0.0010$), with trends for *AvBD4* ($P=0.0758$), *CLDN1* ($P=0.0763$), and *OCLN* ($P=0.0939$) in the ileum. Spearman's test further revealed correlations between brain and ileal gene expression within groups. In CON, positive correlations were found between *Ghrelin* and *CD3*, *TJP*, *NPY2R* and *Cadherin*, *chB6*; *NPY* and *IL4,* and negative correlations between *Ghrelin* and *ZO1*; *PYY* and *OCLN*. In DHA50, positive correlations were observed between *HTR1A* and *ZO1*, *SOD1*; *NPY2R* and *IL10*; *NPY* and *CD56*, *chB6*, with negative correlations between *Ghrelin* and *Cadherin*; *NPY2R* and *CD3*; *NPY* and *OCLN*. In DHA100, positive correlations were found between *HTR1A* and *JAM2*, *SOD1*; *NPY2R* and *Cadherin*; *NPY* and *IL4*, while negative correlations were found between *PYY* and *chB6*; *Ghrelin* and *ZO1*, *CD56*, *NPY2R*.

Keywords: In ovo, gene expression, gut-brain axis, embryonic development, docosahexaenoic acid

17 Host genetics shape early-life cecal microbiota trajectories following embryonic probiotic exposure in chickens

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Early-life gut microbiota assembly plays a critical role in host health and productivity. However, the extent to which host genetics modulates microbial succession and probiotic responsiveness remains unclear in poultry. We hypothesized that chicken breeds with distinct genetic backgrounds would exhibit differential microbiota trajectories and responsiveness to embryonic probiotic intervention. At embryonic day 17.5, fertilized eggs from two genetically divergent breeds, Arbor Acres (AA) broilers and Beijing You (BJY) chickens, were injected in ovo with either *Lactobacillus reuteri* DSM17938 (*L reuteri*, 10^6 CFU/egg) or sterile saline. Cecal microbiotas were profiled by 16S rRNA gene sequencing at days 1, 3, 7, and 14 post-hatch. AA broilers exhibited rapid cecal microbiota succession, with unique genera declining from 165 on day 1 to 12 by day 14, and 44 core genera consistently detected, indicating early community stabilization. Alpha diversity decreased significantly over time ($P < 0.05$), and PCoA showed strong temporal clustering ($R = 0.7493$, $P = 0.001$). Functional maturation was marked by enrichment of SCFA-associated genera, including *Faecalibacterium*, *Subdoligranulum*, and *Christensenellaceae* ($LDA > 3.5$, $P < 0.05$). In contrast, BJY chickens displayed slower, more heterogeneous microbiota development, with unique genera declining from 151 on day 1 to 4–18 by day 14, and only 8 genera shared across all time points. Alpha diversity remained stable, and PCoA indicated weaker temporal separation ($R = 0.6336$, $P = 0.001$). Enrichment of taxa linked to redox and immune functions, such as *Escherichia–Shigella* and *Oscillospiraceae*, suggested delayed ecological stabilization ($LDA > 3.5$, $P < 0.05$). *L reuteri* accelerated microbiota maturation in both breeds through genotype-specific pathways. In AA, unique genera decreased from 80 to 4 by day 7, with early enrichment of SCFA producers including *Faecalibacterium, Butyricicoccus,* and *Christensenellaceae* ($R = 0.765$, $P = 0.001$; $LDA > 3.5$, $P < 0.05$). BJY showed slower convergence, maintaining 11 shared genera and persistence of immune-modulatory taxa such as *Coriobacteriaceae_UCG-002* and *Anaerofilum* through day 14 ($R = 0.612$, $P = 0.001$; $LDA > 3.5$, $P < 0.05$), consistent with delayed functional transition. These findings demonstrate that host genetic background fundamentally shapes both the trajectory of microbial assembly and its modulation by probiotic intervention, emphasizing the need for genotype-specific microbiome strategies in poultry production.

Keywords: In ovo administration, Lactobacillus reuteri DSM17938, Cecal microbiota dynamics, Breed-specific microbial response, 16S rRNA gene sequencing

Student Competition: Poultry Management, Waste, and Well-Being

18 *Pectoral* myopathy as a contributor to broiler carcass condemnation: assessment of associated risk factors

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This study aimed to determine the prevalence of hardened *pectoral* myopathy (characterized by wooden breast syndrome) identified at the condemnation stage in a commercial broiler abattoir and to examine its associations with other rejection reasons and potential risk factors. We hypothesized that hardened myopathy shares common risk factors with other conditions, and that these can be linked to flock management practices. Wooden breast has emerged as an unintended consequence of genetic selection in broilers. Though often considered a quality defect, it may reflect impaired welfare during broiler growth. Abattoir condemnation offer valuable insight into health issues that manifest at the end of the production cycle. We analyzed three-years of truckload-level monitoring data to explore the contribution of hardened myopathy to carcass condemnation and its links to on-farm conditions and transport-related factors. The dataset included 13,849 truckloads, representing 93.7 million broilers processed at a federally inspected abattoir in western Canada (April 2021-March 2024). For each load, rejection rates for 10 conditions were recorded: dead-on-arrival (DOA), respiratory, subcutaneous, leg, skin, ascites, liver, emaciation, dark-colored carcasses, and *pectoral* myopathy. Additional variables included average age, weight, count, transport distance, proportion of small and uneven birds, antibiotic-free status (RWA), thinning/reloading, stunning method, region, and season. Descriptive statistics characterized the prevalence. Association rules mining assessed co-occurrence patterns of rejection causes. Multivariable mixed-effects models evaluated the impact of risk factors on rejection rates; outcomes were log-transformed due to skewed distribution. Hardened myopathy (0.18%) ranked third among rejection reasons, after subcutaneous condition (0.77%) and DOA (0.36%), and ahead of ascites (0.11%). Truckloads with high incidence of myopathy often showed elevated respiratory and liver lesions, with all three condition significantly increased under RWA system ($P<0.01$) and in cold seasons ($P<0.001$). Both greater average weight and small bird proportion were positively associated with DOA, ascites, myopathy, and respiratory issue, showing comparable effect estimates ($P<0.001$).

Keywords: Myopathy, Condemnation, Risk factors, Weight variation, RWA

19 Effects of different types of methionine on intestinal health of broilers and related mechanisms

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Improving the intestinal health and immune level of broilers by means of nutritional regulation is of great significance to the development of the current breeding industry. As a functional amino acid, the effects of different sources and different levels of methionine on the intestinal health status of broilers remain to be explored. In this experiment, the intestinal organoid model was used to explore the effects and possible mechanisms of adding different types and concentrations of methionine on intestinal health of broilers under TNF-α-induced intestinal injury. The results showed that direct addition of 100 and 150 μmol/L DL-methionine (DL-Met), L-methionine (L-Met), methionine hydroxyl analogue (MHA, 88 %, mainly active as 2-hydroxy-4-methylthiobutanoate) significantly inhibited the growth of intestinal organoids ($P < 0.05$). The intestinal organoid injury model of broilers was constructed by using 300 μg / L TNF-α, and the cells were treated with 25,50,100 μmol/L DL-Met, L-Met and MHA for 24 h. It was found that with the increase of methionine concentration, the growth of intestinal organoids in broilers was inhibited to varying degrees. Compared with DL-Met and L-Met, 25 μmol/L MHA significantly improved the intestinal injury induced by TNF-α ($P < 0.05$). Moreover, compared with the TNF-α treatment group and the control group, 25 μmol/L MHA could significantly increase the relative expression of ZO-1, Claudin-1 and Lgr5 in intestinal organoids ($P < 0.05$). Compared with the TNF-α treatment group, the expression level of SOX9 was significantly increased, and the relative expression of TNF-α was significantly lower than that of 25 μmol/L DL-Met and L-Met addition groups ($P < 0.05$) ; compared with the blank control group, the expression of Occludin in 25 μmol/L DL-Met, L-Met addition group and 300 μg/L TNF-α treatment group increased significantly ($P < 0.05$), while the expression of 25 μmol/L MHA addition group did not change significantly compared with the blank control group ($P > 0.05$). All the above data were analyzed using one-way analysis of variance. The above results indicate that MHA can alleviate TNF-α-induced intestinal injury and promote the intestinal growth of broilers by promoting the proliferation of intestinal stem cells. The recommended dose is 25 μM.

Keywords: broilers, methionine, TNF-α, intestinal health, intestinal organoids

20 Study on the mechanism of heat stress affecting the growth and development of intestinal organs in broilers through Hippo pathway

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With the intensification of global warming in recent years, intensive and large-scale broiler farming mode, heat stress has become a key factor affecting the development of modern broiler farming. Heat stress can significantly reduce the performance and feed conversion rate of broilers, and disrupt the intestinal barrier function of broilers, which can lead to an imbalance of intestinal flora and trigger intestinal inflammation. The aim of this article is to investigate the role of heat stress on the growth and development of broilers and their intestinal organs and related mechanisms. A total of 72 1-day-old AA broilers were selected for the 42-day animal testing period and randomly divided into three treatment

groups: control group, paired-feeding group, and heat stress group. The heat stress group was treated with 34°C for 8 h during the day and fed at night according to the temperature specified in the feeding standard. The test cycle of class organs was 3 days in total, and three treatment groups were set up: normothermic control group (CON group), with the temperature controlled at 37 ± 1°C; chronic heat stress group (HS group), which was treated at a fixed time every day for 1.5 h at 43°C; and acute heat stress group (AHS group), which was subjected to heat stress treatment (43°C) on the last day. The results of one-way ANOVA showed that compared with the control group, the cell area and number of intestinal organoids in broilers in the HS group decreased significantly ($P < 0.001$) after 1.5 h of heat stress treatment; and heat stress significantly down-regulated the relative expression of YAP1 ($P < 0.05$) and up-regulated the relative expression of HSP70 ($P < 0.05$) in the Hippo pathway. Heat stress decreased the expression of ZO-1, Claudin1, and Ki67 ($P < 0.001$) and up-regulated the relative expression of MUC2, SOX9, LGR5, and OLFM4 mRNA ($P < 0.05$). In animal experiments, heat stress treatment significantly up-regulated the mRNA expression of PEPT1 ($P < 0.001$) and Occludin ($P < 0.05$) in the jejunum of broiler chickens, while the mRNA expression of GLUT1 ($P < 0.05$) was significantly down-regulated. In conclusion, heat stress would damage the intestinal barrier of broiler chickens, reduce the expression of intestinal villus cuprocyte genes, increase intestinal permeability, inhibit the proliferation of intestinal stem cells to promote stem cell differentiation, and at the same time would reduce the feed intake of broiler chickens, so that glucose absorption would be inhibited.

Keywords: Heat stress, Hippo pathway, Intestinal organoids, Mechanism

Poultry Management, Waste, and Well-Being I

21 Monitoring welfare and behaviors of cage-free hens with deep learning methods

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The US egg production is shifting from conventional cage to cage-free (CF) due to concerns of animal welfare. Primary food chains have pledged to source only CF eggs by 2025 or 2030. The market share of CF eggs has increased from 10% in 2015 to 40% in 2025. However, the current CF housing system is facing many challenges. For instance, CF hens tend to have higher injury rates or health issues such as severe pecking (cannibalism), footpad lesion or dermatitis, and keel bone damages than conventional caged hens. Besides, mislaying behavior results in floor eggs, which could lead to increased labor cost and foot safety concerns. The objective of this study was to develop and optimize deep learning models to monitor welfare and behaviors of CF hens for improving egg production efficiency and animal wellbeing. The YOLO (You Only Look Once) family models have gained significant prominence due to their exceptional speed and accuracy in object detection tasks in recent years. In this study, two deep learning models, i.e., YOLOv5x-pecking and YOLOv5s-pecking, were developed and tested with up to 85% of precision in monitoring CF hens' pecking behaviors and damages. For bumblefoot detection (BFD), the performance of three newly developed deep learning models (i.e., YOLOv5s-BFD, YOLOv5m-BFD, & YOLOv5x-BFD) were compared. The result shows that the YOLOv5m-BFD model had the highest precision (93.7%) and F1-score (89.0%) compared with other models. For footpad lesion detection (FLD), OLOv8 models (YOLOv8n, YOLOv8s, YOLOv8m, YOLOv8l, and YOLOv8x) and YOLOv7 models (YOLOv7 and YOLOv7x) were comparatively evaluated for predicting footpad scores. The results show that the YOLOv8l outperformed other models, with higher recall (96.6%) and F1-score (95%). Those new deep learning methods provide new solutions for proving animal behavior and welfare monitoring and management in CF housing systems.

Keywords: cage-free housing, animal welfare, layer behavior, precision farming, machine vision

22 Lycopene Ameliorates Atrazine-Induced duodenal damage in laying hens by restoring intestinal barrier function

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Atrazine, a widely used triazine herbicide, threatens intestinal health as its residues enter poultry through contaminated-feed[1]. The duodenum, crucial for digestion and absorption, relies on structural integrity and barrier function for optimal production performance. Thus, finding a safe and effective antagonist to counteract atrazine-induced toxicity is vital. Lycopene, a natural antioxidant with anti-inflammatory, anti-apoptotic, and cytoprotective properties, is a promising candidate due to its availability and safety. In this study, 75 Hy-Line white variety chickens were randomly divided into five groups: control, vehicle control, atrazine exposure (100 mg/kg ATR), lycopene supplementation (5 mg/kg LYC), and atrazine combined with lycopene (100 mg/kg ATR + 5 mg/kg LYC), aiming to investigate the toxic effects of atrazine on the duodenum of laying hens and the antagonistic effects of lycopene. The results showed that atrazine severely compromised duodenal integrity by reducing villus height and crypt depth, downregulating tight junction proteins (ZO-1, Occludin), and activating the TLR4/NF-κB pathway, which elevated pro-inflammatory cytokines (IL-1β, TNF-α). Gut microbiota profiling demonstrated that atrazine disrupted microbial equilibrium, marked by a drastic increase in the Firmicutes/Bacteroidota ratio and enrichment of pathogenic genera (Enterococcus_E, Clostridium_T), while suppressing beneficial taxa (Limosilactobacillus). Lycopene intervention restored villus structure, enhanced tight junction expression, inhibited inflammatory signaling, and partially normalized the thick-walled bacillus/anabolic bacillus phylum ratio, reshaping the flora balance and systematically alleviating atrazine-induced duodenal injury. The present study reveals the molecular basis of lycopene antagonizing atrazine toxicity by alleviating duodenal physical, chemical, immune and biological barrier damage, which provides an important theoretical and practical basis for the improvement of poultry intestinal health and production performance.

Keywords: Atrazine, Lycopene, Laying Hens, Gut microbiota, Intestinal barrier

23 Full repertoire of Pekin duck vocalizations & effects of specific vocalizations on conspecifics suggest alarm or calming calls

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Pekin ducks are exposed to various stressors throughout their lives, but stressor identification usually happens in reaction to decreased production rather than identifying in real time. Decades of wild bird literature have shown that a duck's vocalization reflects their welfare. If we are able to understand the information portrayed by ducks' vocalizations, then they could be used to identify stressors monitoring vocalizations in real-time. To accomplish this, we first developed a vocal repertoire for Pekin ducks. We used a total of 29 adult Pekin ducks and placed 1 to 4 ducks of varying sexes into a sound chamber (~30min) with various positive and negative stimuli and their responsive vocalizations were recorded. Vocalizations were then clipped and named based on a predetermined naming system. We found that Pekin ducks produce up to 16 different vocalizations along with 4 egg laying vocalizations. Next, we determined how specific vocalizations affect the physiology of conspecifics. We tested Pekin ducks with playbacks of 5 different vocalizations from the repertoire, with a no noise and white noise as controls (N = 15 ducks/sex/treatment). These vocalizations were as follows: (1) Am Long (2) Honk (3) Pip (4) Harmonic stack and (5) Egg laying squiggle. Trials consisted of an initial recording in a closed off room with 5 consecutive measurements of heart rate (HR), blood pressure (BP), and respiratory rate (RR). A specific playback was then played on repeat while 8 more measurements of HR, BP, and RR were taken. After the playback, 5 measurements of HR, BP, and RR were taken. Ducks were randomly selected, and the playbacks were given at random. Data from all BP, HR, and RR were subject to a principal component analysis (Proc Princomp in SAS

9.4). The significant principal components (Prin 1 loaded strongly with BP and Prin 2 loaded strongly with HR and RR) were then analyzed by ANOVA with repeated measures (Proc Mixed SAS 9.4). A $P \leq 0.05$ was considered significant. Our results showed that ducks experienced an increase in blood pressure when played back the honk vocalization ($P = 0.0453$) and the white noise control ($P = 0.0309$) when compared to the no sound control. Specifically, the egg vocalization significantly decreased the hens' blood pressure when compared to the two controls ($P = 0.0255$ vs no sound; $P = 0.0237$ vs white noise). This study represents a critical steppingstone toward using vocalizations as a real-time flock welfare indicator.

Keywords: anechoic chamber, flock management, calls

24 Effect of the cage size and stocking density on the natural behavior expression, serum biochemical indices, and productive performance of laying hen

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This study aimed to investigate the interactive effects of cage size and stocking density on laying hen welfare, physiology status, and productive performance to optimize cage design in intensive poultry production. A total of 720 Jingfen laying hens (18 week old) were randomly assigned to a 3×3 factorial design: three cage sizes (Small, SC: 0.45m×0.60m; Medium, MC: 0.90m×0.60m; Large, LC: 1.35m×0.60m) and three stocking densities (Low, LSD: 540 cm^2/hen; Medium, MSD: 450 cm^2/hen, High, HSD: 386 cm^2/hen), with four replicates per group. Formal experiment was performed from 20 to 31 weeks of age. Measured parameters included natural behaviors (feeding, drinking, preening, standing, walking, resting), daily step counts (using a pedometer), laying rate, egg quality and serum biochemical indices (FSH, AKP, MDA, T-AOC, IgA, IgG). Data were analyzed using two-way ANOVA (cage size, stocking density) in SAS 9.3. Treatment means were compared via Tukey's test. Behavior & Welfare: LC and MC significantly increased the frequency of feeding, drinking, preening and walking, as well as daily step counts compared to SC at the same density ($P < 0.05$). LSD significantly increased the frequency of preening and walking compared to MSD and HSD under the same cage size. Significant interaction effects of cage size and stocking density on feeding, preening, and walking behaviors were observed ($P < 0.05$). Physiology status: LC and MC had significantly higher levels of FSH, AKP, T-AOC and IgA ($P < 0.05$), and had lower MDA levels ($P < 0.05$) compared to SC at the same density. MSD exhibited higher serum AKP, IgA and IgG levels compared to HSD and LSD ($P < 0.05$) under the same cage size. Significant interaction effects of cage size and stocking density on serum FSH, AKP, and IgA levels were observed ($P < 0.05$). Productive performance: LC and MC significantly increased laying rate (at LSD) and eggshell thickness (at LSD & HSD) compared to SC ($P < 0.05$). Under the same cage size, laying rates were significantly higher in LSD and MSD compared to the HSD ($P < 0.05$), and eggshell thickness was significantly higher in LSD compared to MSD and HSD ($P < 0.05$). A significant interaction effect of cage size and stocking density on the laying rate was observed ($P < 0.05$). The current study investigated the effects of the cage size and stocking density on laying hens. Significant interactive effects were observed between cage size and stocking density for the feeding, preening and walking behaviors; serum levels of FSH, AKP, and IgA; and egg production rate. The results provide valuable insights for the precise optimization of cage design to support sustainable development in the poultry industry.

Keywords: laying hen, cage size, stocking density, natural behavior, laying performance

Metabolism and Nutrition: General Nutrition I

25 Advances in the use of prebiotics in poultry to modulate gut health

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The poultry industry is transitioning toward antibiotic-free production systems, necessitating the development of alternative strategies to enhance gut health and overall bird performance. Prebiotics, among others, have been extensively studied to serve this purpose. Oligosaccharides (like raffinose, xylooligosaccharides, mannanoligosaccharides, and chitooligosaccharides) have emerged as promising prebiotics that produce short-chain fatty acids (SCFAs), enhance gut barrier integrity, selectively stimulate beneficial gut microbiota, and modulate immune responses, thereby improving intestinal health and overall poultry performance. Prebiotics are resistant to endogenous enzymatic digestion in the upper gastrointestinal tract, allowing them to reach the ceca, where they undergo fermentation by beneficial bacteria such as Lactobacillus and Bifidobacterium species. This fermentation process produces SCFAs, including acetate and butyrate, which serve as energy sources for intestinal epithelial cells and play crucial roles in maintaining gut integrity and modulating immune responses. Recent studies have demonstrated that both *in ovo* and dietary supplementation with prebiotics can lead to significant improvements in gut morphology, including increased villus height and a higher villus-to-crypt ratio, which are indicative of enhanced nutrient absorption capacity. Additionally, prebiotics have been shown to modulate the gut microbiota composition, promoting the growth of beneficial bacteria while inhibiting the proliferation of pathogenic microorganisms such as *Escherichia coli*. The immunomodulatory effects of oligosaccharides are also noteworthy. Our ongoing research focuses on optimizing prebiotic use through various strategies, including *in ovo* administration and combination with other feed additives, such as enzymes and probiotics. These approaches aim to enhance further the efficacy of prebiotics in promoting gut health and reducing reliance on antibiotics in poultry production. Despite these promising findings, the efficacy of prebiotics can vary based on factors such as dosage and degree of polymerization, poultry age, and dietary composition. Therefore, further research is essential to optimize supplementation strategies and fully elucidate the mechanisms underlying their beneficial effects.

Keywords: prebiotics, poultry, intestinal health, immune response, microbiota

26 Managing egg size in broiler breeders through nutritional manipulation

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In broiler breeders, egg weight plays an important role. Initially the egg weight is around 50 gm but at the end of production cycle it tends to cross 73 gm. If egg size exceeds the threshold of 70 gm it can be considered a problem by both farm and hatchery managers. Large eggs tend to have thinner shells and poorer shell quality and as a consequence higher incidence of cracks and rejected eggs. Hatchery managers may also notice an increase in contamination rate and the number of shell cracks due to larger eggs that do not properly fit into standard setter trays. Oversized eggs could also force hatchery managers to use non standard setter trays containing fewer eggs and a result hatchery capacity would be reduced having a possible negative economic impact. Reduction in dietary crude protein, methionine and linoleic acid is often used to control the egg size. But these measures are producing mixed results. So this study aimed to investigate how the further manipulation of crude protein, methionine and corn can control the egg size. A study of 294 days duration was carried out on vencobb 430 hens to assess the effect of partial substitution of corn with broken rice and gradual reduction of crude protein and dietary methionine on egg weight. Total 10000 numbers of vencobb 430 hens were divided into 2 groups T1 and T2. Each group consisted of 5000 hens. The trial started at 23 rd week and ended at 66th week. T1 was kept as control and was fed standard recommended. In T2 corn was partially substituted with broken rice and dietary crude protein and methionine was gradually but significantly reduced compared with T1. Result showed that while the performance parameters like no of hatching eggs per bird remained same in both the groups the egg weight in T2 was considerably lesser than the T1.

Keywords: broiler breeders, Hen Day production, hatching eggs selection

27 Evaluation of near-infrared calibration on nutritional composition and processing conditions in mechanical-extracted soybean meal

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Mechanical-extracted soybean meal (MESBM) is a commonly used feed ingredient in poultry diets, known for its high protein and fat content. It is produced by extracting oil from raw soybeans through processes such as conditioning, expanding, and expelling. MESBM typically exhibits great variability in nutrient composition, levels of antinutritional factors, and degree of heat damage. This variability can lead to over- or under-formulation of feed, potentially compromising poultry performance. Near-infrared (NIR) calibration offers a rapid alternative for assessing the nutritional composition and processing conditions of soybean products, including MESBM, compared to traditional wet chemistry analysis. This study compares the analytical results of proximate composition, trypsin inhibitor (TI), and KOH protein solubility (KOHPS) obtained from NIR predictions with those from wet chemistry analysis. The hypothesis posits that the analytical results from NIR predictions will not differ significantly from those obtained through wet chemistry. In Experiment 1, 20 MESBM samples were collected from various production batches at a soybean meal crushing facility. These samples were analyzed using Evonik's NIR calibration (AMINONIR® Prox + RED, Evonik Operations GmbH) for moisture, crude protein, crude fat, TI, and KOHPS. Parallel analyses were conducted at two commercial laboratories for the same parameters. In Experiment 2, 11 samples, comprising raw soybean meal, Intermediate product, and finished product samples, were collected and analyzed using NIR and wet chemistry for the same parameters as in Experiment 1. Results for moisture, crude protein, crude fat, and KOHPS from both experiments were statistically analyzed using one-way ANOVA and T-tests in JMP 17. Correlations between different methods for TI were also examined. In Experiment 1, no significant differences were found in moisture, crude protein, and KOHPS. However, crude fat levels from NIR analysis were significantly higher than those reported by the two commercial labs ($P<0.05$). In Experiment 2, no significant differences were observed in moisture, crude protein, crude fat, and KOHPS between NIR and wet chemistry methods ($P<0.05$). A strong correlation was found between NIR and wet chemistry results for TI when combining data from both experiments. These findings indicate that AMINONIR® Prox + RED predictions for proximate analysis, TI, and KOHPS are reliable and can be effectively utilized to assess the nutritional value and quality of MESBM.

Keywords: near-infrared spectroscopy, mechanical-extracted soybean meal, trypsin inhibitor activity, heat damage, proximate analysis

28 Comparison of serum bile acid composition among different species and the effect of taurochenodeoxycholic acid on regulating abdominal fat deposition in broilers

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Bile acids (BAs)are major regulators of host lipid homeostasis. It was reported that hyocholic acid exhibited strong effects on glucose regulation and had exceptional resistance to type 2 diabetes. We speculate there might be a specific bile acid in chickens that could develop a special function. Therefore, serum BAs compositions were compared among different species. The results indicated that the serum BA profile in chicken is relatively simplistic, with taurochenodeoxycholic acid (TCDCA) being the most abundant component in laying hens (92%) and broilers (88%). The predominant BAs in goat were cholic acid (CA, 57%), deoxycholic acid (DCA, 13%) and taurocholic acid (TCA, 10%), while the BAs pool in mice is characterized by various forms of CA, with lithocholic acid (LCA) being the most prevalent (20%). In the human BAs pool, glychenodeoxycholic acid (GCDCA) and LCA represented the highest proportions (both about 25%). RNA-seq from the NCBI database revealed that CYP8B1 expression seemed to be deficient in chicken when compared with goat, pig, mouse and humans. Western blot results showed that BAs synthase expression in the alternative pathway was higher than that in the classic pathway in chicken, while the opposite phenomenon was found in goat, pig and mouse. Considering TCDCA as the most abundant BAs in chicken, experiments were carried out *in vivo* and *in vitro* to evaluate the effect of TCDCA on lipid deposition in broilers. Results showed that 4, 8 and 12 μM TCDCA addition could promote oile acid-induced cell differentiation and increase triglyceride contents in immortalized chicken preadipocytes after 12 h treatment. 200 mg TCDCA daily perfusion during 14 to 28 days significantly increases abdominal fat percentage in broilers at 28 days as well as serum TG, TC and total BAs levels. Serum TCDCA level was higher in the TCDCA group, while TCA level was lower. In addition, hepatic CYP7A1, CYP27A1, BAAT and BSEP mRNA abundance were increased in TCDCA group. Gene expression in abdominal fat tissue related to lipid biosynthesis was improved in TCDCA group, such as SREBP, C/EBPα and ELOVL6; while ATGL and CPT1 mRNA expression was down-regulated. TCDCA perfusion also down-regulated FXR, VDR, SHP and FGF19 expression in abdominal fat tissue. On the other hand, TCDCA perfusion decreased Shannon and Simpson indexes and increased the ratio of *Firmicutes*/*Bacteroidetes* in cecal microbiota. In conclusion, most proportion of TCDCA in chicken might be the result from CYP8B1 deficiency. Our findings indicated that TCDCA could promote lipid deposition, which might be the reason for the susceptibility to lipid metabolism disorders in chicken.

Keywords: broilers, lipid metabolism, gut microbiota, bile acids

29 Comparative study of pelleted diet with corn meal vs corn grit on growth performance, gizzard and intestinal development in white-feathered broilers

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This experiment was conducted to comparative study of pelleted diet with corn grit vs corn meal on growth performance, gizzard and intestinal development in white-feathered broilers. A total of 1,152 fifteen-day-old Cobb broilers with the body weight about 524 grams were randomly divided into 2 groups, with 12 replicates per group, and 48 broilers per replicate (half male and half female), and the trial lasted for 24 days. From 15 to 38 days of age, broilers were fed pelleted diet containing corn meal (1.8-2.0 mm sieve aperture) and corn grit (6-8 mm sieve aperture), respectively. At 38 days of age, growth performance, gizzard weight, and small intestine length and weight were measured. The results showed that: Corn grit pelleted diet significantly increased average daily gain (ADG) ($P<0.05$) and reduced feed to gain ratio (F/G) ($P<0.05$) during 15-21 days of age. No significant effects on growth performance were observed during 22-28 days. However, during 29-38 days, the corn grit pelleted diet significantly decreased F/G ($P<0.05$). Throughout the entire experimental period, the corn grit pelleted diet showed no significant effects on body weight, ADG and average daily feed intake (ADFI) ($P > 0.05$). However, it significantly improved F/G ($P<0.05$) during the 15-38 day period. In addition, the corn grit pelleted diet significantly increased gizzard weight ($P<0.05$) and gizzard index ($P<0.05$), while reducing gizzard lesions ($P<0.05$), but had no significant effects on small intestinal length, relative small intestinal length, or small intestinal weight ($P > 0.05$). The results from the commercial-scale feeding trial (N=32,000 Ross 308 broilers, two group, with 3 replicates per group) demonstrated that the corn grit pelleted diet significantly increased final body weight, ADFI, and ADG of broilers ($P<0.05$), while markedly reducing the F/G ($P<0.05$). Furthermore, the corn grit pelleted diet significantly increased gizzard weight ($P<0.05$) and showed a tendency to enhance the gizzard index（$P=0.065$）. However, it resulted in a significant decrease in both small intestinal length and relative small intestinal length ($P<0.05$). In conclusion: the corn grit pelleted diet primarily enhances nutrient digestibility by promoting gizzard development, thereby reducing the F/G in broilers.

Keywords: FCR, ADG, Corn Grit, Corn meal

Student Competition: Processing, Products, Meat and Egg Quality

30 Hydroxy-methionine manganese supplementation improved eggshell quality and laying performance in laying hens with translucent eggs

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Translucent eggs, a common defect in commercial egg production, negatively impact eggshell quality and diminish consumer purchasing preference. Nutritional intervention represents an effective strategy for improving egg quality. This study investigated the effects of hydroxy-methionine manganese (MnHMet) on laying performance, egg quality, eggshell quality, and shell gland function in hens with translucent eggs. A total of 400 42-week-old Lohmann pink laying hens were randomly assigned to individual cages and provide with a standardized diet. Eggs were collected daily for 2 consecutive weeks and subjected to translucency scoring by using standardized grading criteria (0 = clear, 5 = severe opaqueness). Subsequently, 120 hens were selected and divided into 3 treatment groups: negative control (NC, score = 0.87), positive control (PC, score = 2.87), and MnHMet group (score = 2.73, basal diet + 60 mg/kg MnHMet + 60 mg/kg $MnSO_4$,). Each treatment had 10 replicates (3 hens per replicate), and the trial was conducted for 12 weeks under standardized management conditions. Data were analyzed using one-way ANOVA and Turkey's multiple comparison test in SAS 9.2. Results showed that MnHMet supplementation significantly reduced translucency score compared to the PC group ($P < 0.05$). The PC group showed significant decreases in laying rate and egg mass, along with increase in feed conversion ratio ($P < 0.05$). Notably, dietary MnHMet significantly improved laying performance ($P < 0.05$). Compared with PC group, the eggshell strength was lower in NC and MnHMet groups ($P < 0.05$). Eggshell quality further analysis revealed that eggshells from the NC and MnHMet groups exhibited significantly lower moisture content and mammillary layer thickness but higher calcium content ($P < 0.05$). Shell gland from MnHMet groups showed significantly increased levels of glutathione (GSH), glutathione s-gransferase (GST) and total antioxidant capacity (T-AOC), while malondialdehyde (MDA) content was significantly decreased ($P < 0.05$). Moreover, dietary supplementation with MnHMet significantly increased the expression levels of calcium transportation-related genes (*Carbonic Anhydrase* and *CaBP-D28k*) of shell gland in hens with translucent eggs ($P < 0.05$). Additionally, metabolomic analyses highlighted amino acid metabolism and ABC transporter pathways as the primary distinct metabolic pathways between PC and MnHMet groups. In summary, dietary supplementation with MnHMet effectively improved eggshell quality and laying performance in hens with translucent eggs by enhancing shell gland function.

Keywords: laying hens, translucent eggs, eggshell quality, MnHMet, shell gland

31 Astaxanthin: King of carotenoids pigment preserve frizzle chicken meat quality under heat stress

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Global climate change has intensified heat stress (HS), negatively affecting poultry performance, immune function, and meat quality. HS disrupts water retention, texture, and oxidative stability, leading to economic losses. Astaxanthin (AST), a red carotenoid pigment with antioxidative and anti-inflammatory properties, may enhance poultry resilience under HS conditions. This study evaluates AST's effects on Frizzle chicken meat quality, focusing on antioxidant defense, muscle fiber integrity, and inflammation regulation. Three Yellow curly feathered female chickens were assigned to five experimental groups(A–E). Group A was thermoneutral control group, while Groups B, C,D and E experienced HS (32°C ± 1°C) for two weeks. AST supplementation was provided at 20 mg/kg, 40 mg/kg, and 80 mg/kg to Groups C, D, and E, respectively. Meat quality parameters such as pH, color, shear force, cooking loss, and drip loss were analyzed alongside texture profile assessments. Muscle fiber integrity was examined via hematoxylin-eosin staining, and gene expression related to oxidative stress, lipid metabolism, and inflammation (SOD, CAT, GPX, FAS, ACC, PPAR-α, NF-κB, MyD88, TLR-4, IL-6, TNF-α) was quantified using RT-qPCR. The results showed that HS significantly increased shear force and cooking loss, negatively affecting meat texture, particularly in Group B. Group D (AST 40 mg/kg) exhibited improved shear force and moisture retention, enhancing tenderness compared to the heat-stressed control. No significant variations were observed in pH and drip loss among groups, though Group E showed a slight increase in cohesiveness. Muscle fiber analysis revealed notable atrophy in Group B with reduced cross-sectional area compared to Group A. Group D demonstrated restored fiber structure, reinforcing AST's muscle-protective effects. AST supplementation significantly modulated gene expression under HS conditions. Group D exhibited upregulated antioxidant enzyme activity in muscle and liver, with increased GPX and SOD expression, indicating improved oxidative defense. Heightened inflammatory responses in Group B were mitigated in Group D, particularly through modulation of NF-κB and TLR-4 expression. Additionally, AST supplementation in Group D normalized lipid metabolism genes, preventing excessive fat accumulation while maintaining meat quality. These findings suggest that AST supplementation at 40 mg/kg effectively mitigates HS-related impacts, improving antioxidant defense, regulating fat metabolism, and suppressing inflammatory responses. This study provides valuable insights into enhancing poultry resilience through dietary interventions, offering strategies for optimizing feed additives under heat stress conditions.

Keywords: Climate change, Heat stress, Astaxanthin, Meat quality, Poultry

32 In ovo taurine feeding: enhancing broiler resilience to cyclic heat stress at market age

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The rise in global temperatures each year has made poultry production even more challenging, as heat stress is a major menace to the poultry industry. In ovo feeding could be a cheap and effective way to produce chicks with inherently improved heat tolerance. Taurine is a sulphur-containing amino acid which could reduce reactive oxygen species generation and improve the growth and production of broilers. Hypothesising that in ovo feeding of taurine will mitigate detrimental effects of heat stress, this study was designed to determine the effects of in ovo taurine on epigenetic modifications and antioxidant enzyme functions in broilers. Fertile eggs (n = 350) were divided into four treatment groups according to the concentration of taurine injected in ovo (0.6 mL of 0%, 1%, 3% or 5% taurine) at embryonic day 17.5. The chicks hatched were reared under standard temperature guidelines until day 28. During the 5th week of rearing, birds were either raised at standard temperature (NT) or subjected to cyclic heat stress (HS) at 31°C ± 1°C for 6 hours per day and grouped as: (a) 0TAU-NT; (b) 0TAU-HS; (c) 1TAU-HS; (d) 3TAU-HS; and (e) 5TAU-HS. At the end of the HS period, 6 birds per group were slaughtered to collect liver and blood samples. Growth parameters and rectal temperature (RT) were recorded, plasma antioxidant status was estimated, and hepatic gene expression was studied for several antioxidant, DNA-methylation, and demethylation-related genes. Data was analysed using one-way ANOVA, Pearson's correlation, and polynomial regression using IBM SPSS statistics software. Multivariate analysis of variance (MANOVA) and planned contrast were performed in SAS software. In ovo taurine improved the average daily gain (ADG) of birds under HS linearly ($p = 0.032$). Final RT ($p = 0.032$) and plasma malondialdehyde content ($p = 0.001$) were higher in 0TAU-HS, 1TAU-HS and 5TAU-HS compared to 0TAU-NT, while 3TAU-HS was statistically similar to 0TAU-NT. Further, the free radical scavenging capacity of plasma (DPPH-RSA%) was significantly reduced in the 0TAU-HS and 1TAU-HS groups compared to others ($p = 0.001$). The NADPH-oxidases (NOX1 and NOX4) were positively correlated with the expression of DNA-demethylation-related genes ($p < 0.05$). Nuclear factor erythroid 2 (NRF2) was found to be significantly correlated with the epigenetics-related genes ($p < 0.05$). MANOVA revealed that DNA-demethylation-related genes were upregulated in 5TAU-HS compared to 0TAU-NT ($p = 0.030$).

Keywords: in ovo feeding, taurine, heat stress, broilers, DNA-demethylation

33 Not Presented

34 Genomic profiling reveals the role of HOXA13 in inhibiting adipocytes proliferation and differentiation of chicken

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Excessive accumulation of abdominal fat tissue (AFT) in broilers poses a significant industrial challenge, making it imperative to elucidate the mechanisms underlying abdominal fat deposition. In this study, 12 broilers were randomly selected to be euthanized for sampling abdominal fat tissue at D3, D14, D28, and D42. The immortalized chicken preadipocytes (ICP2) were induced differentiation with 200 μM oleic acid for 24 hours to collect samples. RNA-seq, ATAC-seq, ChIP-seq, and IP-MS methods were employed to investigate the epigenetic mechanisms of AFT deposition aiming to excavate the key regulatory factors. Results from RNA-seq and ATAC-seq of AFT between D3 and D14 showed that HOXA13 was overlapped between differential genes and DNA motifs derived from differential peaks. The protein levels of HOXA13 decreased with age in AFT, as well as oleic acid-induced ICP2. ATAC-seq results for ICP2 showed that HOXA13 was predicted based on DNA motif analysis of differential peaks. Moreover, HOXA13 possesses multiple predicted binding sites within the promoter regions of lipid metabolism-related genes, including PPARγ, CPT1A, FABP4, and PPARα. Interference with 70% of HOXA13 significantly increased triglyceride content and lipid droplet deposition in oleic acid-induced ICP2, and upregulated the C/EBPα and FABP4 protein levels, while inhibited CPT1A protein abundance. Conversely, an opposite phenomenon was found when HOXA13 was overexpressed. Furtherly, flag-HOXA13 labeled ChIP-seq analysis identified 1,978 gain and 478 loss peaks in oleic acid-induced ICP2 when compared to the control, corresponding to 1,756 gain and 454 loss genes. The combined results of ATAC-seq and ChIP-seq revealed that chromatin accessibility and HOXA13 binding were enhanced in the promoter or the gene body regions of ACSL4, CPT1A, and MGLL genes. HOXA13 labeled IP-MS suggested that HOXA13 may interact with USP1, MAP4K4, CPT1A, MYO1C, and MYOM3 proteins. Collectively, this study demonstrates that HOXA13 might function as a repressor, inhibiting abdominal adipogenesis through transcriptional and epigenetic remodeling mechanisms.

Keywords: broiler, abdominal fat, HOXA13, epigenetic mechanism

35 Elucidating the role of DMRT1 in germ cell differentiation in chickens

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Birds have a unique sex determination system, with males being homogametic (ZZ) and females heterogametic (ZW). In chickens, recent studies have shown that the expression level of the Z-linked transcription factor Doublesex and Mab-3 Related Transcription Factor 1 (DMRT1) regulates gonadal sex and somatic masculinization. However, the function of DMRT1 in germ cells remains unknown, and this is a major obstacle to understanding avian sex differentiation mechanisms. In this study, we focused on the role of DMRT1 in germ cells and performed in vivo analysis of the differentiation of DMRT1 knockout primordial germ cells (PGCs) knocked out by genome editing. Fluorescently labeled DMRT1-KO or normal PGCs were transplanted into the subgerminal cavity of the blastoderm in chicken embryos. Gonads were collected from embryos on the 14th day of incubation, when PGCs differentiate into spermatogonia. Germ cells were sorted from the gonads using FACS and quantified. RNA-seq analysis was subsequently performed on the sorted cells. Cell count analysis revealed a significant reduction in the number of DMRT1-KO PGCs compared to normal PGCs ($p < 0.01$). RNA-seq analysis identified approximately 2,500 differentially expressed genes (DEGs), including downregulation of some growth factors (e.g., EGF, BMP4) and upregulation of pluripotency markers such as Nanog and SOX2 ($\log FC > 3$, $p < 0.05$). Gene ontology analysis of DEGs revealed significant enrichment of genes related to signal transduction pathways ($p < 0.05$). These results indicate that DMRT1 deficiency impaired proliferation of PGCs and impaired differentiation into spermatogonia. It also appears that cytokines and transcription factors that control differentiation are involved. These findings suggest that DMRT1 may function as an important regulator of germ cell differentiation in chickens. In this study, we analyzed the function of DMRT1 in the process of germ cell differentiation of chicken PGCs. As a result, it was revealed that the deletion of DMRT1 in PGCs affected their proliferation and differentiation. These results indicate that DMRT1 plays a role as a key regulator of germ cell differentiation in chickens.

Keywords: Chicken, Germ cell, Development, Genome editing, Sex differentiation

36 Effect of FABP4 gene on intramuscular fat deposition in Muscovy duck in vitro and in vivo

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Intramuscular fat (IMF) consists of lipids deposited within muscle tissue. Increasing IMF content is a key strategy for enhancing meat quality. Fatty acid-binding protein 4 (*FABP4*) regulates IMF metabolism by reversibly binding to fatty acids, playing a crucial role in fat deposition. This study aimed to analyze the temporal expression patterns of IMF content and *FABP4* gene mRNA expression in Muscovy ducks at the individual level, as well as to evaluate the role of *FABP4* in lipid accumulation within primary muscle cells at the cellular level. Healthy Muscovy duck hens aged 20, 40, 60, and 80 days, along with fertilized embryos, were collected from breeding farms at Hainan Chuanwei Muscovy Duck Breeding Company. Two muscle tissue samples were obtained from ducks at each growth stage. One sample was used to prepare oil red O frozen sections for IMF detection, with quantitative lipid analysis performed using ImageJ software. The second sample was used for RT-qPCR analysis to measure relative *FABP4* mRNA expression, using GAPDH as a reference gene. Primary myogenic cells were isolated from 17-day-old embryos to develop an oleic acid-induced lipid deposition model. The constructed

pCDNA-3.1-FABP4 plasmid was transfected into cells to establish an *FABP4* overexpression (OE) group, while pCDNA-3.1 served as the negative control (NC) group. After four days of lipid induction, cellular triglycerides, fatty acids, and malonyl-CoA levels were measured using biochemical kits, and expression levels of lipid metabolism-related genes (*PPARγ, FASN, FABP4, GPAT, PLIN1*) were analyzed via RT-qPCR. Data were processed using SPSS 26.0 and Graphpad Prism 9.0, and statistical significance was set at $P < 0.05$. The results indicated that *FABP4* mRNA expression and IMF content in leg muscle increased significantly with age ($P < 0.05$) at the individual level Whereas FABP4 promote fatty acid and triglyceride accumulation ($P < 0.05$) at the cellular level, but did not affect malonyl-CoA levels. RT-qPCR results showed significantly upregulated expression of *FASN, GPAT, FABP4,* and *PPARγ* ($P < 0.001$). This study suggested that *FABP4* expression in duck leg muscle is positively correlated with IMF deposition, increasing with age. Additionally, *FABP4* enhances lipid accumulation in muscle cells without influencing the de novo lipid synthesis pathway. As *FABP4* is predominantly expressed and secreted by adipocytes, this study provides a foundation for understanding myocyte–adipocyte interactions in IMF regulation.

Keywords: Muscovy duck, Intramuscular Fat, FABP4, Lipid Deposition

37 Construction and mechanism analysis of the sex reversal model of chicken gonadal somatic cells

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The unclear regulatory mechanisms of avian sex determination impede poultry sex control technology development, challenging efficient production. To address this, we established an in vitro sex reversal model using E18.5 chicken gonadal somatic cells, isolating male (SOX9+) and female (FOXL2+) cells. To validate the reliability of the sex reversal model, we conducted hormonal treatments on the isolated cells. In female cells, a short-term (3 days) exposure to 50 nM fadrozole (FAD) significantly upregulated male markers (SOX9, AMH; $P < 0.001$), while a prolonged (13 days) treatment induced an intersex state with co-expression of male and female genes ($P < 0.01$). In male cells, 50 nM estradiol (E2) induced bisexual characteristics within 3 days ($P < 0.0001$), and 100 nM E2 triggered sex reversal by day 4 (CYP19A1, FOXL2, WNT4; $P < 0.01$), though extended treatment reverted to an intersex phenotype. Flow cytometry analysis confirmed the hormone-induced protein-level changes in sex-related genes, further validating the model's accuracy. Preliminary receptor dynamics analysis showed that in female cells, 50 nM FAD transiently activated ESR1 (0-7 days), inhibited ESR2/AR, and induced AR-dependent male gene expression (8-13 days). In male cells, E2 repressed AR for 14 days and activated ESR1/ESR2 in phases, correlating AR inhibition with male gene peaks. 10x Genomics single-cell sequencing technology with PAGA analysis provided deeper insights. In FAD-treated female cells, granulosa cells transformed into Sertoli cells via interstitial cells, forming a new Sertoli cell cluster enriched in AP-1, PPAR, and Foxo pathways, suggesting PPARG and FOS as key male sex regulators. Female-specific granulosa cell clusters persisted, enriched in Insulin and PPAR pathways. In E2-treated male cells, Sertoli cells transformed into granulosa cells through epithelial or interstitial intermediates, accompanied by a new granulosa cell cluster. Male-specific Sertoli cell clusters remained, with genes enriched in oxidative stress, Jak-stat, and P53 pathways. The discovery of the bidirectional transformation trajectories of gonadal cells provides a novel perspective on the cellular plasticity during sex determination. These findings not only deepen our understanding of the genetic and cellular mechanisms underlying avian sex determination but also offer a solid theoretical basis for constructing a comprehensive sex regulation network. Ultimately, this research paves the way for the development of precise sex control technologies in poultry, with the potential to revolutionize the poultry breeding industry.

Keywords: Chicken gonadal somatic cells, Sex reversal model, Single-cell sequencing, PAGA analysis, Sex determination mechanism

38 Investigation of the molecular genetic mechanisms underlying feed efficiency during the extended laying period in laying hens

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Extending the laying cycle of hens has become a major focus in poultry production and research. However, prolonged laying is often accompanied by declining feed efficiency. This study aims to identify causal variants, genes, and key metabolites associated with feed efficiency in aged laying hens by integrating genomic, multi-tissue transcriptomic, and metabolomic data, providing a theoretical basis for genetic improvement. A total of 248 purebred Rhode Island Red hens were used, provided by Beijing Huadu Yukou Poultry Co., Ltd. Feed intake, body weight, and egg production were recorded at 70–71 and 99–100 weeks. Residual feed intake (RFI) and feed conversion ratio (FCR) were calculated. GWAS was conducted using univariate linear mixed models. eQTL mapping followed the ChickenGTEx pipeline. SMR was applied to GWAS summary data for 70wRFI and 100wRFI to identify candidate genes. TWAS fine-mapping was performed using the GIFT method. GSMR was used to explore causal relationships between phenotypes and metabolites. Estimated heritabilities for 70wRFI and 100wRFI were 0.32 and 0.24, indicating moderate genetic contribution. Fine-mapping of GWAS data identified credible sets with non-zero effect SNPs: lead SNP rs3384506997 (Chr1:57481513) for 70wRFI and rs316881285 (Chr9:358753) for 100wRFI. SMR identified 45 and 23 candidate genes for 70wRFI and 100wRFI, respectively. TWAS fine-mapping via GIFT yielded smaller causal sets. PCCB was consistently identified in multiple tissues and positively correlated with 100wRFI. In liver and cecal tissues, PCCB co-occurred with GPR17 and NGEF. GSMR revealed a bidirectional causal relationship between hepatic malate levels and 100wRFI. Malate was negatively correlated with 100wRFI and positively with PCCB expression. Feed efficiency is tightly linked to energy metabolism. PCCB encodes the β-subunit of propionyl-CoA carboxylase, which catalyzes propionyl-CoA into methylmalonyl-CoA, feeding into the TCA cycle. Elevated PCCB expression may reflect metabolic stress in aged hens, potentially leading to inefficient energy use and higher feed intake. Malate, a TCA cycle intermediate, may enhance energy production but also indicate compensatory metabolic shifts. The positive correlation between PCCB and malate suggests increased propionate metabolism demands higher malate levels, yet overall metabolic efficiency may still decline, resulting in higher RFI. This study provides insight into the molecular basis of feed efficiency in aged hens.

Keywords: laying hens, late laying period, feed efficiency, genetic variation, multi-omics

39 Investigation of the mechanisms underlying the blood and meat spots formation in chicken eggs by integrating multi-omics data

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In this study, continuous phenotypic tracking throughout the laying period revealed that Rhode Island Red hens exhibited a consistently high incidence of blood and meat spots, while the occurrence in White Leghorn hens remained extremely low. 16S rRNA sequencing of both albumen and yolk demonstrated that microbial diversity in spotted brown-shelled eggs was significantly lower than that in normal brown- and white-shelled eggs. Notably, the microbial compositional differences between spotted and normal brown eggs exceeded those observed between brown and white egg types, suggesting that the presence of spots exerts a stronger influence on the microbiota than breed divergence. Histological examination (H&E staining) revealed breed-specific physiological inflammation in the ovary and magnum of Rhode Island Reds compared to White Leghorns. Subsequent transcriptomic analysis of these tissues identified 1,226 and 828 differentially expressed genes, 201 and 268 differentially expressed lncRNAs, and 60 and 59 differentially expressed miRNAs, respectively. Functional enrichment analysis implicated these transcripts in immune response and inflammatory signaling pathways. Genome-wide association analysis (GWAS) identified a significant locus on chromosome 2 (15.56–16.26 Mb). Integrative analysis with transcriptomic data pinpointed *MKX*, *MASTL*, and *APBB1IP* as key candidate genes within this region. Chromatin accessibility and epigenetic landscape profiling via ATAC-seq, H3K27ac, and H3K27me3 ChIP-seq revealed regulatory signatures associated with transcriptional activation and repression within the candidate region. Hi-C-based 3D genome analysis further identified compartment switching and altered TAD boundaries in this locus, aligning with observed gene expression dynamics. Together, these results highlight a coordinated multi-layered regulatory mechanism underpinning the formation of blood and meat spots in eggs and provide potential targets for genetic improvement in laying hens.

Keywords: Hen, Egg quality, Blood and meat spot eggs, Multi-Omics

40 Not Presented

41 Sequence identification, physiological function and molecular mechanism of chicken leptin

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Leptin, as a key appetite-regulating factor, has been extensively studied in mammals. However, due to limitations in sequencing technologies and the high GC content of avian leptin sequences, the complete leptin sequence and its functions in birds have remained insufficiently characterized over the past two decades. Chicken is the most populous avian specie and a major human protein source, therefore, clarifying the functions of chicken leptin is crucially important for advancing biological research and improving chicken breeding. In this study, we determined the full chicken leptin coding sequence using high-depth sequencing and PCR. Protein docking by Z-dock and co-IP experiments in DF-1 cells validated the biological function of chicken leptin to bind its receptor (LEPR). Tissue expression analysis in 6-week-old White Leghorn and Cornish roosters showed high leptin expression in the central nervous system (CNS). We generated primary neural cells from chicken embryos with leptin overexpression and performed transcriptome sequencing, the results showed that differentially expressed genes were primarily enriched in fatty acid metabolism pathways. To clarify whether chicken leptin directly participates in appetite regulation and energy metabolism, we conducted fasting-refeed experiments using 2-week-old White Leghorn roosters and WOD 188 commercial male broilers. The results from both broiler and layer chickens consistently showed that leptin expression levels in the hypothalamus decreased during feed deprivation and increased during feed rehabilitation. Finally, to directly validate the physiological function of leptin in vivo, we generated leptin-overexpressing transgenic chickens using lentiviral vectors. At 10 weeks of age, the body weight of leptin-overexpressing transgenic chickens were significantly lower than those of the control group ($p<0.05$). Lipidomics analysis showed that compared with the control group, differential lipids in leptin-overexpressing transgenic chickens were mainly enriched in pathways related to fatty acid synthesis. In conclusion, this study identifies the complete coding sequence of chicken leptin, confirms its concentrated expression in the central nervous system, and validates the important role of chicken leptin in chicken growth and energy metabolism through overexpression experiments at both cellular and individual levels.

Keywords: chicken, leptin, leptin receptor, appetite, growth

42 Chicken LRP1 protein mediates the in vitro infection process of Marek's disease virus

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Chicken Marek's disease (MD) is one of the key diseases to be prevented and controlled in the poultry industry, which is caused by chicken Marek's disease virus (MDV) infection. The process by which Marek's disease virus infects the host has not been clearly defined. The aim of the present study was to screen and identify host genes that play an important role in the early stages of viral infection. This assay was developed by comparing the in vitro MDV infectivity of primary chicken embryo fibroblast CEF and chicken embryo fibroblast cell lines, followed by proteomic screening for candidate proteins, and then an in vitro assay to determine if there is an interaction between the candidate protein and the viral protein. The cells used in this experiment included

CEF and DF1. MDV weakly recombinant fluorescent strain 814-GFP was obtained from the Harbin Institute of Veterinary Medicine, Chinese Academy of Agricultural Sciences. Screening of candidate proteins by proteomics. Infection rates were identified by flow cytometry. The knockdown assays were performed using siRNA and ShRNA strategies. Q-PCR and WB were used to analyse gene expression and IP-MS was used to identify interacting proteins. Co-expression and co-IP was used to verify the interaction. The trail data were analyzed using student's t test or one-way ANOVA. P value ≤0.05 means statistically significant difference, P value ≤0.01 means statistically highly significant difference. DF1 and CEF cells showed highly significant differences in MDV infection rates, and LRP1 was screened as a candidate protein through proteomics. After knocking down LRP1 in vitro, the infection rate of MDV was significantly reduced (31.9%_vs_3.01%). IP-MS results suggested that LRP1 potentially interacts with the viral glycoprotein gE, which has been reported to be closely related to MDV infection in vitro. LRP1 consists of four structural domains, and the expression vector was constructed by truncating these four structural domains and then co-expressed with the gE overexpression vector. The co-expression results showed that domains 3 and 4 interact with gE. The results show that domains 3 and 4 interact with gE. In this study, we identified a candidate protein LRP1 associated with early viral infection, which significantly down-regulated viral infection after in vitro knockdown and interacted with viral glyproteins gE, which is a viral glycoprotein directly associated with viral infection, suggesting that LRP1 mediates the process of viral infection, and further experiments are needed to verify the functional mechanism of LRP1 in viral infection.

Keywords: Marek's disease, Vitro infection, Proteomics, LRP1, Viral glyprotein

Processing, Products, Meat and Egg Quality

43 Combination of Hot Water Spray (HWS) and Subzero Saline Chilling (SSC) improved chilling efficiency, bacterial decontamination, and meat tenderness of broiler carcasses

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Poultry became the world's most consumed meat in 2019 as consumers recognize poultry products are nutritious, healthy, tasty, and affordable. Despite these advantages, the poultry industry continues to face significant challenges related to food-borne pathogens and meat toughness – both of which reduce food safety and product quality. These issues are particularly exacerbated when boiler carcasses are poorly chilled. Over the last ten years, our laboratory has developed a subzero saline chilling (SSC) technique that significantly enhances chilling efficiency, microbial safety, meat tenderness, and sensory attributes. More recently, combining SSC with hot water spray (HWS) has further improved outcomes, achieving greater reductions in microbial populations and blood/gut contamination compared to traditional water immersion chilling (WIC) or SSC alone. The combination of SSC/HWS creates a hostile environment for bacteria by applying a sequence of heat shock (from hot water), cold shock (from subzero temperatures), and saline shock (from the salt solution), effectively reducing bacterial activity and attachment to carcass surfaces. Additionally, subzero saline chilling minimizes exposure of broiler carcasses to the temperature ranges that induce rigor and cold shortening, thereby maintaining tenderness and allowing deboning earlier. Given the industry's increasing demand for cut-up parts and further-processed meats – often requiring deboning within 2 hours postmortem – SSC combined with HWS offers a promising solution. This approach not only improves chilling efficiency and meat quality but may also reduce the need for chemical antimicrobials such as peracetic acid or chlorine in chilling systems.

Keywords: sub-zero saline chilling, hot water spray, chilling efficiency, bacterial decontamination, meat tenderness

44 The role of bacteriophages to control Salmonella in poultry meat

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According to the WHO, unsafe foods cause annually 600 million cases of foodborne outbreaks and more than 400,000 deaths, with South-East Asia the second most affected region after Africa. Salmonella in poultry meat is often incriminated, due to contamination on farms, during processing as well during meal preparation. The presence of Salmonella on meat products is also a trade barrier, restricting the potential export of Thai products to Western countries and Japan. Bacteriophages, as natural killers of bacteria, may be a sustainable option to eliminate the contamination of poultry meat and reduce the prevalence of bacterial foodborne diseases. A cocktail of phages, previously identified for its activity against the main Salmonella serovars was used in a series of tests to investigate its efficacy at 2 x 10^5 PFU/ml to control Salmonella (S. enteritidis, S. typhimurium and S. Agona) on various chicken parts (mixed, boneless leg and skinless breast parts). Chicken parts, either spiked with a combination of Salmonella serovars at a rate of 10^3 CFU/kg or left uncontaminated, were kept at room temperature for 30 mins before applying 50 ml of the phage cocktail. After 1 hour at 4C, viable Salmonella titers were reduced by 96% (mixed parts), 84.7% (boneless leg) and 86.5% (skinless breast). Other studies, investigating the same cocktail applied at 10^7 PFU/ml, demonstrated excellent activity against the top serovars of concern in Europe, a key export market for Thai poultry processors (S. enteritidis, S. Hadar, S. infantis, S. typhimurium and S. Virchow at 10^4 CFU/g). Spiked chicken parts, treated with the phage cocktail or left as control, were kept in MAP at 4C for 3 days. The bacterial load was reduced by 1 Log after 24 hours and by 4 Log after 48 hours. A meat sensory quality, using the 9-point Hedonic scale, confirmed similar organoleptic characteristics between phage-treated and untreated meat parts. A third series of studies, using a dipping method, evaluated the efficacy of the phage cocktail on various meat parts spiked with S. enteritidis (10^3 CFU/25 g). Samples were kept either at 4C or -20C for up to 5 days and analyzed for the presence of Salmonella. While untreated spiked meat parts were all positive at each sampling time (3 hours, Day 2 and Day 5), S. enteritidis was not detected at any time in any of phage-treated meat parts samples. In addition to the treatment with phages of live birds, the application of phages in processing plants, using dipping or spraying techniques, reduces significantly the presence of Salmonella on meat products. With no effect on sensory evaluation, the use of bacteriophages creates an opportunity to increase the export of fresh meat products from Thailand.

Keywords: Bacteriophages, Poultry meat, Salmonella, Food safety, Meat processing

45 Hyaluronic acid and chondroitin sulfate influence on calcium carbonate crystallization

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During the entire process of biological mineralization, organic matrices play a very important role. Thus, they control the microstructure and properties of inorganic mineral phases. Shell calcification is the most rapid form of biological mineralization in nature. It is essentially the phenomenon where calcium ions and bicarbonate ions in the eggshell gland deposit calcium carbonate crystals in a regular pattern through the eggshell membrane as a carrier. The entire process is regulated by many organic components in the uterine fluid. This experiment aims to study the effects of the organic matrices hyaluronic acid and chondroitin sulfate on the crystallization behavior of calcium carbonate under in vitro conditions. The gas diffusion method was used to simulate the conditions for calcite formation in vitro, and 32 and 64 μg/ml of hyaluronic acid, chondroitin sulfate, and their combined treatment solutions were added to the reaction solution. The reaction was conducted for 1, 3, and 12 hours, and the products were analyzed using scanning electron microscopy (SEM) and X-ray powder diffraction (XRD). The results show: 1) Short-term treatment with hyaluronic acid can reduce the amount of CaCO3 formation, while long-term treatment can increase the particle size of CaCO3, but there is no concentration-dose effect. 2) The treatment with chondroitin sulfate has no significant effect on the particle size of CaCO3; however, it can change the morphology of CaCO3, making the calcite edges less distinct, and has a concentration-dose effect. The higher the chondroitin sulfate concentration, the rounder the calcite edges. 3) The combined treatment of hyaluronic acid and chondroitin sulfate can superimpose their effects, changing the morphology of CaCO3 while promoting an increase in particle size. 4) Hyaluronic acid, chondroitin sulfate, and their combined treatment will all induce amorphous calcium carbonate to directly transform into calcite without forming other crystal forms of CaCO3. Thus, hyaluronic acid and chondroitin sulfate have a very significant impact on the morphology and crystal form of CaCO3 crystals. This research result is helpful for understanding the mechanism of the influence of organic matrices hyaluronic acid and chondroitin sulfate on calcium carbonate crystallization in the uterine fluid and provides new research ideas for regulating eggshell quality.

Keywords: Eggshell

46 Photoclick chemistry-enabled synthesis of silver-based bimetallic nanoparticles for multifunctional sensing applications in food quality monitoring

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This study explores the synthesis of silver-based bimetallic nanoparticles (Ag-Bi NPs) using photoclick chemistry, leveraging edible tyrosine as both a photoinducer and surface ligand. The synthesis process is green, efficient, and safe, aligning with sustainable development goals. Tyrosine, a natural amino acid, serves dual roles: as a photoinducer to initiate the reaction under visible light and as a surface ligand to stabilize the nanoparticles. This method eliminates the need for toxic reducing agents and high-temperature conditions, offering a simple, one-step synthesis process that is both environmentally friendly and cost-effective. The synthesized nanoparticles, including Ag-Cu, Ag-Au, and Ag-Pd NPs, were characterized using TEM, infrared spectroscopy, particle size, and zeta potential measurements, confirming their successful fabrication and distinct properties. The Ag-Bi NPs were applied in the freshness monitoring of various meat products (pork, beef, mutton, lobster, and fish) and the detection of phosphorus-containing pesticide residues in fruits and vegetables, as well as alcohol content detection. For instance, in chicken samples stored at 4°C, the Ag-Cu NPs detected a significant increase in TVB-N levels (exceeding 15 mg/100g) within 6 days, indicating spoilage. Notably, the detection time was accelerated to 4 days when the storage temperature was raised to 10°C, highlighting the temperature-dependent sensitivity of Ag-Cu NPs in spoilage detection. In the detection of pesticide residues, the Ag-Au NPs showed a linear response with a detection limit of 0.1 ppm for glyphosate in spinach. The nanoparticles exhibited high sensitivity and selectivity, with response times under 10 minutes in most applications. The photoclick chemistry approach ensures a green, efficient, and safe synthesis process, aligning with sustainable development goals. This research demonstrates the potential of Ag-Bi NPs as versatile sensing materials for food quality monitoring, offering rapid, accurate, and on-site detection capabilities. Future work will focus on optimizing nanoparticle formulations and integrating them into smart packaging systems for broader industrial applications.

Keywords: Photoclick Chemistry, Silver-Based Bimetallic Nanoparticles, Food Quality Monitoring, Freshness Detection

Metabolism and Nutrition: Amino Acids, Vitamins and Minerals I

47 Ca & P nutrition as influenced by vitamin D

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Calcium and phosphorus each play numerous critical physiological roles in the body, but quantitatively, are most important in bone structure. Bone acts as a reserve of Ca and P to maintain homeostasis. The intestinal absorption of both minerals can take place via regulated, transcellular pathways at low levels of intake relative to the requirements, and via unregulated, paracellular pathways at high levels of intake relative to the requirements. Because the vast majority of Ca and P is found in the bone, dietary requirements of growing birds and non-egg-producing mature birds reflect the composition of bone mineral, which is mainly made up of hydroxyapatite (Ca 10 (PO 4) 6 (OH) 2). The ratio is heavily skewed towards calcium in egg-laying birds, reflecting the large amount of additional Ca required to support eggshell formation. The plasma level of each mineral is highly regulated primarily through the action of parathyroid hormone and vitamin D. Blood Ca is regulated by balancing uptake from the intestine, reabsorption from the kidney, and deposition or mobilization from the bone. Blood P is regulated primarily through reabsorption from the kidney. Initially, vitamin D was known to be involved in intestinal Ca and P absorption. Since then, it has become clear that vitamin D plays important roles in other physiological functions such as immunity, muscle growth and cell growth regulation. The ability to produce the first metabolite of vitamin D, 25-OH D 3 is limited in very young birds. This is likely due to low lipase and bile salt production in the intestine, resulting in limited uptake of lipid-soluble material including vitamin D, and a limited ability of the liver to convert vitamin D 3 to 25-OH D 3 . Conversely, in older birds, renal conversion of 25-OH D 3 to the active hormone 1α,25(OH) 2 D 3 is limited. Therefore, vitamin D supplementation strategies may change throughout the life of the bird. In addition to the systemic effects of vitamin D mediated through renally-produced 1α,25(OH) 2 D 3, several extra-renal cell types and tissues can produce the active hormone for local use. These local functions can have effects on inflammation and immunity, gut epithelial barrier function, myogenesis, angiogenesis and fertility. The interaction of vitamin D, Ca and P in the bird are critical for not only skeletal health, but many other aspects of poultry health and productivity.

Keywords: broiler chicken, laying hen, vitamin D, calcium, phosphorus

48 The effect of bio-available silicon on layer performance and egg quality

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The requirements of poultry for microminerals are well-established, with their physiological functions and deficiency symptoms profoundly identified and demonstrated. These micro minerals are available in different forms which also determine their bioavailability. At present, there is no known minimum requirement for Silicon, likewise, its deficiency symptoms. Silicon is an essential mineral in the macrostructure of collagen, a structural protein that is present in connective tissues, organs, blood vessels and intestinal lining. Silicon is present in most plant-derived feed materials, but the bioavailability is almost negligible. With an initiative to explore the relevance of Silicon and its bioavailability in mineral nutrition, a four-week feeding trial was conducted to evaluate the effect of a novel bio-available Silicon (bio-available Si, Poulsil) on the performance of laying hens and egg quality. One thousand-one hundred-seventy Rhode Island hens were randomly distributed to three dietary treatments (0, 150, and 450 grams per ton bio-available Si) and assigned to 26 replicate cages with 15 birds per cage. Laying hens were fed layer mash feed from 26-30 weeks of age. Egg production rate, feed consumption and mortality were recorded daily while egg size and egg quality traits such as shell strength (static stiffness and fracture force), shell proportion, index and thickness, Haugh units, and vitelline membrane strength (rigidity and rupture force) were gathered on a weekly basis. Data analysis was performed using SAS software at alpha risk 5%. Results showed a significant ($P<0.05$) increase in egg weight and vitelline membrane strength as demonstrated by significantly ($P<0.05$) higher vitelline membrane rigidity and rupture force with 150 or 450 g/tons bio-available Si supplementation. Moreover, hens fed layer diet with bioavailable Si at 150 or 450 g/ton had significantly ($P<0.05$) heavier body weight at 30 weeks and a significant ($P<0.05$) increase in daily feed consumption of hens supplemented with bio-available Si. This study concluded that bio-available Si positively influenced egg weight and egg quality which are important economic traits in commercial egg production. With the information derived from this study, it is recommended to conduct further studies on bio-available Si in poultry breeders and other species.

Keywords: silicon, mineral, layer, egg quality, collagen

49 Cyclic heat stress increased basal ileal endogenous amino acid losses and standardized ileal amino acid digestibility in 22-day-old broiler chickens

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The objective of this study was to investigate the effects of cyclic heat stress and diet quality on basal ileal endogenous amino acid losses (BIEAAL) and standardized ileal amino acid digestibility (SIAAD) of two corn samples in 22-d-old broiler chickens. A total of 384, d-old male by-product Cobb breeder chicks were weighed on d 0 and randomly assigned in a randomized complete block design to 3 dietary treatments in a 2 (temperature [thermoneutral, TN at 29 °C vs. cyclic heat stress [CHS] at 34 °C for 6 hr/day from d 7 to 22) x 3 (diets: nitrogen-free diet [NFD] and two semipurified diets containing either the regular corn or mycotoxin contaminated corn fines [CF]. Each temperature was replicated twice with 4 replicate cages per treatment per room resulting in 8 replicate cages per dietary treatment. Each replicate cage contained 8 birds. A standard corn-SBM-based diet that met or exceeded nutrient and energy requirements was fed to all birds until they were placed on their respective experimental diets. The NFD was fed for four days (d 18-22) while the two semipurified diets containing either the regular corn or the CF were fed for 5 days (d 17 to 22). Each of the two semipurified diets contains 65% of their respective corn. Data from the ileal digesta from birds fed the NFD was used to determine the effect of CHS on BIEAAL which was eventually used to correct apparent ileal digestibility (AID) of amino acids to generate SIAAD values. Data were analyzed using the Proc GLM procedure of SAS with BIEAAL analyzed using the one-way ANOVA and the AID and SIAAD data analyzed using two-way ANOVA (2 temperature x 2 corn quality). Heat stress did not influence the intake of the NFD, but it resulted in an increase ($P < 0.05$) in BIEAAL. Heat stress decreased ($P < 0.05$) the intake of the semipurified diets but corn quality did not influence feed intake. Apparent ileal digestibility of N and AA was influenced by corn quality with CF having lower ($P < 0.05$) AID of N and AA. Interactions ($P < 0.05$) between corn quality and temperature were observed for AID of Arg, Lys, Met, Val, Asp, and Gly. In each case, the AID values for the regular corn, irrespective, of the room temperature were higher ($P < 0.05$) than that of CF. For SIAAD, significant interactions between corn quality and room temperature were observed for Arg, Ile, Lys, Phe, Val, Asp, and Gly. The SIAAD value of these AA showed that birds under CHS-fed diets containing the regular corn had higher ($P < 0.05$) SIAAD values compared to that of the remaining treatments. Results from this study showed that CHS influence on nitrogen (31%) loss is high. Corn fines decreased AID and standardized digestibility of N by 23 and 21%, respectively.

Keywords: amino acid, corn, corn fines, cyclic heat stress, endogenous amino acid

50 Mechanisms of AM/AP ratio and amino acid levels in corn substitution using different starch types on broiler growth and development

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Synchronized intestinal amino acids (AAs) and glucose supply is essential for optimal growth and development in broilers. This study aimed to evaluated the effects of varying carbon and nitrogen release rates in low-protein (LP) diets on broiler growth. A 3×3 factorial design was employed, in which birds were fed nine dietary combinations comprising three diets with different starch digestion rates—prepared using waxy corn, corn, and peas, with AM/AP ratios of 0.19, 0.29, and 0.41, respectively—and three levels of AA, based on SID Lys at 1.00%, 1.20%, and 1.40%. Each treatment consisted of 6 replicates with 10 birds per replicate. The experimental period was 4-6 weeks of age. This study demonstrated significant interactive effects of dietary AM/AP ratios and SID Lys levels on BW, BWG, and protein deposition in broilers ($P<0.05$). The highest BW, BWG, and protein deposition in broilers occurred in the group with dietary AM/AP ratio of 0.19 and SID Lys level of 1.20% (1.20 RDS), and the broilers had a late stage F/G of 1.51 and a full stage F/G of 1.46. Conversely, the lowest performance was observed in the group with a dietary AM/AP ratio of 0.41 and SID Lys level of 1.40% (1.40 SDS). Compared with the 1.40 SDS group, broilers in the 1.20 RDS group showed significantly increased serum IGF-1 levels, jejunal Na^+K^+-ATPase, amylase, tryptase, and chymotrypsin activities, and upregulated phosphorylation of AKT, mTOR, and p70 protein in breast muscle ($P<0.05$). Concurrently, reductions were observed in serum uric acid and urea nitrogen concentrations, liver LKR activity, and the expression of Foxo1, FBXO32, and TRIM63 protein in breast muscle ($P<0.05$). Transcriptomic analysis further revealed that the 1.40 SDS group exhibited upregulated expression of genes involved in lysine catabolism, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation pathways, relative to the 1.20 RDS group ($P<0.05$). In conclusion, during the grower phase, broiler diets formulated with waxy corn and 1.20% SID Lys were more effective in promoting the synchronous metabolism of glucose and AAs in the intestine, thereby enhancing growth performance. This effect may be attributed to improved AA transport, absorption, and utilization, reduced AA oxidative catabolism, and enhanced breast muscle protein deposition via activation of the Akt-mTOR-p70 signaling pathway.

Keywords: Grain types, Amino acid metabolism, Protein metabolism, Transporter protein, Broiler

51 High amylose to amylopectin ratio in a low-protein diet promoted growth performance and protein deposition of breast muscle in goslings

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For low-protein diets in poultry feed industry, it is becoming increasingly important to study the correlation between starch and protein digestion kinetics in poultry. This experiment was designed to investigate the effects of increasing amylose to amylopectin ratio on the growth performance and muscle development of goslings fed a low-protein diet. A total of 240 healthy commercial male Jiangnan white goslings with similar body weight at 35 days of age were randomly divided into 4 groups (6 replicates/group and 10 birds/replicate) with a 2 × 2 factorial design having 2 protein levels (16%, 14%) and 2 AM/AP ratios (0.34, 0.44) as dietary treatments. The experimental duration was 21 d. The results showed that: 1) Lowering dietary protein level increased average daily feed intake ($P < 0.05$), and increasing the AM/AP ratio uplifted the body weight of goslings at 56 d, improved average daily weight gain and decreased feed to gain ratio ($P < 0.05$). 2) Increasing the AM/AP ratio led to an increase in crude protein content in the breast muscle ($P < 0.05$). 3) The Thr and Phe content in the liver of goslings was improved after reducing the dietary protein level ($P < 0.05$), while increasing dietary AM/AP ratio enriched the amino acids composition in the liver and muscles, specifically elevated the Lys content in the liver and leg muscles, however, Glu, Leu, Lys and Pro content in the breast muscles ($P < 0.05$). 4) Breast muscle weight at 56d and the protein deposition from 52 d to 56 d of goslings was improved with increasing dietary AM/AP ratio ($P < 0.05$). 5) Low-protein diet with high AM/AP ratio also accelerated the protein fractional synthesis rate and protein fractional growth rate in breast muscles. 6) High AM/AP ratio in the diets caused the leg muscle fibers to swell and thicken. In conclusion, increasing AM/AP ratio in low-protein diets was beneficial to the growth of goslings from 35d to 56d and promoted protein deposition in breast muscles. Based on this study, it is suggested to use a diet with a protein level of 14%, and an AM/AP ratio of 0.44 for goslings after 35 d.

Keywords: low-protein diet, amylose to amylopectin ratio, growth performance, protein deposition, gosling

52 Effects of dietary low protein levels on reproductive performance and offspring muscle quality of yellow feathered broiler breeders

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The formation of poultry meat is a complex biological process, and the meat quality traits are influenced by a combination of genetic, nutritional, and environmental factors. This study aimed to investigate the effects of low-protein diets in yellow feathered broiler breeders on their reproductive performance and the muscle quality of their offspring, and to explore the underlying mechanisms, particularly how maternal nutrition influences protein utilization efficiency and muscle development in the offspring.A total of 300 11 week old yellow feathered broiler breeders with similar body weights were randomly assigned to two treatment groups: Normal protein (NP) and Low protein (LP), with 10 replicates of 15 breeders in each treatment, and reared in individual cages. The test period was 23 weeks. The LP group received diets with 3% less crude protein than the NP group during the breeding, pre-laying, and laying stages, while both groups were provided with equivalent energy levels and balanced supplementation of essential amino acids. Breeders were artificially inseminated at peak laying, and fertilized eggs were incubated. A total of 182 offspring chicks per group were reared under controlled conditions for evaluation of growth and muscle quality. All data were organized using Excel 2021 and statistically analyzed with SAS 9.4. Chi-square tests were used for reproductive performance data. Results showed that the LP diet had no significant effect on breeder body weight, daily weight gain, egg production, or feed-to-egg ratio ($P > 0.05$), but it significantly increased eggshell thickness and yolk weight ($P < 0.05$), while decreasing albumen weight ($P < 0.01$). It reduced the fertilization rate and healthy chick rate ($P < 0.05$) but had no effect on the hatching rate ($P > 0.05$).Compared with the group of breeder hens fed the NP diet, breeder hens fed the LP diet significantly ($P < 0.05$) decreased the initial body weight of the offspring chicks at 1 day of age, decreased the pH_{45min} of the breast muscle of the offspring broilers ($P < 0.05$). Feeding of low protein diets to breeder hens did not significantly ($P > 0.05$) affect the crude protein and amino acid content of breast muscle of offspring broilers compared to the group of breeder hens fed normal protein diets. Embryonic gene expression analysis revealed downregulation of *MSTN*, *MURF1*, and *FBXO32* ($P < 0.05$), suggesting reduced inhibition of muscle development. In summary, maternal low-protein diets impair reproductive outcomes but do not compromise muscle quality in offspring. Additionally, maternal nutrition may regulate embryonic muscle development through changes in egg composition and gene expression.

Keywords: Low-protein diet, broiler breeders, reproductive performance, muscle quality, gene expression

Student Competition: Animal Health & Immunology

53 Impacts of Eimeria vaccination on microbiome composition and function

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Eimeria infection causes a significant state of disease that disrupts microbiota and metabolic function. This study aimed to characterize effects of multi-species turkey Eimeria vaccination on the microbiome of turkey poults. The experiment comprised two treatments: Control (CON) and Vaccinated (VAC), with 20 poults per treatment. Poults in the VAC group received 1x dose of commercial vaccine on the day of hatch, and cecal samples were collected at 4, 9, and 14 days of age for Illumina NextSeq 2000 PE250 platform processing. Data were processed in QIIME 2 (2024.5) with DADA2 for quality filtering, error correction, and chimera detection. Alpha diversity and Beta diversity indices were analyzed using Mann-Whitney/Kruskal-Wallis statistical methods with a significance threshold of $p<0.05$. Significant differences in cecal alpha diversity were observed, including the Shannon index ($p=0.007$), observed OTUs ($p=0.004$), and Chao1 ($p=0.003$), indicating shifts in microbiota diversity and richness over time. At d9, the VAC group exhibited a substantial decline in cecal bacterial diversity with a Shannon index of 1.65 vs. 2.6 in CON, observed OTUs of 35 vs. 54 in CON, and Chao1 index of 35 vs. 54 in CON, suggesting transient dysbiosis, and ileal microbiome showed a similar pattern. Beta diversity indexes, including Bray-Curtis ($p=0.001$), Jaccard ($p=0.001$), Weighted UniFrac distance ($p=0.001$), and Unweighted UniFrac distance ($p=0.002$), showed a significant distance of the microbiome samples on d9 VAC group from the CON. Also, a higher abundance of potentially pathogenic bacteria, Enterobacteriaceae, Enterococcaceae, Clostridiaceae, and Peptostreptococcaceae, were observed in VAC ceca on d4 and d9 compared to CON. By d14 cecal microbiome indicated recovery by the presence of increased levels of beneficial bacteria such as Lachnospiraceae and Butyricicoccaceae. However, A higher abundance of potentially pathogenic bacteria, Streptococcaceae and Clostridiaceae, was observed in the ileum of VAC group on day 14. Functional metabolic pathways analysis using PICRUST 2 and the MetaCyc database revealed a decrease in pathways correlated with energy metabolism, amino acid biosynthesis, and fermentation in VAC sample, indicating a potentially less functional microbiome. Predictive functional analysis showed a significant ($p > 0.05$) VAC upregulation of pathways relating to stress adaptive responses of bacteria in both ceca and ileum. These findings underscore that, even though live Eimeria vaccine is critical for protection against disease, it concurrently leads to a temporal microbial shift towards stress-induced and potentially harmful bacterial taxa with decreased metabolic function.

Keywords: Broiler

54 The relationship between blood parameters and hepatic lipid deposition in laying hens during the late laying period

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Fatty Liver Hemorrhagic Syndrome (FLHS) is a metabolic disorder characterized by excessive hepatic triglycerides (TG) accumulation, predominantly affecting caged, high-producing laying hens. In surviving hens, clinical symptoms of FLHS are often subtle and difficult to detect. This study aimed to investigate the relationship between various blood parameters and hepatic fat deposition in late-phase laying hens under both fasting and fed conditions, with the goal of identifying reliable biomarkers for the diagnosis of fatty liver. A total of 69-week-old Jingfen No. 6 laying hens maintained under normal feeding conditions were selected for analysis. Hepatic TG levels and a wide spectrum of blood parameters-including glucose andlipid metabolites, inflammatory markers, hormones, and free amino acids-were measured. Based on blood TG concentrations, hens were grouped into a low serum TG group (LSTG, TG < 15 mmol/L) and a high serum TG group (HSTG, TG ≥ 15 mmol/L). According to liver TG content, hens were also categorized into a normal liver group (NORM, TG < 0.3 mmol/g prot) and a fatty liver group (FL, TG > 0.45 mmol/g prot). Interestingly, liver TG levels were not significantly correlated with classical blood lipid indices such as serum TG, total cholesterol (TCHO), glucose (GLU), and high-density lipoprotein (HDL) ($P > 0.05$), contrary to previous assumptions. Under fasting conditions, hepatic TG content was positively correlated with albumin (ALB), total amino acids (TAA), and leucine (Leu) ($P < 0.05$), while negatively correlated with α-aminobutyric acid (α-ABA), β-alanine (β-Ala), and α-aminoisobutyric acid (β-AiBA) ($P < 0.05$). Alanine (Ala) exhibited a highly significant negative correlation with liver TG ($P < 0.001$). Under fed conditions, hepatic TG was significantly positively correlated with alanine aminotransferase (ALT), the ALT/aspartate aminotransferase (AST) ratio ($P < 0.05$), and ALB ($P < 0.001$). Untargeted metabolomics analysis identified 57 differentially expressed metabolites (VIP > 1, $P < 0.05$) between the NORM and FL groups, including 28 upregulated (e.g., taurodeoxycholic acid, L-tyrosine, D-tryptophan) and 29 downregulated metabolites (e.g., chenodeoxycholic acid, PC(18:0/14:0), PC 32:0). Functional enrichment analysis indicated significant involvement of these metabolites in 67 metabolic pathways, particularly those related to protein digestion and absorption, amino acid biosynthesis, and bile secretion. In conclusion, aged laying hens with fatty liver exhibited distinct alterations in amino acid metabolism. Blood levels of TAA, Ala, and Leu under fasting conditions, and ALB under fed conditions, may serve as promising biomarkers for the early identification of FLHS in laying hens.

Keywords: FLHS, Biomarkers, Jingfen No. 6 laying hens, Amino acids, Untargeted metabolomics

55 Evaluating commonly used anticoccidial drugs against *Eimeria tenella* field isolates in Bangladesh

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Frequent applications of anticoccidial drugs have resulted in the development of drug resistance in *Eimeria* parasites. In this study, seven *E. tenella* field isolates were investigated to evaluate drug resistance against commonly used anticoccidials. For each of the isolates, a total of 112 chicks were divided into seven groups of 16 chicks each at 12 days of age. The chicks in all the groups, except group 7, were infected with 7.5 x 10^4 sporulated oocysts of *E. tenella* at day 14. Following manufacturer dose, from day 12 to day 21, the chicks of group 1 were treated with amprolium, group 2, 3, 4, and 5 were medicated with maduramicin, sulphaclozine, toltazuril and amprolium+sulfaquinoxaline, respectively. Birds of group 6 served as infected, non-medicated control (INC), and group 7 was kept as a non-infected, non-medicated control (NNC). The drug resistance of each isolate of *E. tenella* against the respective anticoccidial drug was determined by calculating the global index (GI) that includes weight gain, FCR, mortality%, lesion score and oocyst index. Mean values of ANOVA analyzed data obtained on various parameters were compared by Duncan's Multiple range test through SPSS. The %GI for sulfaclozine in case of isolate-I was 33.91% indicates resistance. While in isolate-II, isolate-III, isolate-IV, isolate-V, isolate-VI and isolate-VII, the %GI were 54.49%, 65.23%, 52.19%, 58.05%, 63.26%, and 68.40% respectively demonstrating partial resistance. The %GI for maduramicin for isolate-III, isolate-V and isolate-VII were 76.58%, 70.09% and 73.18% respectively exhibiting limited efficacy, while for isolate-I, isolate-II, isolate-IV, and isolate-VI, %GI were 68.42%, 51.23%, 58.77% and 68.67% respectively indicating partial resistance. The study depicted that, the *E. tenella* isolates showed varying degree of resistance against sulfaclozine, whereas limited efficacy to partial resistance were observed against maduramicin. On the other hand, amprolium, showed good efficacy against all the field isolates, whereas amprolium+sulfaquinoxaline depicted good to very good efficacy. However, toltazuril showed very good efficacy against most of the field isolates.

Keywords: Anticoccidial drugs, Eimeria tenella, Resistance, Efficacy, Field isolates

56 Gut-metabolism-bone axis dysregulation in broiler skeletal pathogenesis

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Various skeletal disorders, especially of the leg bones, are one of the major problems facing intensive broiler farming. In order to investigate the mechanisms and analyse the potential causes of skeletal abnormalities in caged broilers. The present study was conducted to initially screen based on gait scores, and to achieve grouping of Normal and Abnormal skeletal broilers based on calcium - phosphorus product in serum. Skeletal phenotypes were used to determine the validity and variability of the groupings, serum metabolites, gut microbes and their correlations were compared, and finally, the characteristic metabolites were targeted and analysed. Broiler rearing was conducted at Xinjiang Chuangyu Poultry Breeding Co., Ltd. Upon reaching a body weight of 3 kg, 60 birds were selected based on gait scoring and subsequently slaughtered for sampling. Independent samples t-test was performed between groups and data were expressed as mean ± SEM. non-normally distributed data were supplemented with non-parametric tests (Mann-Whitney U test). The Abnormal group exhibited deteriorated bone phenotypes with significant alterations in geometry and microstructure ($P<0.05$). Elevated bone resorption markers (+ osteoclast genes *TRAP/CTSK/MMP9*) and reduced osteogenic markers (+ osteoblast genes *Runx2/ALP/OCN*) indicated enhanced resorption and impaired formation ($P<0.05$). Serum metabolomics revealed TCA cycle disruptions (KEGG enrichment $P<0.01$), while glycolysis-related genes were upregulated ($P<0.05$), suggesting osteoclast activation via metabolic reprogramming. Harmful bacteria increased, and energy-metabolizing taxa (*Phascolarctobacterium, Dysosmobacter*, etc.) strongly correlated with bone/metabolite markers. Targeted analysis confirmed depleted TCA intermediates (fumarate/malate/succinate; $P<0.05$), with fumarate+malate combination yielding ROC AUC=0.89 for skeletal abnormality detection. Dysbiosis of the intestinal flora inhibits the synthesis of TCA cycle intermediates, triggering compensatory activation of glycolysis and oxidative phosphorylation, which ultimately leads to enhanced bone resorption, inhibited bone formation, and the formation of a vicious cycle of bone disease.

Keywords: Broiler, bone abnormalities, intestinal flora, bone metabolism, energy metabolism

57 Dynamic immune responses to avian pathogenic Escherichia coli infection in chicks

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Avian pathogenic Escherichia coli is an extra-intestinal pathogen causing a high incidence and mortality rate, which leads to huge economic losses in the poultry industry. This study investigated the systemic immune responses of chicks during APEC infection, which focused on survival rates, organ index, histopathological injury and blood physiological indicators. A total of 350 1-day-old Xueshan chicks were randomly divided into a non-infection group (n = 270) and a infection group (n = 80). The infection group were intramuscular injection with 1.0 × 10^7 CFU/mL of E. coli O18K1, while the non-infection group was injected with saline. Samples were collected at 1, 2, 3, 5, 7, and 9-day post-infection (dpi). The dynamic immune responses from acute to recovery phase was performed by the Mann-Whitney U test. The Kaplan-Meier analysis confirmed that APEC infection significantly reduced survival rates compared to the non-infection group, with peak mortality occurring in the first 2-days after infection, but without death after 7 dpi. Meaning while, the body weight, liver index and and bursa of Fabricius index was significantly decreased in infected chicks compared with non-infected chicks, while the heart index and spleen index was significantly increased. In addition, it was shown that no significant differences were observed in the heart index, liver index and spleen index at 9 dpi. During the infection period, a similar changing trend was observed in the histopathological score. Although no *E.coli* was detected in the blood after 5 dpi, the levels of the pro-inflammatory cytokine IL-1β and the chemokine CCL4 were significantly decreased and the level of the anti-inflammatory cytokine IL-10 was significantly elevated until 5 dpi. Moreover, blood physiological indicators related to liver injury, ions and energy metabolism were detected. It was shown that *E.coli* infection significantly decreased plasma enzyme activities of AST and ALP, as well as the levels of TG, NEFA, serum iron and albumin, while significantly increased the levels of globulin. Among these indicators, only ALP, TG, NEFA, serum iron and globulin in the infection group recovered to the normal level as the uninfected group. This study elucidates that systemic lipid metabolism and iron homeostasis could be crucial immunomodulators, which provides important insights into immune defence during APEC infection.

Keywords: Avian pathogenic Escherichia coli, Chicks, Histopathological injury, Immune responses, Serum iron

58 Early feed restriction affects broiler immune function through gut flora and the Wnt/β-catenin signaling pathway but is reversed by late *ad libitum* feeding

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Restricted feeding is often used to reduce the risk of metabolic and other diseases in broiler chickens. Recently, with changes in feed prices, the focus has shifted to the ability of restricted feeding practices to reduce feed costs. Gut homeostasis and microbiota are strongly related to animal health. The aim of this study was to reduce the cost of broiler production without any adverse effects. Experiments were conducted at the experimental station of the Shihezi University, China. A total of 480 1-day-old male, yellow-feathered broilers were randomly divided into four groups with six replicates and 20 broilers per replicate, and one replicate placed in each cage. On days 1-21 of the experiment, Birds were fed ad libitum for 24, 20, 16, and 12 h/d in groups C, F, E, and T, respectively. All groups were fed ad libitum for 24 h per day on days 22-70 of the experiment. The experiment lasted for 70 d, and 20 broilers were placed in each cage. The growth performance of the broilers was measured from days 1–21 and 22–70. The immune organ indices, serum immune factors, changes in intestinal morphology and flora, and the expression of intestinal immune-related genes and Wnt/β-catenin signaling pathway genes and proteins were also measured at days 21 and 70. Measurement data are expressed as means ± standard error of mean (SEM). One-way analysis of variance, followed by Duncan's test, was used for group comparisons using SPSS 26.0. $P < 0.05$ was considered statistically significant. Early feed restriction (**EFR**) in broilers decreased the BW, ADG, ADFI and bursa of Fabricius index ($P < 0.05$). Moreover, the Firmicutes to Bacteroidetes (**F`/B**) ratio was lower for EFR broilers ($P < 0.05$) and the inhibition of Wnt/β-catenin signaling pathway in the gut exacerbated the inflammatory response, leading to decreased immunity. This was improved by all-day *ad libitum* feeding (**ADF**) for a period (22-70 d). Growth performance and immune organ indices recovered after ADF and were similar in the treated and control groups ($P > 0.05$). Butyric acid (**BA**) was remarkably higher in the treated groups ($P < 0.05$). Consistently, some BA-producing gut bacteria were also significantly increased in the treatment groups and the intestinal Wnt/β-catenin signaling pathway was activated. The abundance of beneficial bacteria increased in the treatment groups at the ADF stage. A period of ADF reversed the EFR-induced inflammatory response in broilers, possibly by increasing the beneficial intestinal flora and activating the gut Wnt/β-catenin signaling pathway. This feeding practice helps reduce the cost of production when feeding broilers.

Keywords: early feed restriction, ad libitum feeding, yellow-feathered broilers, immune function, gut microbiota

Student Competition: Metabolism and Nutrition: Enzymes and Feed Additives

59 Tributyrin restored growth performance in broilers offered low protein diets based on high protein wheat

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Low protein diets led to reduced nitrogen excretion, reduced soybean meal usage and improved litter quality which is beneficial for bird welfare and health. However, birds fed low protein diets based on maize- or sorghum-generated better body weight gain and FCR than birds offered low protein diets based on wheat. This poses a challenge, as wheat is the predominant grain used in Australia, Canada and part of Europe and Asia. Tributyrin may be utilised as an energy source of enterocytes, and it was reported to support better gut health and ultimately enhance growth performance. Therefore, this study tested the hypothesis that supplementation of tributyrin would restore growth performance in birds fed low protein diets based on high crude protein wheat. The experimental consisted of a 2×2 factorial array of treatments with two wheat protein content (HCPW 153 versus LCPW 107 g/kg) and with the presence or absence of tributyrin (0 versus 500 mg/kg). Experimental diets were offered to mixed-sex Ross 308 broiler chickens from the grower phase (10 to 24 days) and finisher phase (25 to 36 days post-hatch). All four diets had energy densities of 3050 for grower and 3100 MJ/kg for finisher. The experimental data were analysed by two-way analyses of covariance (ANCOVA). Male ratio, while is the percentage of male bird numbers in total bird numbers in each cage, was used as the covariant. Treatment interactions were observed for BWG and FCR in the grower, finisher, and the overall growing phases. When birds were fed LCPW diets, growth performance was comparable with or without the supplementation of tributyrin during the overall phase. However, when birds were fed HCPW diets, tributyrin increased BWG by 25.9% (2021 versus 1605 g/bird), FI by 12.3% (3160 versus 2813 g/bird), and improved FCR by 11.1% (1.563 versus 1.758). Supplementation with tributyrin increased *Pectoralis* major yields by 4.5% (186 versus 178 g/kg) regardless of wheat type. At 36 days post-hatch, gizzard pH was negatively correlated with FCR and was significantly lower in birds offered HCPW relative to those offered LCPW. Supplementation with tributyrin numerically restored pH by 12.4% (3.08 versus 2.74). in conclusion, tributyrin supplementation restored growth performance in broilers offered diets based on high crude protein wheat in low crude protein diets.

Keywords: broiler, protein, wheat, starch, tributyrin

60 Effects of curcumin on growth performance, antioxidant capacity and mitochondrial biosynthesis in broiler breeder hens

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This research was performed to investigate the effects of dietary supplementation with different levels of curcumin (CUR) on production performance, Breeding performance, antioxidant capacity and mitochondrial biosynthesis in broiler breeders. Two hundred and eighty-eight 20-week-old Qingyuan jatropha parent breeder hens were allocated randomly into 4 treatments, with 6 replicates per treatment, and 12 birds per replicate cage. Birds in the control group (CON) was provided with a basal diet, while others were fed a basal diet supplemented with 100, 200, or 400 mg/kg CUR (CUR100 CUR200, and CUR400), respectively. The experiment lasted for 18 weeks. The results were showed that: compared with birds in CON,1)supplementation with CUR had no significant effects on laying rate, average daily egg weight and feed-to-egg ratio among of breeder hens, but decreased the percentage of abdominal fat($P<0.05$).2)supplementation with CUR increased ($P<0.05$) the hatchability of eggs increased (P <0.05) the number of dominant follicles (CUR100) in broiler breeder hens;and increased ($P<0.05$) the level of LH (CUR200, CUR400) in ovarian tissue.3)the levels of triglyceride, total cholesterol, and low-density lipoprotein cholesterol in plasma were decreased in birds treated with CUR ($P<0.05$).4)In liver, supplementation with CUR increased the activity of SDH (CUR100, CUR200), and decreased($P<0.05$) the activity of MDH (CUR200). 5)Supplementation with CUR increased($P<0.05$) the activities of T-SOD, T-AOC and GSH-Px (CUR200) in ovary, and increased ($P<0.05$) the activity of GSH-Px in plasma (CUR100). 6)Studies on the expression level of apoptosis-related genes in ovary found that supplementation with all levels of CUR upregulated ($P<0.05$) the relative expression of *SIRT1, SIRT3, SIRT5*,while decreased ($P<0.05$) the expression of *SIRT2* and *Bax*. In addition, supplementation with 200 mg/kg CUR upregulated ($P<0.05$) the relative expressions of *HO-1, NRF2, Bcl2*, and down-regulated ($P<0.05$)the relative expression of *Caspase-9*. 7)Furthermore, supplementation with 100 mg/kg CUR up-regulated ($P<0.05$) the relative expression levels of mitochondrial biosynthesis-related genes (*AhR, GCLC, MFN1, NRF1, OPA1, TFAM*, and *MFN2*) in liver. In conclusion, the dietary supplementation with curcumin had no adverse effect on the production performance of broiler breeder hens; while it regulated lipid metabolism by enhancing mitochondrial biosynthesis, increasing mitochondrial-related enzyme activity, inhibiting apoptosis, and enhancing antioxidant function, thus having an ameliorative effect on the health of breeder hens. Under the conditions of this experiment, the recommended supplemental level of curcumin is 100 to 200 mg/kg.

Keywords: curcumin, broiler breeder hens, antioxidant capicity, lipid metabolism, mitochondrial biosynthesis

61 Effects of dietary gallic acid at varying levels on growth performance, gut health, and meat quality in broiler chickens challenged with Lipopolysaccharide

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This study was aimed to investigate the ameliorative effects of gallic acid (GA) on growth performance, intestinal health, and meat quality of lipopolysaccharide (LPS)-challenged broilers. A total of 750 1-d broilers were allocated into 5treatments, with 6 replicates per treatment, and 25 birds per replicate. Birds in the control group (CON) and the challenged group (CHA) were fed a basal diet, while others were fed basal diet supplemented with 150, 300, or 450 mg/kg GA (GA150, GA300, GA450), respectively. On days 14, 18, and 21, birds inCHA, GA150, GA300, and GA450 received an intraperitoneal injection of LPS at 0.5 mg/kg body weight (BW), while those in CON received an equal volume of saline. The experiment lasted for 50 days. The results showed that: 1) At the 1 to 21 days of age, compared with CON, lipopolysaccharide challenge significantly decreased ($P < 0.05$) the final BW, average daily feed intake (ADFI) and average daily gain. Compared with LPS, GA treatments (GA150, GA300, GA450) increased all those parameters ($P < 0.05$). Additionally, the ADG of birds in GA150 and GA300 was significantly increased from 1 to 50 days of age. 2) Dietary supplementation with GA significantly ameliorated ($P < 0.05$) LPS-induced reduction in ileal villus height and the villus height to crypt depth ratio, and the increase in crypt depth in 21-d birds. bird3) Compared with birds inCHA, dietary GA up-regulated ($P < 0.05$) the expression of Occludin in the ileum of 21-d birds; Furthermore, the expression of ZO-1 in the jejunal mucosa was significantly increased ($P < 0.05$) in birds from GA300. 4) Dietary GA significantly improved the LPS-induced reduction in the relative abundance of Bacteroidetes at phylum level (GA450) and Bacteroides at genus level (GA150, GA300). 5) Compared with birds in LPS, GA450 significantly increased ($P < 0.05$) the b^* value of the breast muscle at 45 min and 24 h post slaughter in 50-d birds. 7) Compared with challenged-birds, all GA treatments decreased ($P < 0.05$) the relative expression of myosin heavy chain (MyHC) IIx in the breast muscle of 50-d birds. Additionally, supplementation with 300 mg/kg GA increased ($P < 0.05$) the relative expression of MyHC I and MyHC IIa. In conclusion, the dietary supplementation with GA alleviated the LPS-induced decline in growth performance of broilers by modulating the expression of tight junction proteins, improving intestinal barrier function, and modifying the microbiota structure. Supplementation with GA also regulated the expression of MyHC, and improved meat quality of broilers. Under the conditions of this experiment, the recommended supplemental level of GA is 300 mg/kg.

Keywords: gallic acid, broilers, lipopolysaccharide, intestinal health, meat quality

62 Effect of exogenous bile salts supplementation on the performance and hepatic lipid metabolism in aged laying hens with fatty liver syndrome

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Fatty liver syndrome (FLS) is a common metabolic disorderin laying hens, particularly during the late laying period. Bile acids (BAs), known for their role in emulsifying fats and enhancing lipid digestion and absorption, have been explored as functional feed additives in poultry production. In this study, we investigated the effects of dietary BAs supplementation on alleviating FLS in aged laying hens, with a focus on the hepatic lipid metabolism. A total of 384 75-wk-old Hy-line Brown hens with relative lower laying rate (around 70%) were randomly assigned to receive either a control diet (Normal, 2700 kcal/kg, 16.5% CP) or a high-energy, low-protein (HELP, 3000 kcal ME/kg, 13.0 % CP) diet, with or without supplementation of 0.1 g/kg BAs. For the variables a two-way ANOVA model was used to assess the main effects of diet and BAs, as well as their interactions. The results showed that BAs supplementation significantly improved ($P < 0.05$) average daily egg production, feed efficiency, and eggshell specific gravity in hens fed the HELP diet. Moreover, BAs markedly reduced ($P < 0.05$) hepatic lipid accumulation, including lipid droplets, triglyceride, and total cholesterol levels. At the biochemical level, BAs enhancing carnitine palmityl transferase (CPT1) activity in the liver of hens fed the HELP diet while suppressed fatty acid synthase (FAS) activity ($P < 0.05$). Gene expression analysis revealed that BAs down-regulated ($P < 0.05$) key lipogenic genes such as acetyl coenzyme A carboxylase (*ACC*) and *FASN* in hens fed the HELP diet, while down-regulated ($P < 0.05$) the expression of peroxisome proliferator-activated receptor γ (*PPARγ*), L-carnitine palmityl transferase 1 (*L-CPT1*), very low density lipoprotein receptor (*VLDLR*), cytochrome P450 family 7 subfamily A member 1 (*CYP7A1*), small heterodimeric chaperone receptor 1 (*SHP-1*), and liver receptor homologue 1 (*LRH-1*). Additionally, BAs enhanced the hepatic protein expression of phosphorylation-AMP-activated protein kinase (p-AMPK), phosphorylated-ACC (p-ACC), and VLDLR compared with HELP group ($P < 0.05$). In conclusion, dietary BAs supplementation effectively mitigates HELP-induced FLS in aged laying hens by suppressing hepatic lipogenesis and promoting fatty acid oxidation. These metabolic improvements are closely linked to enhanced laying performance. Future studies should focus on the role of BAs in regulating extrahepatic lipid transport mechanisms.

Keywords: bile acids, liver, lipid metabolism, fatty liver syndrome, laying hens

Metabolism and Nutrition: Amino Acids, Vitamins and Minerals II

63 Effects of starch structure on the absorption of encapsulated amino acids in laying hens fed a low-protein diet

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The successful application of low-protein diet needs the supplementation of synthetic amino acids (AAs). The absorption of crystalline AAs is faster than that of protein-derived AAs and could be slowdown by encapsulated treatment. Here, we investigated the effect of starch structure (amylose-to-amylopectin ratio, AR) on laying performance and absorption of encapsulated AAs in hens fed a LP diet. Six hundred and seventy-two 34-week-old Jingfen No. 6 laying hens, were used for the experiment. The hens were randomly allocated to seven groups and subjected to one of the following dietary treatments: a corn-soybean meal-based layer diet (16% CP; Control), and one of the five experimental diets (13% CP) with different amylose-to-amylopectin ratios (AR: 0.128, 0.228, 0.328, 0.428, and 0.528) that supplemented with encapsulated amino acids. The laying performance and ileal digestibility of starch and amino acid were determined. the activities of lactase, maltase and sucrase and the expression of genes related to glucose and amino acid transportation were measured in duodenum, jejunum, and ileum. Hens fed the AR0.428 diet had higher ($P < 0.05$) body weight gain, laying rate, and egg mass compared with control. The feed efficiency and egg quality were not altered by dietary treatment ($P > 0.05$). Compared with control, the apparent ileal AA digestibility was decreased ($P < 0.05$) by AR0.128, whereas apparent ileal CP digestibility was increased ($P<0.05$) by AR0.428. The ileal digestibility of amylopectin, amylose, and total starch was decreased with elevated AR. Plasma uric acid level was decreased by AR0.428 and AR0.528 groups. Compared to control, maltase activity was increased by AR0.328 and AR0.528 in duodenum, decreased by AR0.228 and AR0.528 in jejunum, and decreased by AR0.328 in ileum. Compared to control, the mRNA expression of *EAAT3* was increased by AR0.428 and AR0.528 in jejunum and ileum and $y^{+}LAT1$ in ileum ($P<0.05$). The expression of *SGLT1* was decreased by AR0.128 whereas increased by AR0428 and AR0.528 in ileum, compared to control. Hens fed low-protein diet of AR0.428 supplemented with MAAs has a comparable laying performance with control hens fed a normal diet (16% CP).

Keywords: Low-protein diet, amylose-to-amylopectin ratio, starch, amino acid, laying hen

64 Nutritional evaluation of wheat and wheat bran for Jingfen No. 6 laying hens and white-feather broilers

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This experiment aimed to study the standard ileal digestibility of amino acids and net energy values of wheat and wheat bran in laying hens and broilers, to obtain the specific energy values of raw materials for laying hens and broilers, and to design the lowest-cost feed formula to meet the energy requirements of animals. 1. Three hundred 16-day-old Jingfen 6 laying hens and two hundred 16-day-old AA broilers with similar body weights were selected. Laying hens and broilers were each divided into three treatments, with six replicates per treatment and twelve chickens per replicate. The diets included a nitrogen-free diet, a wheat diet, and a wheat bran diet. There was a three-day adaptation period and a five-day formal period. On the 23rd day of the experiment, the test chickens were sacrificed and the ileal chyme at the terminal ileum was collected. 2. Fifty-four 16-day-old Jingfen 6 laying hens and broilers with similar body weights were selected and randomly divided into three groups, which were respectively fed: basal diet, wheat diet, and wheat bran diet. They were placed in a respiration calorimeter for a three-day adaptation period and a five-day formal period. The results showed that: 1. The SIDAA of wheat for laying hens during the brooding period was 94.61%, and that of wheat bran was 82.37%. The SIDAA of wheat for broilers was 96.22%, and that of wheat bran was 79.45%. 2. The apparent metabolizable energy and net energy of wheat for laying hens during the brooding period were 13250.14 KJ/kg and 7951.34 KJ/kg, respectively. The apparent metabolizable energy and net energy of wheat bran were 5511.49 KJ/kg and 4256.45 KJ/kg, respectively. The apparent metabolizable energy and net energy of wheat for broilers were 11742.46 KJ/kg and 8122.62 KJ/kg, respectively.

Keywords: Laying hen, broiler, wheat, wheat bran, SID, NE

65 Effects of cholecalciferol and 25-hydroxycholecalciferol on reproductive performance, egg quality and plasma biochemical indices on aged duck breeders

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This study aimed to evaluate the effects of supplementing two different vitamin D sources on reproductive performance, egg quality, and plasma biochemical indices of aged duck breeders, and to estimate vitamin D requirements and relative bioavailability from these two sources. A total of 180 laying Pekin ducks (64 wk of age) were randomly allotted to 9 treatments with 10 replicate pens of 2 birds each. The birds were fed a basal diet with no vitamin D supplementation or supplemented with cholecalciferol (**VD$_3$**) or 25-hydroxycholecalciferol (**25-OH-D$_3$**) at 250, 500, 1000 or 2000 IU/kg of feed for 15 wk. Compared with ducks fed the basal diet, supplemented with either VD$_3$ or 25-OH-D$_3$ increased egg production (9 to 15 wk), hatchability (7 to 10 wk), egg Haugh unit, plasma 25-OH-D$_3$ concentration, ovarian weight, ovarian index, weight and number of dominant follicle, and large yellow follicle of duck breeders ($P < 0.05$). Furthermore, calcium and phosphorus levels in plasma were increased in the 25-OH-D$_3$ treatments ($P < 0.05$). Compared to VD$_3$, diets with 25-OH-D$_3$ resulted in greater ovary weight, the number and weight of dominant follicle, and plasma 25-OH-D$_3$ concentration in ducks ($P < 0.05$). According to the quadratic broken-line model, the VD$_3$ requirements of duck breeders in terms of enhancing egg production, ovarian weight, and dominant follicle number were 906, 359, and 730 IU/kg, respectively, whereas the 25-OH-D$_3$ requirements were 260, 324, and 308 IU/kg, respectively. Based on slope ratio comparison from multiple linear regressions of ovarian weight, dominant follicle number, and plasma 25-OH-D$_3$ concentration, the bioavailability of 25-OH-D_3 were 147%, 191%, and 211%, respectively, relative to VD_3.

Keywords: duck breeder, vitamin D3, 25-hydroxycholecalciferol, requirement, relative bioavailability

66 Study on the net energy and standard ileal digestibility of soybean meal and fermented soybean meal in Jingfen No. 6 laying hens

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This study aimed to investigate the net energy and standard ileal digestibility of soybean meal and fermented soybean meal in laying hens. In Experiment 1, 54 25-day-old Jingfan 6 laying hens of similar weight were selected and randomly assigned to three groups using a completely randomized design. The control group was fed a basal diet, while the experimental groups were fed diets with 20% substitution of soybean meal and fermented soybean meal for the basal diet components, respectively. They were placed in the metabolic chambers of the respiration calorimeter for 8 days: 3 days of adaptation, 3 days of respiration calorimetry, and 2 days of fasting calorimetry. In Experiment 2, 300 Jingfan 6 laying hens of similar weight were divided into three treatments, with six replicates per treatment and 12 hens per replicate. The diets included a nitrogen-free diet, a soybean meal diet, and a fermented soybean meal diet. The adaptation period was 3 days and the formal feeding period was 5 days. On the 23rd day of the experiment, the hens were sacrificed and the ileal chyme at the terminal ileum was collected. The results of this experiment showed that the apparent metabolizable energy and net energy of soybean meal in laying hens were 10527.02 KJ/kg and 6956.59 KJ/kg, respectively, and those of fermented soybean meal were 11681.51 KJ/kg and 7005.04 KJ/kg, respectively.

Keywords: Soybean meal, Fermented soybean meal, Net energy, Standard ileal digestibility

67 Influence of age and dietary supplementation of phytase on apparent and standardized ileal amino acid digestibility palm kernel meal in broiler chickens

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The aim of this study is to evaluate the effect of age and dietary phytase supplementation on ileal digestibility of palm kernel meal (PKM) in broilers. A total of 162 12-day-old and 72 32-day-old AA chicks has been used. These selected birds, with the same health status and a similar initial weight, were randomly divided into three groups with six repetitions of nine birds at 12 days old and 4 birds at 32 days old. The group P0 received a diet without nitrogen, then group P1 received an experimental diet with palm kernel meal as the sole nitrogen source, and finally group P2 received a diet without mineral phosphorus while supplemented with 1000 FTU phytase/kg diet. The subjects were fed ad libitum, then fasted for six hours. After three hours, they were given ad libitum access to the test diet. On days 20 and 40, all subjects were taken for each repetition. They were asphyxiated by carbon dioxide. The abdominal cavity was opened, and the small intestine was quickly separated. The portion of the small intestine extending from the yolk sac diverticulum to nearly 40 mm at the ileocecal junction was cut out to collect its contents. We determined the amino acid content of the feed and chyme using an automatic amino acid analyzer (Hitachi L-8900). Results for the 17 amino acids were expressed on a dry matter basis. The GraphPad Prism 8.0.2 software was used to analyze the data. The experimental data were processed using a two-way ANOVA test followed by a Tukey post-test.The results showed that feed consumption remained consistent over time. As for endogenous losses, there was no significant difference according to animal age ($P > 0.05$). However, there was an interaction ($P < 0.05$) between bird age (21 vs. 41 days) and feed treatments (P1 and P2) for the apparent ileal digestibility (AID) and the standard ileal digestibility (SID) of most amino acids (AAs), except for the methionine and aspartate AID values and the methionine, aspartate, and cysteine SID values. The effect of age on amino acid (AA) digestibility was examined, and the effect of diets on AA digestibility was similar to the apparent ileal digestibility (AID) of AA values. Based on our results, we can conclude that PKM can be improved in broiler diets by using it in small amounts.

Keywords: Amino acid, AID, Growing stage, PKM, SID

68 Dietary arginine supplementation increases growth performance, enhances immune function and antioxidant function, alleviates intestinal injury of broiler chickens challenged with *Clostridium perfringens* via activating CASTOR1/SLC38A9-mTOR signaling pathway.

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The study was conducted to investigate the effects of arginine on the growth performance, immune function, antioxidant capacity, and intestinal health of broiler chickens challenged with *Clostridium perfringens* (CP), and explore the underlying mechanisms. The experiment was designed as 2×2 factorial arrangement with arginine level (1.22 % or 1.52 %) and CP challenge (with or without CP challenge). The results showed that arginine supplementation increased the average daily gain (ADG) from day 1 to day 13. On day 14-20, CP challenge significantly decreased the ADG, while this effect was significantly reversed by arginine supplementation. Birds fed arginine-supplemented diet without CP challenge have the lowest ratio of feed to gain among the four groups. Arginine supplementation significantly increased the decreased serum levels of immunoglobulin (Ig)A, IgG and IgM induced by CP challenge. CP challenge significantly decreased the serum T-AOC and T-SOD activity, while arginine supplementation significantly increased the serum activities of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px). CP challenge significantly decreased the total antioxidant capacity (T-AOC), the activity of T-SOD and GSH-Px in jejunal mucosa, while arginine supplementation alleviated the decrease result from CP. Arginine addition alleviated the increased mRNA expression of *IL-6*, *IL-1β*, *TNF-α*, and *IFN-γ* induced by CP challenge. Arginine supplementation up-regulated the mRNA expression of antimicrobial peptide *AVBD1*, *AVBD2*, *CATH3* and *LEAP2*. Arginine supplementation down-regulated the mRNA expression of pro-apoptotic genes *caspase-3* and *caspase-9*, and autophagy genes *ATG5* and *beclin-1*. Arginine supplementation increased the *Nrf2*, *SLC38A9* and *mTOR*, while decreasing the mRNA expression of *NF-κB*, *CASTOR1* and *ARG2*. In conclusion, arginine supplementation increased the growth performance, immune function and antioxidant capacity, alleviated the intestinal inflammation, apoptosis and autophagy in broiler chickens challenged with CP, which may be contribute to the activation of CASTOR1/SLC38A9-mTOR signaling pathway.

Keywords: Arginine, Clostridium perfringens, Broiler, Intestinal injury, CASTOR1/SLC38A9-mTOR signaling pathway

69 Effects of dietary low protein levels and amino acid patterns on production performance, egg quality and intestinal function in laying hens

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This study aimed to investigate the effects of different low-protein (**LP**) levels and amino acids (**AAs**) patterns on production performance, egg quality and intestinal function of laying hens. A total of 384 (33-week-old) Hy-Line hens were randomly assigned to control group (**CONT,** crude protein (**CP**) 15.91% + 4AAs), **LPF** group (CP 14.53% + 4AAs), **LPS** group (CP 14.58% + 8AAs) and **LPH** group (CP 13.05% + 8AAs),The pre-feeding period lasted 2 weeks, and the experiment period was 20 weeks (from 33 to 55 weeks of age). The results showed that the LPF and LPH groups significantly increased feed conversation ratio (**FCR**) ($P < 0.05$), the LPS group decreased FCR ($P < 0.05$). The LPH group significantly decreased laying rate and egg mass ($P < 0.05$). The plasma reproductive-related hormones anti-Mullerian hormone **(AMH),** follicle stimulating hormone **(FSH)** levels significantly decreased in the LPH group ($P < 0.05$), and Leptin **(LEP)** significantly decreased in LP diets($P < 0.01$). Intestinal morphology and function analysis indicated that the LPH group significantly enhanced duodenal villus height-to-crypt depth ratio (**V/C**) ($P < 0.05$), up-regulated the expression levels of *oligopeptide transporter 1* (***PEPT1***) and *Solute Carrier Family 7 Member 1* (***SLC7A1***) ($P < 0.05$). The LPH group had lower short chain fatty acids (**SCFAs**) content, such as acetic acid and isovaleric acid ($P < 0.05$). Additionally, different LP levels and AA patterns significantly altered the cecal microbial composition in phylum and genus levels. At the genus level, *Desulfovibriowere* was negatively correlated with acetic acid ($r = -0.48$; $P < 0.01$), *Fournierella* were positively correlated with LEP ($r = 0.46$; $P < 0.01$), while *Methanocorpusculum, Desulfovibrio* and *Synergistes* were negatively correlated with LEP ($r = -0.46, -0.51, -0.45$; $P < 0.01$), *Rikenellaceae_RC9_gut_group* were negatively correlated with FCR ($r = -0.47$; $P < 0.01$), and *Methanocorpusculum* was negatively correlated with EP ($r = -0.46$, $P < 0.01$). In conclusion, the LP diet (CP 14.58% + 8 AAs) improved egg production performance without negatively affecting egg quality, while maintaining intestinal function in laying hens.

Keywords: Low-protein diet, Amino acids, Egg production performance, Egg quality, Intestinal function

70 Effects of protease on performance and hepatic lipid metabolism in laying hens fed low-protein diet with sustained-release amino acids

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The objective of this study was to evaluate the ability of protease supplementation to mitigate hepatic lipid accumulation and maintain performance in laying hens fed low-protein diets supplemented with sustained-release amino acids. A total of 504 36-week-old Nongda No. 5 laying hens were fed low-protein diets with sustained-release amino acids for eight weeks. The birds were randomly assigned to six treatments (six replicates each): a control diet (CON; CP 16%, ME 2,700 kcal/kg); low-protein diets (LP; CP 13%, ME 2,700 kcal/kg) with crystalline amino acids (LP-CAA) or microencapsulated amino acids (LP-MAA); and low-protein diets supplemented with protease (LP-P; CP 13%, ME 2,700 kcal/kg) with similar supplementation (LP-CAA-P and LP-MAA-P). The crystalline amino acids included Lys, Met, Thr, Arg, Ile, and Val. The sustained-release form consisted of 45% amino acids, 5% polyethylene glycol, and 50% lipid-based coating (hydrogenated palm oil and glycerol monostearate). The protease complex contained protease enzymes compounded with zeolite powder and exhibited a guaranteed activity of at least 100,000 U/g with a moisture content of no more than 8.0%. A two-way ANOVA followed by a Tukey test evaluated the main and interaction effects of amino acid forms and protease supplementation on production performance, egg quality, and fat metabolism-related parameters. The results indicated that low-protein diets had no significant impact on production performance ($P > 0.05$). LP-CAA significantly increased the liver index ($P < 0.05$) and the presence of hepatic lipid vacuoles and lipid droplets. It also increased hepatic TG, TCHO, and FFA ($P < 0.05$) as well as hepatic GSH-Px, GSH, and CAT ($P < 0.05$) and serum GSH-Px, GSH, and SOD ($P < 0.05$). Sustained-release amino acids significantly reduced these indicators ($P < 0.05$). LP-CAA down-regulated hepatic *CPT-1* expression ($P < 0.05$), while LP-MAA up-regulated *AMPK* ($P < 0.05$) and down-regulated *SREBP-1c* ($P < 0.05$). In conclusion, reducing dietary crude protein by three percentage points did not significantly impact production performance, but it did exacerbate hepatic lipid metabolism disorders. Low-protein diets supplemented with sustained-release synthetic amino acids alleviated hepatic lipid deposition. This was potentially achieved through the *AMPK-mTORC1* pathway by regulating the expression of *AMPK*, *SREBP-1c*, and *CPT-1* to suppress hepatic lipogenesis and enhance lipolysis. However, protease supplementation showed no significant impact on hepatic lipid metabolism in low-protein diets.

Keywords: Amino acid, Laying hen, Lipid metabolism, Low protein diet, Protease

71 Study on amino acid balance of low protein diet for Chinese domestic broilers aged 1–30 days based on response surface model

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The objective was to determine the optimal ratio among lysine (Lys), total sulfur amino acid (SAA), and threonine (Thr) in Chinese domestic broilers (Mahuang Chicken) based on response surface model (RSM). This trial was designed based on Box-Behnken Design (BBD), with 3 factors (Lys, SAA, and Thr) and 3 levels (80%, 100%, and 120% of recommended requirement of each tested AA). A total of 312 1-day-old female birds were allotted to 12 dietary treatments, with 6 replicates (cages) for each treatment and 4 birds per cage. Additionally, 6 cages were set as the response surface center points. The diets were formulated in accordance with Nutritional Requirements for Yellow-feathered Broilers (NY/T 3645-2020), and dietary protein was reduced from 21% to 19% as low protein diets. The average daily gain (ADG), feed to gain ratio (F:G), and feed intake were measured, and a 3-day total excreta collection was carried out from 14 to 16 days to estimate daily nitrogen retention (NR). The statisitics was carried out by R program (4.4.1). ANOVA was performed by using "aov" function. Specifically, based on the installed package "rsm", dietary level was designed by "bbd", and multivariate quadratic regression was performed by using "rsm". Additionally, "persp" and "image" function were used to obtain 3-D plot and contour plot. Optimal points were estimated based on "steepest" and "canonical.path". X_1, X_2, and X_3 are in relation with Lys, SAA, and Thr as follows: X_1=(Lys-0.98)/0.2; X_2=(SAA-0.71)/0.14; X_3=(Thr-0.66)/0.13; Results indicated that multivariate quadratic regression on ADG was $ADG=0.148\times X_1+0.175\times X_2-0.146\times X_1^2-0.326\times X_2^2-0.296\times X_3^2+15.795$ ($R^2 = 0.760$; $P = 0.006$); Multivariate quadratic regression on F:G was $F{:}G=-0.036\times X_1-0.050\times X_2+0.037\times X_3^2+2.018$ ($R^2 = 0.539$; $P = 0.057$) Multivariate quadratic regression on daily nitrogen retention was $NR=0.165\times X_1+0.206\times X_2-0.183\times X_1^2-0.310\times X_2^2-0.165\times X_3^2+0.293\times X_2X_3+2.143$ ($R^2=0.791$; $P=0.004$) To conclude, during 1-30 days, starter broilers achieved the optimal ADG of 15.86 g/d when dietary Lys, SAA, Thr were 1.09%, 0.75%, and 0.66%, respectively. The optimal F:G of 1.98 was obtained when dietary Lys, SAA, Thr were 1.16%, 0.83%, and 0.63%, respectively. The optimal daily nitrogen retention of 2.22 g/d was achieved when dietary Lys, SAA, Thr were 1.05%, 0.78%, and 0.62%.

Keywords: Response surface model, amino acid balance, low protein diet, nitrogen retention, Chinese domestic broilers

72 Maternal Selenium nanoparticle supplementation ameliorates intestinal health in the chicken offspring challenged with lipopolysaccharide

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This study evaluated how in maternal diets affect the jejunum's resistance to LPS in chicken offspring. A total of 450, 18-week-old Hy-Line Grey hens and 50 18-week-old Hy-Line Grey breeders were randomly allocated into five groups, with five replicates (18 hens and 2 roosters/replicate). The birds were fed either a non-supplemented basal diet (control) or the same basal diet supplemented with 0.3 mg/kg sodium selenite (SS), 0.3 mg/kg Selenium nanoparticles (Nano-Se), 0.3 mg/kg selenocysteine (Se-C), 0.3 mg/kg selenomethionine (Se-Met), for 8 weeks. Fertilized eggs were collected and incubated in the final week of the experiment. After hatching, chicks were fed a uniform diet for 7 days and then injected intraperitoneally with 1 mg/kg LPS solution for 3 consecutive days. The non-supplemented basal diet treatment was separated into two treatments, control and injected LPS. Compared to the control, LPS challenge resulted in a reduction of mRNA expression levels of tight junction and marker genes in intestinal cells, while elevating mRNA expression levels of apoptosis-related genes, NLRP3 signaling pathway genes, and ROS concentration in the jejunum. Supplementation with 0.3 mg/kg Nano-Se significantly enhanced the number of goblet cells and mRNA expression of tight junction and marker genes in intestinal enteroendocrine and Paneth cells . Maternal 0.3 mg/kg Nano-Se and 0.3 mg/kg Se-Met supplementation increased mRNA levels of intestinal *Muc2* and goblet cell differentiation factors *Klf4*. Additionally, 0.30 mg/kg Nano-Se reduced mRNA levels of apoptosis-related genes, lowered ROS concentration, and down-regulated NLRP3 signaling pathway. A stable in vitro culture system for 1-day-old chicken intestinal organoids was successfully established for evaluating the development of goblet cells, with optimized conditions for growth, passage, and cryopreservation. Nano-Se significantly increased the buds rate of organoids and increased the mRNA expression levels of *Muc2* and *Klf4* . The mRNA transcriptome sequencing showed that Nano-Se significantly changed the mRNA expression of genes related to inflammatory response pathway and MAPK signaling pathway in chicken intestinal organoids . q-PCR showed that Nano-Se significantly reduced the mRNA expression levels of *Tnf-α*, *IL-6*, *IL-8*, *Nlrp3*, *MAPK8*, *DUSP-1*, and *DUSP7* in chicken intestinal organoids. In conclusion, Maternal 0.3 mg/kg Nano-Se supplementation could ameliorate intestinal mucosal damage and alleviate intestinal inflammation in the chicken offspring challenged with LPS. It promotes the proliferation and differentiation of intestinal goblet cells by inhibiting the NLRP3-MAPK-DUSP signaling pathway, enhancing the formation of the intestinal mucus layer.

Keywords: chicken offspring, Nano-Se, goblet cells, NLRP3 signaling pathway, intestinal organoids

73 Not Presented

Metabolism and Nutrition: Enzymes and Feed Additives I

74 Feed additives: Their role in poultry productivity, welfare, and environmental sustainability

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As the leading source of animal protein for humans, poultry production has been on an upward trajectory. This growth is associated with increased challenges associated with resource use, production efficiency, bird welfare, environmental sustainability, and government regulations. Efficient poultry production is intricately linked with the genetics of the birds, the composition and quality of the diet, as well as the environment where the birds are raised. Feed additives comprise of a broad array of nonnutritive substances that are added to the diet of poultry to improve performance and health of the bird, as well as to enhance sustainable poultry production. The list includes, but is not limited to, exogenous enzymes, pre-, pro-, and post- biotics, phytogenic compounds, and antioxidants. The choice of what, when, and how it is supplemented and its effects on poultry may vary depending on the circumstance. However, there is a preponderance of information showing that feed additives do work and have contributed immensely to feed cost reduction, increased productivity, improvement in birds' health and welfare, as well as enhancements in environmental sustainability. Based on the preference of current consumers of poultry meat and egg, the role of feed additives in poultry production has become even more important. For a more efficient use of feed additives, an understanding of the mechanism through which they exert their effects on poultry production is important. As poultry production becomes more sustainable, the influence of consumers on poultry production such as the removal of antibiotic growth promoters from poultry diet as well as an increasing demand for organic poultry products have introduced another set of challenges that make pathogen control and environmental sustainability more difficult to achieve. These challenges, however, come with opportunities to further improve poultry production through a more efficient resource use with the use of feed additives to improve efficiency, health, welfare, and environmental sustainability. Some of these challenges could be mitigated through a better understanding of the potential roles these feed additives play in immune enhancement via microbiome modulation.

Keywords: environmental sustainability,, exogenous enzymes, feed additives, poultry, production efficiency

75 Supplementation effects of mepeta cataria root powder on production performance, biochemical parameters and egg quality in older laying hens

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This study focused on the effects of *Nepeta cataria* root powder supplementation on production and biochemical performance and egg quality in old laying hens. To conduct this experiment, 324 70-week-old Hy-Line Brown laying hens with a similar laying rate of (76.28 ±0.87 %) were divided into 3 treatment batches of 108 with six replicates of 18 hens each. These birds were housed in cages equipped with automatic drinkers and feed for 10 weeks on a reference layer feed supplemented at a rate of 0.75% for batch 1 (T1) and 1.5% for batch 2 (T2), but batch control (T0) did not receive *Nepeta cataria* powder. The temperature of the enclosure was the daily room temperature with 16 hours light per day. At the end of the 10th week of the trial, twelve treatment birds (2 per replicate) were randomly selected and fasted 12 hours before sampling. They were weighed and blood samples were taken from each hen via the brachial vein using a vacuum blood collection tube.All data obtained will be processed by the Graph pad Prism software using the one and two factor ANOVA (Analysis of Variance) test.The results showed that the egg-laying rate in the group that received 0.75% *Nepeta cataria* supplementation was higher than in the control group and those who received 1.5% *Nepeta cataria* during the 10-week trial. Supplementation of *Nepeta cataria* powder to 0.75% also had a significant effect on oviduct length, ileum weight, and SWF. At the 79th week of the trial, serum concentrations of luteinizing hormone, estrogen, and progesterone were significantly higher in the group supplemented with *Nepeta cataria* powder at 0.75% compared to the other groups. The biochemical parameters of laying hens that received *Nepeta cataria* supplementation improved. The 0.75% supplementation level affected certain egg quality parameters, namely shell thickness and yolk colour. In summary, supplementation of *Nepeta cataria* root powder at 0.75% improved egg production and quality in older laying hens.

Keywords: egg quality, laying hens, Nepeta cataria, production performance, supplements

76 The effects of polysaccharides from *Hypsizygus marmoreus* on intestinal barrier function and microbial diversity in broilers

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The gut is a critical site for nutrient digestion/absorption and an essential part of the poultry immune system, serving as a barrier to maintain internal environmental balance. Intestinal health has long been a key focus in poultry farming, making the discovery of green natural products for improving it highly significant. Polysaccharides from edible-medicinal fungi, as natural active metabolites, show diverse biological effects and hold broad prospects for enhancing poultry intestinal function. Mycelia polysaccharides (MPS) were extracted and purified from *Hypsizygus marmoreus* through a series of processes, including liquid fermentation, optimization of extraction parameters, purification, and freeze-drying. A total of 360 one-day-old broilers were randomly allocated into three groups, with each group consisting of eight replicates (15 birds per replicate). The chicks were fed a basal diet supplemented with 0, 500 or 1000 mg/kg body weight of MPS for three weeks (from day 1 to day 21). Dietary supplementation with 500 and 1000 mg/kg of MPS significantly increased jejunal villus length, the villus length-to-crypt depth ratio ($P < 0.05$). Additionally, these dosages significantly upregulated the protein expression of TLR4 and

NF-κB p65 in the jejunum (P < 0.05), along with the mRNA expression of *IL-8* (P < 0.05). Furthermore, MPS supplementation notably enhanced the mRNA levels of *CAT, SOD1, and GPX7* in the jejunum (P < 0.05) and the serum CAT content (P < 0.05). Supplementation with 1000 mg/kg of MPS significantly increased the mRNA expression levels of jejunal *Claudin-1* and *ZO-1* (P < 0.05) and mucosal *MUC2* in the duodenum and ileum (P < 0.05). Metagenomic analysis of cecal microbiota showed that 1000 mg/kg MPS supplementation regulated intestinal microbial diversity in broilers. The MPS group was dominated by *Bifidobacterium* and *Lactobacillus*. At the phylum level, it increased the abundances of *Bacteroidetes* and *Proteobacteria*, possibly due to polysaccharides serving as fermentation substrates and energy sources. At the genus level, it reduced the relative abundances of *Angelakisella* (*P* < 0.05) and *Erysipelatoclostridium*. EC enzyme library analysis revealed high expression of redox enzymes and glycosyltransferases in cecal contents, which may be associated with MPS fermentation and utilization by *Bifidobacterium* and *Lactobacillus*.

Keywords: Broiler, Polysaccharides, Intestinal Health, Gut Microbiota, Feed Additive

77 Not Presented

78 Not Presented

Animal Health

79 Exploring multiomics approaches for One Health-driven poultry health

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The continuing expansion of the poultry industry worldwide offers significant opportunities to improve food security and economic growth. However, intensification of production brings heightened risks at the intersection of poultry health, human health, and environmental health, including the spread of zoonotic pathogens, the emergence of antimicrobial resistance, and increased resource demands. Addressing these challenges requires a One Health–driven approach that integrates animal, public, and environmental health objectives into disease prevention and production strategies. This leadoff presentation will inform the construct of a multiomics-based framework for improving poultry gut health through targeted interventions, applied in health and disease- challenge conditions. The talk will provide details on how omics-informed insights can identify repeatable indicators of gut health and metabolic resilience from the context of recent investigations employing targeted probiotics and phytogenics. The speaker will also provide details on significant perturbations in the cecal metabolome and microbiome observed in Salmonella-challenge studies, which were effectively restored to near homeostasis, alongside upregulated networks and pathways linked to gut health and resilience. The talk will connect these findings to One Health sustainability goals, highlighting how novel insights can be scaled to diverse production systems, enabling the production systems to meet the demands of a growing global population without compromising animal and environmental health.

Keywords: broiler

80 Field observations on Necrotic Enteritis in broiler breeders in India

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Necrotic enteris (NE) is a serious intestinal disease of commercial broilers worldwide. The disease is characterized by necrosis and inflammation of gastrointestinal tract (GIT). The primary causative agent of NE is clostridium perfringens. C. perfringens is a Gram –positive aerobic spore - forming bacterium which is able to produce several enzymes and toxins responsible for causing disease. C perfringens is naturally found in the intestines of the healthy chickens. However, when exposed to certain conditions, it can multiply and produce toxins. These toxins cause necrosis of the intestine, hemorrhaging perforation of the intestine and eventual death from the septic shock. Outbreaks of NE are sporadic and the clinical form of NE short with birds dying within a day of when initial clinical signs are first observed. The most common clinical signs observed include ruffled feathers, depression and diarrhea. Most of the scientific literature available on NE is related with broilers. A very scanty literature is available on NE in broiler breeders. We addressed several issues of NE in broiler breeders raised in cages. In many cases direct relation was noticed between NE and peak production. In some cases oyster soiled shell grit awas identified as the source of clostridial infection. In one case limestone powder proved to be the source of infection. In all the cases tetracycline through drinking water proved to be the drug of choice and helped to control the mortality. Lincomycin and avilamycin proved to be a good choice to prevent the infection in the subsequent flocks.

Keywords: broiler breeders, necrotic enteritis, clostridia perfringens, tetracycline, lincomycin

81 The alleviating effects and mechanism of oleic acid on intestinal leakage caused by high-fat diet in Pekin ducks

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In our previous study of Zhang et al.(2025), we found dietary supplementation with oleic acid (OA), especially at a level of 0.50%, may decrease the serum TG content and promote lipid deposition in the jejunum in Pekin ducks. Many reports have been shown that high-fat diet (HFD) can cause intestinal leakage in poultry. However, there are no information about the effect of dietary OA on intestinal leakage in poultry. Therefore, the current study aims to explore whether OA can alleviate HFD-caused intestinal leakage in Pekin ducks by promoting the esterification of excess free fatty acids (FFAs) into lipid droplets (LDs) to avoid lipotoxicity. This study randomly divided 240 fourteen-d-old male Pekin ducks with an average body weight of 512.08±3.51 g into the following three groups: control group (Con, add 3% palm oil), High-fat group (HFD, add 9% palm oil), HFD plus 0.50% OA groups (HFD-OA). Each group comprised 8 replicate cages, each housing 10 ducks. The experiment lasted 28 days. On 42d, the meat ducks were subjected to a 12-hour fasting period prior to sampling. All the statistical analyses utilized the GLM procedure of SAS 9.2 software. The results showed that both Con and HFD-OA groups demonstrated notable reductions in serum FITC-dextran concentration compared to the HFD group ($P <$ 0.05); Concurrently, these two groups exhibited upregulated mRNA expression of tight junction proteins (*Occludin*, *Claudin*, and *TJP1*) along with downregulated *MLCK* expression in ileal mucosa ($P < 0.05$), which suggested OA supplementation improved intestinal barrier function and decrease intestinal leakage. OA supplementation showed enhanced utilization of dietary ether extract, Saturated fatty acid (SFA), C16:0 and C18:0 as well as serum FFA levels compared to the HFD group ($P < 0.05$). After fasting, the HFD-OA group exhibited significant downregulation of jejunal mucosal genes associated with LD formation (*PLIN2* and *DGAT2*) compared to the HFD group ($P < 0.05$). In contrast, LD degradation analysis revealed that the Con group showed suppressed *LPL* and *ATGL* expression ($P < 0.05$), whereas the HFD-OA group demonstrated a significant upregulation for both *LPL* and *ATGL* ($P < 0.05$). In conclusion, these results suggest that : Supplementation of OA in HFD can enhance the utilization efficiency of dietary SFA, it effectively reduces lipotoxicity of SFA while strengthening mucosal barrier integrity of Pekin ducks; At the same time, during the fasting period, the HFD_OA group may release FFAs

through the lipolysis of stored LDs in the jejunum to meet the ducks's energy demands, which could be an important reason for reducing intestinal permeability.

Keywords: oleic acid, intestinal barrier, utilization of fatty acid, free fatty acids, lipid droplets

82 Dietary resistant starch protects against antibiotic-induced intestinal damage by restoring microbial homeostasis and alleviating intestinal inflammation in meat duck

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Resistant starch (**RS**) is recognized as a nutritional strategy that supports gut and overall host health by modulating gut microbiota. Antibiotic-induced microbial dysbiosis has been widely used to understand the microbiota-host interactions following microbial disruption, and strategies for remediating these perturbations. Thus, to directly assess the effects of RS on gut microbiota and its role in improving intestinal barrier function in meat ducks, this study first established an antibiotic-induced microbial dysbiosis model (ampicillin 1.0 g/L and neomycin 0.8 g/L in drinking water supplied daily), which was characterized by reduced gut microbial diversity, intestinal dysfunction, and an inflammatory outburst following antibiotic exposure. Whereafter, in addition to the control group, ducks treated with antibiotics for 7 consecutive days were further allocated to two groups and fed the basal diet and RS diet that derived from 12% raw potato starch (Type 2 RS in native granular form) until 21 d. At 21 d of age, one bird per cage was selected and sampled. One-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was conducted to examine statistical significance. The results demonstrated that dietary RS supplementation reversed the antibiotic-induced reduction in microbial diversity and restored the Firmicutes-to-Bacteroidetes ratio. *Additionally, RS inclusion enriched beneficial bacterial genera, including Coprobacter, Odoribacter, and Faecalibacterium* (LDA score > 3). Post-antibiotic intervention led to a reduction in villus density and muscular thickness, accompanied by a significant downregulation ($P < 0.05$) of *zonula occludens-1* and *mucin-2* expression, along with increased serum pro-inflammatory cytokine levels ($P < 0.05$). Notably, dietary RS supplementation significantly enhanced ($P < 0.05$) the expression of *glucagon-like peptide receptor* and the anti-apoptotic factor *Bcl-2*, while suppressing caspase transcription. This resulted in increased villus height and muscular thickness in the ileum ($P < 0.05$). Furthermore, RS intervention remarkably reduced ($P < 0.05$) pro-inflammatory cytokine levels, particularly interleukin-1β and tumor necrosis factor-α, in both the ileum and serum. These effects were likely linked to alterations in cecal microbiota, including increased abundances of *Barnesiella*, *Ruminiclostridium 9*, *Megamonas, Faecalitalea, Adlercreutzia, Coprobacter* and *Collinsella*. In conclusion, dietary RS supplementation mitigated antibiotic-induced microbial dysbiosis and restored intestinal structure by promoting enterocyte proliferation and reducing apoptosis. Consequently, RS supplementation helped alleviate systemic inflammation in meat ducks following antibiotic treatment.

Keywords: raw potato starch, Gut microbiota, Inflammation, Antibiotic, Meat ducks

83 Not Presented

84 Intestinal stem cell dysfunction contributes to fatty liver hemorrhagic syndrome in laying hens via regulation of serotonin signaling

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Fatty liver hemorrhagic syndrome (FLHS) is a metabolic disorder that severely compromises the productivity and health of laying hens, with a growing incidence in recent years. Serotonin (5-hydroxytryptamine, 5-HT), a peripheral biogenic amine primarily synthesized in the intestine, is known not only for its role in neurotransmission but also for its critical function in regulating energy homeostasis and lipid metabolism. However, the mechanistic involvement of 5-HT in the pathogenesis of fatty liver in laying hens remains unclear. In this study, we established a FLHS model in laying hens to systematically investigate the alterations in gut–liver 5-HT signaling and its role in lipid metabolic regulation. Our results showed that 5-HT levels were significantly decreased in the serum of FLHS-affected hens, along with downregulation of key intestinal 5-HT synthesis genes (e.g., TPH1). Organoid culture experiments further demonstrated that intestinal stem cell proliferation and differentiation were markedly impaired in FLHS hens, potentially affecting gut-derived 5-HT synthesis and secretion. In vitro, 5-HT treatment of hepatocytes significantly downregulated the expression of lipogenic genes such as SREBP1 and FASN, thereby reducing lipid accumulation, while administration of a 5-HT receptor antagonist partially reversed these effects. Collectively, our findings suggest that intestinal stem cell dysfunction in FLHS may disrupt 5-HT production and secretion, subsequently impairing hepatic lipid metabolism. This highlights the gut-derived 5-HT signaling pathway as a key contributor to the pathogenesis of FLHS and a potential target for nutritional intervention.

Keywords: Fatty liver hemorrhagic syndrome (FLHS), 5-hydroxytryptamine (5-HT), Intestinal stem cells, Gut–liver axis, Lipid metabolism

85 The mechanism of mRNA m^{6}A methylation modification in regulating liver fat metabolism in laying hens

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This experiment aimed to investigate the liver fat metabolism and the regulation of m^{6}A methylation modification in normal laying hens and laying hens with fatty liver. Three hundred 78-week-old Jingfen 6 laying hens with similar body weight were selected and divided into two groups based on serum TG levels: the normal group (Con, TG < 7.5 mmol/L) and the fatty liver group (FL, TG > 15 mmol/L). The experiment lasted for 1 week of adaptation and 2 weeks of formal testing. Blood and tissue samples were collected. The results showed that: 1) Compared with the normal group, the fatty liver group significantly increased the content of triglycerides (TG) and total cholesterol in the liver ($P < 0.05$); 2) From HE staining, it could be seen that the liver tissue of the fatty liver group had obvious vacuoles. Oil Red O staining showed that the content of lipid droplets in the FL group significantly increased; 3)There was no significant effect on the expression of lipid metabolism-related genes PPARα, ACC, SREBP-1, ChREBP, LPL, PPAR-γ, ACOX1, IGF2,MTP, FABP4. However, the expression of CPT1, ATGL, VLDLR in the liver of the fatty liver group was significantly decreased ($P < 0.05$), while the expression of FAS was significantly increased ($P < 0.05$); 4)The expression of m^{6}A methylation-related genes WTAP and FTO in the fatty liver group was significantly decreased ($P < 0.05$); 5)The expression of lipid metabolism-related protein FASN in the fatty liver group was significantly increased ($P < 0.05$), while the expression of VLDLR was significantly decreased ($P < 0.05$), and the expression of m6A methylation-related protein FTO was significantly decreased ($P < 0.05$); 6)The total m^{6}A level in the fatty liver group was significantly lower than that in the normal group ($P < 0.05$). It can be seen that fatty liver in laying hens can cause the content of TG and TCHO in the liver to be significantly higher than that in the normal group; the total m^{6}A modification level is decreased; and the lipid metabolism-related genes FASN, CPT1, ATGL, VLDLR and m^{6}A-related genes WTAP, FTO may be involved in m^{6}A modification.

Keywords: m6A methylation, laying hens, liver, fat metabolism

86 Molecular studies of closely related vaccines against the avian coronavirus infectious bronchitis virus may reveal potential virulence determinants

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Infectious bronchitis (IB) is a ubiquitous disease caused by the IB virus (IBV), a member of the family Coronaviridae. Australia constitutes a very particular ecosystem, isolated from the rest of the world by geographical and biosecurity barriers. It offers a perfect environment to study the ecology and evolution of IBV. It has been previously demonstrated that the strains of IBV isolated in Australia are not to be found anywhere else (Quinteros et al. 2015; Quinteros et al. 2016). In this study, the complete genome of three very similar vaccine strains, VicS-v, VicS-del and Inghams where compared. These three vaccines, despite being very similar in terms of genomic sequence, differ in virulence. Previous studies have shown that VicS-del is the most attenuated subpopulation of the VicS vaccine (Hewson et al. 2012). As mentioned by Hewson et al. 2012, the Inghams vaccine (subtype B) has also been demonstrated to be more attenuated than VicS. A multiple alignment of their complete genomes using Clustal Omega revealed a nucleotide similarity of 99.8% between VicS-v and -del, of 99.9% between VicS-v and Inghams and of 99.7% between VicS-del and Inghams. As there are very little nucleotide differences between these vaccine strains, those differences could be easily related with their virulence differences. In addition to the changes previously described for VicS-del in comparison with VicS-v (Quinteros et al. 2015) it is important to mention that, out of the 15 different indels found, five occurred within the 4th gene, with others located within the genes 1ab (which occupies two thirds of the genome) and 2 (the last coding for Sgp, the most variable CDS in the IBV genome). In VicS-del, there is an early stop codon at the 4b peptide open reading frame (ORF), which can lead to a truncation or inhibition of that peptide. In Inghams, beside other point mutations, there was a premature stop codon at the 4c peptide ORF, which could also truncate or inhibit this peptide. According to these results, 4b and 4c peptides play an important role in the attenuation of IBV. Also, they appear as non-essential, as these vaccines have proven to be able to replicate both in vitro and in vivo. No changes were detected in genes 3 (containing the Egp CDS), 5 and 6 (the last containing the Np CDS). Future experiments utilising reverse genetics and deletion mutants generated under laboratory conditions might assist in confirming the role of these peptides in IBV attenuation/pathogenicity.

Keywords: avian coronavirus, infectious bronchitis virus, virulence, vaccines, genomics

87 Dietary supplementation with raw potato starch enhanced tibia mass of meat ducks potentially linked to intestine-derived inflammation

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Gait problem in poultry is a welfare issue with a prevalence of about 21% in commercial meat ducks, which also compromises growth performance by restricting access to feed and water. This study evaluated the effects of raw potato starch (**RPS**) on intestinal barrier function and bone metabolism in both healthy and enteritis-affected meat ducks. Initial characterization revealed that RPS consists of round or oval granules with a polygonal B-type crystalline structure, exhibiting an apparent metabolizable energy value of 2.67 kcal/g for 21-d-old meat ducks. Day-old male ducks (initial body weight was 51.4 ± 1.7 g) were assigned to 4 groups (6 replicates per group with 12 birds each) and fed diets containing 0, 6%, 12%, or 18% RPS until d 21. Linear and quadratic regression was conducted to examine the alterations of interested parameters response to dietary RPS levels. Broken-line regression analysis was used to evaluate the recommended levels of dietary RPS inclusion in diets using the nonlinear regression procedure. Dietary RPS supplementation dose-dependently increased tibia ash content and strength while reducing serum D-lactate levels and systemic inflammation in 21-d-old ducks. Broken-line analysis identified 12% RPS as the optimal inclusion level for improving bone properties and minimizing D-lactate concentrations (both $P < 0.05$). To further investigate the relationship between RPS, intestinal inflammation, and bone metabolism, an enteritis model was established using dextran sodium sulphate (**DSS**). A total of 288 ducklings with 47.8 ± 0.8 g body weight were allocated to a basal group, a basal diet and 12% RPS diet with DSS treatment group until 21 d. Each group had 8 replicates with 12 birds each. one-way analysis of variance followed by Tukey's *post hoc* test was performed to evaluate the effects of RPS and DSS on intestinal barrier function and bone parameters. DSS administration induced systemic inflammation and excessive bone resorption, impairing tibia density ($P < 0.05$) and mechanical properties. Dietary 12% RPS intervention mitigated gut barrier dysfunction and reduced ileal inflammatory cytokines in DSS-treated ducks. Additionally, RPS supplementation altered the gut microbiota by increasing Firmicutes ($P < 0.05$), decreasing Bacteroidetes ($P < 0.05$) and Proteobacteria, and enhancing the production of short-chain fatty acids (acetic acid and butyric acid, both $P < 0.05$). Consequently, RPS improved tibia microstructure and strength in DSS-exposed birds. These benefits were associated with reduced osteoclast activity, lower osteoclast numbers, and elevated serum phosphorus levels.

Keywords: Bone metabolism, Enteric inflammation, Raw potato starch, Meat duck

88 Shifts in the jejunal and cecal microbiota of broilers with subclinical enteric infection

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Even though mild enteric infections do not cause gross lesions or mortality, they can significantly decrease broiler growth performance. In fact, the lack of gross lesions makes these subclinical infections more difficult to diagnose. The aim of this study was to examine the jejunal and cecal microbiota of broilers with a subclinical enteric challenge impacting their performance. Metagenomic profiling was used to identify markers of a disturbed intestinal microbiota in more granular detail. The experimental design included an unchallenged and a challenged group. The challenged group received a tenfold dose of a live coccidia vaccine on day 14, followed by oral administration of *Clostridium perfringens* on day 18. On day 21, jejunal and cecal contents were collected from nine birds per group. DNA was extracted and submitted for shotgun Illumina sequencing. After trimming, raw sequence reads were mapped to the core nucleotide BLAST database. Alpha diversity analyses revealed no significant differences in microbial richness or evenness within the jejuna (Kruskal-Wallis, $P > 0.05$). However, Shannon, Simpson, and Fisher indices indicated significantly reduced microbial diversity in the ceca of challenged birds (Kruskal-Wallis, $P < 0.05$), despite no observable differences in beta diversity in either intestinal segment (PERMANOVA > 0.05). Taxonomic composition was analyzed using Kraken2 and Bracken. Taxonomic profiling using DESeq2 revealed significant compositional shifts in both intestinal segments. Challenged birds demonstrated a marked depletion of beneficial *Lactobacillus* species in the jejuna ($P_{adj} < 0.01$) and a significant reduction of commensal *Bacteroides* in the ceca ($P_{adj} < 0.001$), along with increases in potential opportunistic pathogens like *Pseudomonas savastanoi* and *Flavobacterium* species ($P_{adj} < 0.05$). To complement these findings, functional analysis using HUMAnN3 with DESeq2 revealed significant metabolic shifts. In the jejuna, key fermentation pathways were downregulated, and pyrimidine nucleotide biosynthesis was upregulated ($P_{adj} < 0.001$). The cecum showed increased peptidoglycan biosynthesis and reduced purine and amino acid metabolism, including L-arginine and L-ornithine biosynthesis ($P_{adj} < 0.05$). These findings reveal segment-specific microbial responses to subclinical enteric infection. The jejuna and ceca showed significant taxonomic and functional disruption, with the jejuna particularly affected in *Lactobacillus* abundance and fermentation, while the ceca exhibited broader metabolic dysfunction involving cell wall and nucleotide metabolism. This highlights variation in microbial responses between segments and the importance of site-specific analyses in enteric disease research.

Keywords: functional characterization, intestinal microbiota, broilers, subclinical

89 Not Presented

90 Evaluating the efficacy and economic impact of a phytogenic anticoccidial (COXIELD) alone and in combination with Diclazuril in broiler challenge trials

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Coccidiosis, caused by Eimeria spp., continues to impose substantial challenges in poultry production by impairing growth performance and elevating mortality rates, with global economic losses estimated at USD 14.4 billion annually (Blake et al., 2020). The increasing resistance to synthetic anticoccidials and the limitations of vaccination strategies have intensified the search for effective phytogenic alternatives. This study aimed to evaluate the efficacy and economic impact of COXIELD, a phytogenic anticoccidial, when used alone or in combination with Diclazuril, through two independent challenge trials. In Trial 1, 375 Ross broilers were assigned to five treatment groups (75 birds per group): unchallenged Diclazuril (0.02%), challenged Diclazuril (0.02%), COXIELD (0.02%), phytogenic A (0.015%), and phytogenic B (0.025%). From day 11 to 31, birds were fed respective diets and challenged orally on day 14 with a 20× dose of Livacox-Q vaccine. Body weight (BW) and feed conversion ratio (FCR) were measured on day 31. In Trial 2, 1600 broilers were randomly distributed into four treatment groups (4 replicates × 100 birds): challenged control (basal diet), Diclazuril (0.02%), COXIELD (0.02%), and COXIELD+Diclazuril (0.02% each). Treatments were administered from day 11 to 35. Outcomes included BW, FCR, Production Index (PI), and Income Over Feed Cost (IOFC). All additives were incorporated into the basal diet as follows: Diclazuril (Sigma) 0.02%, COXIELD (CJ BIO) 0.02%, phytogenic A (K) 0.015%, and phytogenic B (E) 0.025%. Birds were reared in floor pens under standard commercial conditions with ad libitum access to feed and water. Data were analyzed using one-way ANOVA. Statistical significance was declared at $p < 0.05$. Results: In Trial 1, birds supplemented with COXIELD achieved a BW of 1303 g and FCR of 1.65, closely approximating the unchallenged Diclazuril group (1321 g, FCR 1.63), and significantly outperforming the phytogenic comparators. In Trial 2, the COXIELD+Diclazuril group showed the most favorable results (BW: 1865 g; FCR: 1.44; PI: 317; IOFC: 25,440 Rp/bird). COXIELD alone improved IOFC by 0.05 USD/bird compared to the challenged control, and co-application with Diclazuril added 0.03 USD/bird over Diclazuril alone. COXIELD demonstrated equivalent efficacy to Diclazuril and outperformed other natural alternatives.

Notably, its combination with Diclazuril provided synergistic improvements in productivity and profitability while potentially contributing to resistance management. These results support COXIELD as a sustainable component of integrated coccidiosis control strategies in broiler production.

Keywords: Coccidiosis, COXIELD, phytogenic feed additive, broiler performance, Diclazuril synergy

Immunology, Microbiology & Physiology

91 Poultry immune responses to enteric challenges

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Historically, the chicken has served as a valuable model organism for studying the early development and delineation of the various arms of the immune system. As we continue to gain better understanding and more knowledge of the bird's fundamental immunology and immune responses, poultry research still lags significantly behind mammalian model animals, especially for tools and reagents that are critical to investigating certain systems. This deficient state presents major immunological challenges, particularly when studying mucosal and enteric immune responses to common poultry pathogens facing commercial production. Central to more recent discoveries have been the characterization of several cytokines and chemokines, as well as research tools to assess intestinal permeability, kinomes and phosphorylation, metabolic responses, organoid cultures, microbiome profiles, etc. Disease outbreaks pose significant challenges across the global poultry industry. While the gastrointestinal system is considered that largest 'immune organ' of the bird, the alimentary canal is the major port of entry for several species of those enteric and food-borne pathogens including Eimeria, Clostridium, Salmonella, and many others. For instance, enteric challenges such as avian coccidiosis and its 'sidekick' necrotic enteritis are two critical diseases affecting chickens and threatening sustainable poultry production. As per any enteric encounter, the bird's immune system responds via both innate and adaptive elements along with an array of non-specific intestinal defenses. Other pathogens, such as Salmonella, are capable of surviving inside the host cells without causing any clinical manifestation to the bird and can do so via sequestration and evasion of the immune system. The complexity of these pathogens necessitates and triggers a myriad of host reactions to the various antigenic molecules that such parasites and bacteria present to the local immune system. Therefore, a single assessment of the immune response is often inadequate to fully understand the true host response in a particular context. Adding to this complexity is the large variation and inconsistency of the immune responses among birds within and across studies as they often tend to be more circumstantial to each research setting and field conditions. This abstract will attempt to dissect some of these issues encountered in poultry immunological research especially those encompassing several enteric challenges as often is the case in commercial settings. It will also review recent tools aiding us to further elucidate such complex responses.

Keywords: immunity, poultry, enteric, tools, immune response

92 *Ex Vivo* chicken intestinal model for studying *Limosilactobacillus reuteri* CCM 9458 effects on gut integrity in salmonellosis

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Foodborne zoonotic infections such as salmonellosis represent a continuous threat to consumers, as the primary source of infection is the consumption of inadequately cooked poultry meat and eggs. Due to the growing issue of antibiotic resistance, the use of natural substances—such as probiotic bacteria—is gaining increasing popularity. In accordance with the 3R principles, *ex vivo* models are being used to evaluate their effects without extensive animal testing. In this study, we investigated the impact of *Limosilactobacillus reuteri* CCM 9458 on the gene expression of selected markers (occludin-1, claudin-1, zonula occludens-1, E-cadherin, LGR-5), as well as the production of the inflammatory biomarker calprotectin in ileal explants from chickens during *Salmonella* Enteritidis PT4 infection. The experimental scheme included four groups of chicken ileal explants prepared in 96-well plates: control, *L. reuteri* CCM 9458 (LR) (1×10CFU/well), *S.* Enteritidis PT4 (1×10CFU/well), and a combined group (LR + SE – co-incubated). The explants were incubated at 37 °C with 5% CO2 for 4 hours, then washed and collected for Real-Time quantitative PCR (to assess relative gene expression) and ELISA analysis (to measure calprotectin production). Statistical analysis of the obtained results was performed using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism version 8.00. Our results demonstrated a protective anti-inflammatory effect of *L. reuteri* CCM 9458, reflected in beneficial modulation of gene expression across all tested parameters and a significant reduction in acute phase protein production. These findings highlight the ability of this probiotic strain to maintain and enhance intestinal barrier integrity during salmonella infection. This work was funded by the Slovak Research and Development Agency under the contract no. (APVV-21-0129) and VEGA 1/0098/22.

Keywords: ex vivo, explant, limosilactobacillus, chicken

93 The Role of TLR4/NF-κB Pathway in the regulation of intestinal barrier function by Hypsizygus marmoreus polysaccharides in broiler chickens

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This study aimed to extract, purify polysaccharides (MPS) from Hypsizygus marmoreus mycelium, characterize their physicochemical properties, and evaluate immunomodulatory effects on primary chicken intestinal epithelial cells via TLR4/NF-κB and antioxidant pathways. The research comprised three phases. First, H. marmoreus was fermented in Potato Dextrose Agar (PDA) and seed medium. After mycelium extraction, the samples underwent degreasing, water bath extraction, alcoholic precipitation, and Sevage deproteinization. Then, DEAE-52 anion-exchange chromatography yielded three fractions: MPS-1, MPS-2, and MPS-3. Physicochemical characterization used high-performance gel permeation chromatography (HPGPC) for molecular weight, trifluoroacetic acid (TFA) hydrolysis with 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatization for monosaccharide composition, and Fourier-transform infrared spectroscopy (FT-IR) for structure analysis. In the functional analysis, cells were treated with MPS fractions, induced by lipopolysaccharide (LPS), and subjected to NF-κB gene knockdown. Experimental groups included blanks, MPS-treated, MPS fraction-treated, LPS-treated, and combined treatment groups. Assays involved CCK-8 for viability, immunofluorescence for NF-κB translocation, Western blot and qPCR for protein/gene expression, and siRNA transfection for NF-κB knockdown. Data analysis applied one-way ANOVA with Tukey's post-hoc test and Student's t-test ($P < 0.05$). Results showed MPS fractions differed in molecular weight (11,055–16,191 Da) and monosaccharide composition. MPS and MPS-1 were rich in glucose and galactose, while MPS-2 had complex sugars. MPS at ≥4 mg/mL reduced cell viability, but 0.5–2 mg/mL had no significant effect. MPS and MPS-2 upregulated Occludin, ZO-1, TLR4, MyD88, and phosphorylated Nrf2/HO-1, inhibited LPS (40 μg/mL)-induced NF-κB translocation. NF-κB knockdown enhanced Claudin-1/Occludin and antioxidant genes when combined with MPS/MPS-2. In conclusion, H. marmoreus-derived MPS modulated the TLR4/NF-κB pathway to relieve LPS-induced inflammation and activated antioxidant pathways via Nrf2/HO-1. Among them, MPS-2 showed the strongest activity, indicating its potential as a functional food additive or intestinal health therapeutic.

Keywords: Hypsizygus marmoreus, Polysaccharide, Immunomodulation, TLR4/NF-κB pathway, Antioxidant

94 Harnessing apidaecin capability to improve intestinal health and inhibit Salmonella Typhimurium transmission in laying hens

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Apidaecin, an antimicrobial peptide, has been demonstrated to suppress *Salmonella Typhimurium* (*S. Typhimurium*) in vitro and improve the growth performance of broiler by intestinal barrier function. This study aimed to assess the effects of apidaecin on the intestinal health, as well as its potential to alleviate *S. Typhimurium* infections on laying hens and reduce egg contamination. A total of 288 45-wk-old Hy-Line grey layers were randomly assigned to 4 groups with 6 replicates (12 birds each replicate). The control group (Con) was fed a basic diet. The Api300, Api500 and Api700 groups were fed a basic diet supplemented with 0, 300, 500 and 700 mg/kg apidaecin respectively for 12 weeks. Based on production performance results obtained at the end of the feeding trial, 18 laying hens were selected from each of the Con and Api500 group for *S. Typhimurium* challenge. The inhibitory effects of apidaecin on *S. Typhimurium* adhesion and invasion were determined using an in vitro cultured intestinal epithelial cell model. For comparisons between two independent sample groups, an independent samples t-test was performed using SPSS 23. For the analysis of the effect of different apidaecin supplementation levels, one-way analysis of variance (ANOVA) was conducted in SPSS 23. Results indicated that daily feed intake, crypt depth and villus height to crypt depth ratio (V/C) of intestinal tract were quadratic functions with apidaecin supplementation ($P < 0.05$). Apidaecin modulated the gut microbiota by increasing populations of beneficial bacteria (e.g., *Phascolarctobacterium* and *Odoribacter*) that produce short-chain fatty acids (SCFAs), while decreasing pathogenic bacteria (e.g., *Actinobacillus*) associated with toxin production ($P < 0.05$). These beneficial effects were maximized at 500 mg/kg apidaecin. Apidaecin improved intestinal health in laying hens challenged with *S. Typhimurium*, as evidenced by increased jejunal and ileal V/C ratios and elevated ileal sIgA expression ($P < 0.05$). In addition, apidaecin reduced *S. Typhimurium* loads in the ileum, spleen, liver, and on eggshells ($P < 0.05$). *In vitro* cell culture assays revealed that apidaecin reduced *S. Typhimurium* adhesion to and invasion of intestinal epithelial cells ($P < 0.05$). Overall, these findings support the potential application of apidaecin in poultry feed formulations to enhance intestinal health and ensure the food safety of egg products.

Keywords: Apidaecin, Intestinal health, Laying hen, Salmonella, Food safety

95 Yeast β-glucan ameliorates uterine inflammation and eggshell defects in laying hens via microbial-derived UDCA and macrophage M2 polarization along the gut–reproductive tract axis

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Uterine inflammation is a critical factor in the decline of reproductive performance and eggshell quality in aging laying hens. This study aimed to establish a reliable inflammatory model and investigate the protective role of yeast β-glucan (BG), focusing on gut microbiota–bile acid interactions. Three independent trials were conducted. In trial 1, intrauterine inflammation was induced by perfusing a mixed bacterial suspension (MBS) in young (26-wk) and aged (72-wk) laying hens. In trial 2, aged hens were fed 0, 400, or 800 mg/kg yeast BG for 12 weeks, followed by MBS perfusion. In trial 3, chicken macrophage cell line HD11 was used to verify the anti-inflammatory mechanism of UDCA following LPS stimulation. Tissue and serum samples were collected post-treatment. Microbiota (16S and metagenomic), serum metabolomics, uterine transcriptomics, bile acid profiling, and gene/protein expression analyses were performed. Results showed that MBS perfusion caused uterine inflammation in both young and aged hens, marked by villus atrophy, epithelial barrier damage (↓Occludin, ZO-1), and elevated uterine/serum pro-inflammatory cytokines. ESS also reduced eggshell calcification thickness and induced structural abnormalities. Serum metabolomics and gut microbiota profiling revealed that MBS suppressed secondary bile acids (↓UDCA, LCA, IsoLCA) and beneficial microbes (↓*Bifidobacterium*, *Lactobacillus*, *Faecalibacterium*), while increasing potentially pro-inflammatory genera (*Sutterella*, *Fusobacterium*). Yeast BG supplementation significantly alleviated MBS-induced uterine injury, restored epithelial barrier function, and reduced inflammatory cytokines (IL-1β, TNF-α, IL-6). It improved eggshell microstructure and thickness. Metagenomic analysis showed that BG altered the microbiota in a glycosidic linkage-specific manner, enriching *Akkermansia*, *Muribaculum*, and glycoside hydrolases GH1, GH3. BG also enhanced microbial bile acid metabolism (↑BSH, ↑7α-HSDH), increasing UDCA and the SBAs/PBAs ratio. Mediation analysis showed that microbial UDCA significantly accounted for 46.3–72.5% of the BG-induced reduction in uterine inflammatory cytokines. In HD11 macrophages, UDCA promoted M2 polarization and suppressed the FXR–NF-κB/NLRP3 signaling pathway, confirming its anti-inflammatory potential. In conclusion, yeast BG mitigates uterine inflammation and restores eggshell quality in aged hens by modulating the gut–reproductive tract axis. Microbial production of UDCA plays a central role in this process, partially through regulating macrophage polarization and inflammatory signaling.

Keywords: uterine inflammation, secondary bile acids, gut–reproductive tract axis, macrophage polarization, yeast β-glucan

96 Novel QTAP-Nanovaccines against emerging avian influenza isolates in chickens

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Avian influenza (AI) remains a significant threat to the global poultry industry and public health authorities, worldwide.Recent isolates of H5N1 causing outbreaks in poultry flocks with lateral transmission to cattle represent a significant development in the evolution of highly pathogenic avian influenza (HPAI). We hypothesize that efficient delivery of immunogenic antigens against HPAI will generate robust, protective immunity against challenge with HPAI. To address this hypothesis, our group designed plasmid vaccine and mRNA constructs adjuvanted with QuilA-based nanoadjuvant system that encodes mosaic hemagglutinin (mHA) antigen.The mHA antigen represented > 20,000 sequence viruses. The designed vaccine constructs were tested in a vaccine/challenge experiment using strains belong to low pathogenicity avian influenza (LPAI) as a first level of screening for vaccine immunogenicity.Birds were vaccinated twice (prime on day 1 – boost on day 14). When tested in day-old chicks, QTAP-nanovaccine constructs directed against AI resulted in significant induction of robust immune responses.Notably, one-Way ANOVA compared to control group indicated that a single subcutaneous (SQ) dose of QTAP-mHA mRNA vaccine elicited significantly elevated hemagglutination inhibition (HI) titers compared to other groups. Our results showed that all QTAP-formulated vaccines elicited high HI titer (~8-16 log2), only 2 weeks after the first vaccine dose. Notably, 2 weeks after the booster dose, HI titers significantly increased (~32-64 log2). Moreover, these HI were similarly high when another antigen (H5N2) was used for the HI titration. Finally, we analyzed the generated cell-mediated immune (**CMI**) responses post-vaccination using chicken IFN-γ ELIspot in splenocytes. At 28 days of age, spleens were collected and processed from vaccinated chickens. ELIspot analysis showed that all QTAP-formulated vaccines stimulated the activity of T-cell inside spleen to various degrees, which differ according to the type of nucleic acid used (DNA or mRNA) and the vaccination route (SC for subcutaneous or IM for Intramuscular). In conclusion, data analyzed so far indicate that QTAP-adjuvanted vaccines could induce early, cross-reactive systemic anti-influenza antibodies. Moreover, both SQ/ON (subcutaneous/oculo-nasal) and SQ/SQ administration of QTAP-mHA induced heightened levels of serum IgY and IgA.Comparisons with existing studies suggest promising prospects for broadening the application of nucleic acid vaccines to combat HPAI and other emerging poultry infections.

Keywords: High pathogenicity avian influenza, Nanovaccines, nucleic acid vaccines, Nanoadjuvant, H5N1

97 The evaluation study on probiotic function and fat deposition regulation of *Streptococcus alactolyticus* in broilers

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Excessive abdominal fat deposition is detrimental to the healthy growth and economic efficiency in broilers chicken production. Our previous study found that the abundance of *Streptococcus alactolyticus* was higher in the cecum of broilers with lower abdominal fat deposition regulated by folic acid. The current study was carried out to investigate the probiotic potential and anti-adipogenic function of *S. alactolyticus*. The whole-genome sequencing of bacteria revealed that *S. alactolyticus* possesses 40.45% GC content and comprises 117,998 scaffolds. A total of 28 virulence-associated proteins and 41 antibiotic resistance-related proteins were predicted. Probiotic efficacy evaluation in vitro revealed that the strain exhibited excellent acid (pH 2-4) and bile salt (0.1%-0.3%) tolerance, showing resistance to trimoxazole and cephalothin, with intermediate resistance to erythromycin and ciprofloxacin. To further evaluate the role of *S. alactolyticus* in regulating fat deposition, a total of 200 one-day-old broilers were divided into control (CON) and *S. alactolyticus* perfusion (SA) groups with 10 replicates. From 14 to 28 days of age, birds in the SA group received oral perfusion of 1 mL live *S. alactolyticus* (1×108 CFU/mL) everyday, while the CON group received an equal volume of PBS. Unpaired student's t test or special omics analysis methods were employed in the study. Results showed that *S. alactolyticus* perfusion significantly increased breast muscle percentage and reduced abdominal fat percentage and adipocyte diameter at 28 days. Moreover, lowered serum glucose and total cholesterol levels were found in SA group as well as downward trend for triglycerides level. In addition, genes expression of CDK1, PCNA, ELOVL6, C/EBPα, FABP4, SREBP1, PPARγ and adiponectin were down-regulated in abdominal fat tissue by *S. alactolyticus* perfusion. Furthermore, *S. alactolyticus* treatment also altered the diversity and composition of cecal microbiota with decreasing the ratio of *Firmicutes*/*Bacteroidetes*. Serum untargeted metabolomic analysis identified 181 differential metabolites including 118 upregulation and 63 downregulation when compared with the control. Lysine degradation, valine, leucine and isoleucine biosynthesis, purine metabolism, biosynthesis of amino acids, lipoic acid metabolism, 2-oxocarboxylic acid metabolism and tryptophan metabolism were significantly enriched based on differential metabolites. Overall, these results implied that *S. alactolyticus* exhibited probiotic potential and could reduce abdominal fat deposition in broilers through inhibiting adipocytes proliferation and differentiation and altering gut microbiota.

Keywords: broiler, abdominal fat, gut microbiota, Streptococcus alactolyticus

98 Breaking the chain: Probiotic strategies to combat *Salmonella* in broilers from hatch to harvest

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Poultry meat is a major source of foodborne salmonellosis, highlighting the need for sustained farm-to-fork interventions. *Salmonella* Enteritidis (SE) can enter the broiler production pipeline through contaminated hatching eggs, infecting hatchlings and facilitating further spread during transport. This early colonization can lead to flock-wide dissemination on grow-out farms. To address this risk, we evaluated a probiotic-based approach targeting three critical stages: (i) egg disinfection, (ii) inclusion in water replacers during transport, and (iii) in-feed supplementation. Probiotic supplementation along the hatchery-to-farm continuum can significantly reduce (i) the delivery of *Salmonella*-positive chicks to grow-out farms and (ii) subsequent flock-wide *Salmonella* spread, thereby enhancing poultry meat safety and improving public health. A total of 820 Ross 308 hatching eggs were inoculated with *Salmonella Enteritidis* (SE; ~7 log CFU/egg) and treated by spraying with either PBS (control), *Lactobacillus rhamnosus* B-442 (LR; ~8 log CFU/egg), *Lactobacillus paracasei* 13076 (LP; ~8 log CFU/egg), or 0.4% peracetic acid (PAA). Eggs and internal contents were sampled throughout incubation to monitor SE populations. After hatching, chicks were held for 24 hours in transport boxes containing water replacers with or without probiotics to simulate transport conditions. Subsequently, chicks were moved to floor pens and fed starter rations with or without probiotics for 10 days. At multiple time points, chicks were sacrificed to quantify SE colonization in the liver, spleen, and cecum. The study followed a completely randomized design, and data were analyzed in R with significance set at $P \leq 0.05$. Application of LR, LP, and PAA significantly reduced SE populations on hatching egg surface versus controls ($P < 0.05$). Although PAA was effective at surface decontamination, it allowed significantly greater trans-shell migration of SE than LR and LP. Eggs treated with probiotics showed markedly lower SE levels in the embryonic gut (~1.75 log CFU/g) at days 18 and 20 of incubation versus control and PAA (~3.5 log CFU/g), indicating probiotics limit early pathogen colonization. This reduction persisted through hatch and transport simulation. Further, in-feed LR and LP supplementation reduced SE by over 2 log CFU/g in chicks during the grow-out phase. These findings demonstrate that a multi-hurdle probiotic intervention across the hatchery-to-farm continuum effectively disrupts SE transmission, offering a sustainable, feasible intervention. Overall, this strategy improves flock health and enhances poultry meat safety, ultimately benefiting public health.

Keywords: Salmonella control, probiotics, Hatchery-to-farm continuum, poultry meat safety

99 Modulation of hepatic lipid metabolism in laying hens by three different polysaccharides via intestinal microbes

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This work aimed to investigate the effects of three structurally distinct soluble polysaccharides, β-glucan, arabinoxylan, and pectin, on hepatic lipid metabolism in laying hens during late-laying period, focusing on their interactions with gut microbiota and associated metabolites. Results: Two hundred and eighty-eight 63-week-old Jing fen No. 6 laying hens were randomly assigned to four groups and fed with one of the following diets: the basal diet (control, CON), the basal diet supplemented with 2 g/kg pectin, Arabinoxylan (AX), or β-glucan. The result shows that the pectin and β-glucan groups significantly increased laying rate ($P < 0.05$), egg mass ($P < 0.05$), and no marked effects on production performance by Pectin, AX, and β-glucan groups at week 8 ($P > 0.05$). Additionally, β-glucan group notably increased eggshell strength ($P < 0.05$) and showed a tendency to decrease cecal weight ($P = 0.079$) at week 8, decreased liver weight at week 4 ($P < 0.05$). At week 4, supplementation of Pectin, AX and β-glucan markedly reduced hepatic triglyceride (TG) content ($P < 0.05$). However, by week 8, significant reductions were observed only in the Pectin and β-glucan groups ($P < 0.05$). Hepatic lipid deposition was significantly decreased in the Pectin, AX and β-glucan groups at week 4 ($P < 0.05$), but only Pectin and β-glucan groups sustained this effect through week 8 ($P < 0.05$). At week 4, the expressions of fibroblast growth factor 19 (*FGF19*), 5-hydroxytryptamine (*5-HT*), glucagon-like peptide 1 (*GLP-1*) and ghrelin related genes in the Pectin and β-glucan groups were significantly increased ($P < 0.05$). However, at week 8, only the β-glucan group notably increased the expression of *FGF19* ($P < 0.05$). In the Pectin group, the abundances of *Paraprevotella* and *Bacteroides-H* were significantly increased ($P < 0.05$), and the content of Isodecanoic acid was significantly increased ($P < 0.05$). Spearman's correlation analysis revealed that *Faecousia* abundance exhibited positive associations with duodenal *5-HT2A* expression ($P < 0.05$) and lipid-related differential metabolites in the pectin/β-glucan groups ($P < 0.05$), while demonstrating a negative correlation with plasma TG content ($P < 0.01$). Concurrently, *Ligilactobacillus* showed significant positive correlations with short-chain fatty acid (SCFAs) ($P < 0.01$) and negatively correlated with hepatic TG content ($P < 0.01$). Conclusions: Dietary supplementation of pectin and β-glucan alleviates hepatic lipid accumulation, with the involvement of altered gut microbiota and metabolite profiles.

Keywords: Laying hens, Intestinal microbes, Polysaccharides, Intestinal hormone, Hepatic lipid metabolism

100 Effect of dietary L-Citrulline on the thermoregulation and metabolic rate of broiler chickens under acute stress conditions

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This study investigated the effects of L-Citrulline (L-Cit) on the thermal physiology of poultry under stress conditions, and elucidates the involvement of nitric oxide (NO) and its actions in the regulation of body temperature by L-Cit. Three hundred and eighty-four, 1-day-old broiler chicks were randomly divided into 4 groups, 8 replicates of 12 chicks, and managed until 20 days old. The birds were fed two dietary treatments of basal diet (CON) or basal diet with 1% L-Cit (LCT), then utilized for subsequent experiments. Broilers were subjected to conditions of acute heat stress and immune stress at 21 days old birds using a 2 by 2 factorial arrangement. Birds pre-fed with CON or LCT diets were randomly divided into 4 treatments, 8 replicates, and 3 chickens for each replicate and managed in a respiratory chamber. In trial 1, broilers were grouped into CON, CON+HS, LCT, and LCT+HS groups, and subjected to acute heat stress. The CON+HS and LCT+HS groups were housed at 35°C, while the CON and LCT groups were maintained at 24°C for 5 hours.Compared with the CON, the LCT increased the oxygen consumption of broilers. Broilers exposed to HT had higher oxygen consumption than TNZ broilers ($P < 0.001$). LCT had a higher carbon dioxide expired than control broilers ($P < 0.01$). TNZ broilers had lower heat production than HT broilers ($P < 0.001$). In the skin tissue, the amount of TRPV1 mRNA increased with LCT diet ($P \leq 0.05$).In the hypothalamus, the mRNA expression levels of TRPV1 and TRPM8 were upregulated under HS exposure compared with TNZ condition ($P \leq 0.05$). In trial 2, broilers were randomly divided into 4 groups of CON, CON+LPS, LCT, and LCT+LPS for the acute immune stress study. The CON and LCT birds were provided with saline (sham control), while the CON +LPS and LCT + LPS groups were injected intraperitoneally with lipopolysaccharide (LPS, 2 mg/kg BW), and monitored at 24°C for 5 hours post-injection.Diet and injection had a significant interaction effect on the core body temperature of broilers ($P = 0.001$), which made the LCT+LPS group significantly lower than the CON+LPS and LCT+saline groups. Additionally, the core body temperature of CON+LPS broilers was higher than that of CON+Saline group ($P < 0.05$). Under heat stress conditions, broilers regulate body temperature by increasing respiratory thermogenesis, and injection of LPS would lead to an increase in broiler body temperature, therefore, it would also increase the thermogenesis of broilers. TRP channels are involved in the regulation of body temperature. Carcinine can reduce the body temperature of bro, and the addition of carcinine will increase the expression of TRPV1 to regulate body temperature.

Keywords: L-Citrulline, Lipopolysaccharide, body temperature

101 Regulation of intestinal phosphorus homeostasis in commercial laying hens throughout an extended egg production cycle

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Commercial layers are kept in production until close to 100 wks of age, exacerbating economic and welfare concerns related to reduced eggshell quality and skeletal integrity in older hens. These changes are partially attributed to alterations in mineral homeostasis that make uptake and utilization of Ca and P less efficient. While most studies have focused on Ca because it is the predominant mineral in eggshells, P regulation is closely linked to that of Ca and it is an important component of the hydroxyapatite layer on the outer eggshell and within medullary bone. Fibroblast growth factor (FGF) 23 mediates P homeostasis by increasing renal excretion when circulating levels are high. Based on FGF receptor (FGFR) expression patterns observed previously, FGF23 could also influence intestinal P uptake. The objective of this study was to investigate how regulation of intestinal P homeostasis changes over an extended egg production cycle. Mucosa from the proximal ileum was collected from Nick Chick hens (H&N International) during early (25 wks), mid (43 wks), late (80 wks), and extended (95 wks) production at hours post-oviposition (HPOP) representing bone mineralization (1.5 HPOP), eggshell calcification (15 HPOP), or transitions between them (6 and 21 HPOP), and distal ileal digesta was collected at 1.5 and 15 HPOP (n=12 hens/HPOP/age). Expression of FGFRs and P transporters was measured by RT-qPCR, and digestibility was determined by ICP-OES using titanium dioxide as an inert marker. Data were analyzed by ANOVA followed by Fisher’s LSD test. Significant main effects of age and/or HPOP were observed for FGFR1–4 and sodium-dependent phosphate transporters *P_it-1*, *P_it-2*, and *NaP_i-IIa* ($P \leq 0.05$). *FGFR1* was lower at older ages, while FGFR2–4 increased from 25 to 43 wks, remained constant at 80 wks, and decreased again at 95 wks. Both *P_it-1* and *NaP_i-IIa* increased between 25 and 43 wks, with *P_it-1* remaining elevated and *NaP_i-IIa* decreasing at 95 wks. *P_it-2* steadily declined between 25 and 95 wks. Levels of *FGFR4* and *P_it-1* were lower at 6 and 15 HPOP than at 1.5 and 21 HPOP. Significant age-by-HPOP interactions were observed for Ca and P digestibility ($P \leq 0.05$), with decreases between 25 and 95 wks for both minerals at 1.5 HPOP. At 15 HPOP, Ca digestibility did not differ with age, but P digestibility declined between 25 and 80 wks. These findings indicate that intestinal P uptake may be regulated by FGF23 and suggest that declines in mineral digestibility during periods of medullary bone repletion contribute to reductions in eggshell quality and skeletal integrity in older hens. Further, they highlight the need for additional investigation into the role that P homeostasis plays in eggshell and bone mineralization.

Keywords: ileum, hydroxyapatite, fibroblast growth factor 23, phosphorus transport, mineral digestibility

102 Potential for vertical transmission of *Enterococcus cecorum* in broilers through microbiome analysis

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Enterococcus cecorum has evolved into a significant pathogen of poultry. Clinical outbreaks in broilers cause septicemia and locomotive disorders such as bacterial chondronecrosis with osteomyelitis and enterococcal spondylitis. While horizontal transmission is documented and *E. cecorum* has been identified in yolk sacs, and the timing of early disease suggests vertical transmission, definitive evidence is lacking. We conducted a study to analyze evidence of vertical transmission by using 16S rRNA sequencing analysis from intestinal and extraintestinal samples collected from ten 47-week-old female broiler breeders and their eggs (Experiment 1), including small intestine, ceca, reproductive tract, cloaca, and eggshell swabs. Additionally, whole intestines of day of hatch (DOH) chicks from a different flock, as well as small intestines at D3 and D10, were evaluated (Experiment 2). Experiment 1 samples were stored in saline at -80°C before DNA extraction, purification, and amplification (16S rRNA V4, F515/R806), followed by sequencing on the Illumina MiSeq platform at UNC Microbiome Core. In Experiment 2, DNA samples were sequenced using Illumina MiSeq with 2 × 300 paired-end reads. Experiment 1 revealed that the *Enterococcus* genus comprised 3.96% of total microbiome from internal hen samples. *Enterococcus_E* was the most prevalent genus, with *Enterococcus cecorum* as the dominant species. *Enterococcus* abundance varied by sample site, highest in the small intestine, followed by cloaca, eggs, then reproductive tissues. ANOVA ($p>0.05$) showed no significant differences, suggesting uniform distribution and that presence at one site does not promote vertical transmission over another. This demonstrates that presence of *E. cecorum* in a breeding system may increase the likelihood of pathogen transmission to broilers. In Experiment 2, the *Enterococcus* genus was present at 3.73% on Day of Hatch (DOH), which increased significantly in the upper intestine (UI) to 53.39% by D3, and remained relatively high at 37.68% on D10, indicating that low-level presence of *Enterococcus* at DOH can lead to significant presence as broilers age. Microbial richness and diversity did not differ between treatments ($p > 0.05$) at D3 and D10. The detection of *Enterococcus* at DOH, along with its presence in the cloaca and eggs, suggests potential vertical transmission. To improve hatchery biosecurity and early disease detection techniques, it is essential to understand the possibility of vertical transmission of *Enterococcus cecorum* in broilers. If maternal transfer contributes to early colonization, breeder-level interventions may reduce *E. cecorum* in offspring, minimizing production losses.

Keywords: Enterococcus, microbiome, broiler, enterococcal spondylitis, vertical transmission

103 Comparative effects of protected butyrate forms on immune and inflammatory responses in broilers

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Protected butyrate in poultry feed can overcome the rapid absorption of free butyric acid, thereby enhancing gut health and systemic immunity. We evaluated three protected forms by measuring antibody titers and key blood markers of immune function. A total of 600 day-old Hubbard broilers were randomly assigned to four dietary treatments (10 pens per treatment) for 35 days: a negative control without acidified products, organic acids or probiotics (NC); NC+70% sodium butyrate protected by MCFA salts (SB); NC+60% di-/triglycerides of butyrate (TB); and NC+54% sodium butyrate protected by buffer salts (BB). All additives provided an equivalent active butyric acid level of 54%. Diets were pelleted and formulated according to FEDNA (2021) guidelines for starter (0–14d), grower (15–27d), and finisher (28–35d) phases. Blood was collected and analyzed for antibody titers and the concentrations of IgG, IgM, IgA, TNF-α, IL-6, and IL-10. Data were tested for normality (Shapiro–Wilk, Kolmogorov–Smirnov) and homogeneity of variance. Based on results, log-transformed ND titers analyzed using the Kruskal–Wallis test, and IBD markers were analyzed by one-way ANOVA with Tukey's test. Additionally, bootstrapping (20,000 iterations) was applied to validate those values. Results showed highest IgG in SB (465.3 ng/mL), followed by TB (443.0), BB (428.2), and NC (328.5) ($p<0.001$), indicating enhanced humoral immunity, especially with SB. IgA followed a similar pattern, highest in TB (617.7), then SB (581.0), NC (473.7), and BB (459.8) ($p<0.001$), suggesting improved mucosal and systemic responses in SB and TB fed birds. IgM remained relatively stable (284.7–305.2), with NC at 291.8 ($p=0.12$). The cytokine profile supported the immunomodulatory role of the additives. TNF-α declined in all treated groups vs. NC (503.2), with the lowest values in SB (358.2), BB (359.5), and TB (388.3) ($p<0.001$), indicating reduced inflammation. Similarly, IL-10 increased across treatments, highest in SB (525.0), followed by TB (493.5), BB (467.2), and NC (438.8) ($p<0.001$), reflecting improved immune regulation. IL-6 showed no notable change ($p=0.23$; range: 448–483). In the case of ND and IBD titers, non-differences among treatments for IBD, and higher values were obtained for SB (3.89±0.15) in ND titers ($P<0.05$) in the first analyses. Additionally, results from bootstrap showed differences among treatments for both traits, with larger values for SB ($P<0.05$) in ND (3.90±0.16) and IBD (9110±440) titers, reinforcing SB effect on humoral immunity. Finally, protected butyrate, especially SB, enhanced humoral responses and modulated inflammatory markers, underscoring the importance of delivery form in maximizing systemic immune benefits.

Keywords: Broilers, Butyric Acid, Immunity, Cytokines, Antibody Titers

Metabolism and Nutrition: Enzymes and Feed Additives II

104 Effects of exogenous protease on postabsorptive energy metabolism of broiler chickens

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Exogenous proteases are widely used enzymes to improve the digestibility of dietary proteins and amino acids in broiler chickens. This study was conducted to investigate the effects of exogenous protease on postabsorptive energy metabolism of broiler chickens. Day old male chicks were purchased from commercial chicken farms and randomly divided into 3 treatment groups with 6 replicates of 8 birds each. Chicks from each treatment group were either fed with the corn-soybean meal basal diet, or the basal diet supplemented with 2500 U/kg protease A or protease B. The feeding trial lasted for 28 days, and feed intake and body weight were recorded. One Day 22, 15 birds having the average body weight of each treatment group were chosen and subjected to indirect calorimetry experiments. Indirect calorimetry experiments were carried out in the self-developed closed circuit respiration chambers. The birds from the same group were randomly divided and allocated to 5 chambers, allowed to adapt for 3 days and then subjected to the calorimetry analysis for 3 days. Oxygen consumption and body weight gain were automatically recorded and analyzed by the Chambers. Carbon dioxide was automatically collected to quantify with the barium chloride method. Feces were also collected to measure metabolizable energy and uric acid. All data was subjected to one-way ANOVA analyses to determine treatment effects and Pearson's correlations to establish the relationship between energy metabolism and growth performance. The results showed that 2500 U/kg protease A and protease B did not significantly alter the body weight and average daily feed intake as well as the digestibility of dietary energy and crude protein ($P < 0.05$) on Day 28. However, protease A and B had different effects on postabsorptive energy metabolism. Protease B tended to have lower total heat production ($P = 0.112$) and heat increment ($P = 0.104$) but higher net energy ($P = 0.069$) than the basal diet. Protease B also significantly increased net energy deposition as fat ($P = 0.042$). In those birds fed with protease A oxidized more glucose for energy supply ($P = 0.031$) than the counterparts fed with the control diet and protease B. Pearson's analyses demonstrate significant correlations between ADG and AME intake ($P < 0.001$, $R^2 = 0.58$) or NE intake ($P < 0.001$, $R^2 = 0.67$). Taken together, exogenous proteases could modulate postabsorptive energy metabolism without changing energy and protein digestion in broiler chickens. Different proteases had different regulatory effects on postabsorptive energy partition and substrate oxidation for energy supply. The study was supported by National Key R&D Program of China (2021YFD130030402).

Keywords: Protease, Postabsorptive metabolism, Energy partition, Substrate Oxidation

105 Not Presented

106 The effects of dietary supplementation with grape seeds and onion peel extracts on the production performance and egg yolk flavor of laying hens during late laying period

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Eggs are a nutritious, affordable and sustainable food and are consumed globally. The flavor of eggs directly affects consumers' perception of quality and their willingness to purchase. In this study, flavoromics was applied to investigate whether dietary supplementation with grape seeds and onion peel extracts (GSOE) could improve the production performance and the formation of egg yolk flavor precursors in late laying hens. A total of 256 healthy 63-week-old Hy-Line Gray laying hens were randomly divided into four groups, with eight replicates per group and eight hens per replicate. The control group was fed a basal diet, while the experimental groups received the basal diet supplemented with 160, 320, and 480 mg/kg of grape seeds and onion peel extracts (GSOE160, GSOE320, and GSOE480, respectively, supplied by Cargill Animal Nutrition (Nanning) Co., Ltd.) for 8 weeks. Compared with the control group, dietary supplementation with GSOE significantly increased the laying rate, total egg production and total egg weight in the entire experimental period. Post hoc multiple comparison analysis showed that supplementation with 160 mg/kg GSOE were significantly increased the total egg production and total egg weight. It was shown that a total of 143 volatile compounds were identified in yolks by a two-dimensional gas chromatography (GC × GC) coupled with time-of-flight mass spectrometry (TOF/MS) , which is mainly fresh, rich and fruity, with a more herbal aroma including 34 specific volatile compounds. Compared with the control group, 2-heptenal, (E)-, 2-octanone, 2, 3-octanedione, dimethyl sulfoxide, 2, 6-lutidine and thiazole were significantly upregulated in GSOE160 group providing a sweeter green aroma for the egg yolk. While benzene, 1,4-dichloro- and undecane were significantly downregulated and reduced the pungent smell of egg yolks. It is worth noting that 1-OCten-3-one mainly provides a fresh grassy aroma, and 2-Octenal (E)- provides a sweet fruity aroma in VOCs. The sensory flavor primarily originates from hydrocarbons, aldehydes, ketones, and organic heterocyclic compounds. Among them, 1-octen-3-one mainly provides a fresh grassy aroma, 2-octenal, (E)- contributes a sweet fruity flavor, and 1-pentanol imparts a mild fatty note. This study demonstrates that supplementing the diet with 160 mg/kg GSOE effectively enhances the laying performance of hens during late laying period. Furthermore, it reveals that GSOE plays a significant role in shaping the sensory flavor characteristics of the yolk, which provides a scientific basis for improving egg yolk flavor.

Keywords: Egg yolk flavor, Grape seeds, Onion peel, Laying hens, production performance

107 Effects of mixed polyphenols or herbal complexes on production performance and oviduct health in laying hens

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Salpingitis is a common disease in laying hens during the late laying phase, posing a severe threat to the economic efficiency of the poultry industry. This study conducted to investigate the effects of mixed polyphenols (MP mangosteen polyphenol: rosmarinic acid=1: 1) or herbal complexes (HC, dandelion: motherwort: codonopsis: caulis spatholobi=4: 2: 3: 1) on production performance, anti-inflammatory and antioxidant capacity, and oviduct health in laying hens, providing experimental evidence for improving production performance and ensuring oviduct health in late-phase laying hens. In the experiment,288 Hy-Line Grey laying hens (55 weeks old) were randomly divided into three groups: CON group (basal diet), MP group (400 mg/kg MP), and HC group (2,000 mg/kg HC), with 8 replicates per group (12 hens/replicate) for an 8-week trial. Results: (1) compared to CON, the MP group significantly reduced feed-to-egg ratio, serum MDA content, and ALP activity ($P \leq 0.05$), while increasing jejunal mucosal mRNA expression of ZO-1, ZO-2, and Occludin ($P<0.05$). (2) the HC group significantly improved production performance, egg quality, serum AST activity, and reduced pro-inflammatory cytokine levels in the jejunal mucosa and oviduct ($P<0.05$). It also increased ovarian GSH-Px activity and isthmus IL-6 content ($P \leq 0.01$). (3) compared to MP, the HC group significantly elevated serum MDA and GSH content ($P \leq 0.05$) while reducing isthmus TNF-α and uterus MDA levels ($P \leq 0.01$).In summary, dietary supplementation with 400 mg/kg mixed polyphenols or 2,000 mg/kg herbal complexes enhances antioxidant capacity, reduces the expression of the pro-inflammatory factor IL-6, and improves production performance in laying hens.

Keywords: Salpingitis, Mixed polyphenols, Herbal complexes, Laying hens

108 Effects of bile acids on growth performance and meat quality of broilers feeding different energy and protein levels

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Reducing metabolizable energy (ME) and crude protein (CP) levels in broiler diets is a common strategy to decrease production costs; however, this practice may adversely affect growth performance and meat quality. Bile acids (BA) are natural compounds known to facilitate lipid digestion and nutrient absorption, which may compensate for nutrient restrictions and improve meat quality in broilers. This study investigated the effects of BA supplementation in diets with different ME and CP levels on breast meat quality of broilers. A total of 1,456 one-day-old male Cobb500 broilers were randomly divided into seven treatments with eight replications each (26 birds/replicate) using a completely randomized design. The experimental diets were corn-SBM basal diets with T1) control diet, T2) basal diet -50 kcal/kg ME, T3) basal diet -50 kcal/kg ME + 200 g/ton BA, T4) basal diet -50 kcal/kg ME in starter and basal diet -50 kcal/kg ME – 1% CP in grower and finisher, T5) basal diet -50 kcal/kg ME + 200 g/ton BA in starter in starter and basal diet -50 kcal/kg ME – 1% CP + 300 g/ton BA in grower and finisher, T6) basal diet -100 kcal/kg ME and T7) basal diet -100 kcal/kg ME + 200 g/ton BA (Runeon, Shandong Longchang Animal Health Product Co. Ltd., China). All chicks were raised in an evaporative cooling system house. Feed and water were provided ad-libitum for 45 days. At the end of the trial, 1 bird per replication was processed to evaluate breast meat quality (pH, drip loss, thaw loss, cooking loss, and shear force). Data was analyzed using one-way ANOVA (SAS software). Under the conditions of the present study, there were no significant differences on growth performance ($P>0.05$). The inclusion of BA in the diet improved FCR, FCG and mortality). The addition of BA significantly decreased drip loss and thaw loss ($P<0.05$), improving water-holding capacity, particularly in diets with reduced energy and protein. Higher pH values at 0 hours were observed in protein-reduced diets ($P<0.01$), indicating improved meat quality. There were no significant differences in cooking loss and shear force ($P>0.05$). It can be concluded that BA supplementation improving growth performance and effectively mitigates the negative effects of reduced dietary energy and protein on broiler meat quality, supporting its potential use in economically efficient poultry feeding programs.

Keywords: Broiler, Bile acid, Meat quality, Metabolizable energy and crude protein reduction

109 Effects and mechanism of carnosic acid on performance and intestinal health of lipopolysaccharide-challenged broilers

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This research was performed to investigate protective effects of carnosic acid (CA) on growth performance, intestinal barrier, and cecal microbiota of lipopolysaccharide-challenged broilers. Three hundred 1-day-old yellow-feathered broilers (male) were allocated randomly into 5 treatments, with 6 replicates per treatment, and 10 birds per replicate cage. Birds in both the control group (CON) and the lipopolysaccharide-challenged group were provided with a basal diet, while others were fed a basal diet supplemented with 20, 40, and 60 mg/kg CA (CA20, CA40, CA60), respectively. At 17, 19, and 21 days of age, birds were injected intraperitoneally with lipopolysaccharide (500 μg/kg body weight), except those in CON, which were injected with saline. Compared with challenged birds, the CA20, CA40, and CA60 increased ($P < 0.05$) the final body weight, average daily gain, and average daily feed intake, as well as decreased diarrhea rate in CA40 and CA60. Compared with challenged birds, CA reduced ($P < 0.05$) plasmal levels of *D*-lactic acid and endotoxin, increased ($P < 0.05$) the villus height to crypt depth ratio, and the number of goblet cells in duodenum. The CA40 and CA60 elevated ($P < 0.05$) relative expression of cell junction proteins (*Claudin-1/-2* and *ZO-1/-2/-3*) and *MUC-2* in duodenum, while decreased ($P < 0.05$) relative expression of *TLR2*, *TLR4*, and the concentrations of IL-6, IL-10, TNF-α, TGF-β1 in duodenum. Compared with challenged birds, in duodenum, 20 or 40 mg/kg CA

decreased the MDA level, increased the activity of T-AOC and CAT, furthermore, 40 mg/kg CA also increased the activities of T-SOD and GSH-Px ($P < 0.05$). Compared with challenged birds, 40 mg/kg CA significantly upregulated the relative expression of mitochondrial antioxidant enzymes (*Trx2*, *TrxR2*, *Prx3*, and *Mn-SOD*). CA40 also increased ($P < 0.05$) the α-diversity of the cecal microbiota and boosted ($P < 0.05$) the relative abundance of beneficial phyla and genera, particularly Firmicutes, *Anaerofilum*, and *Papilibacter*. In conclusion, dietary supplementation with CA showed protective effects on the growth performance and intestinal health in LPS-challenged broilers by inhibiting the production of inflammatory cytokines, strengthening the tight junction in intestinal epithelial cells, increasing the antioxidant capacity and enhancing the diversity of microbiota and the relative abundance of beneficial bacteria. When supplemented to diet of broilers, 40 mg/kg CA was recommended.

Keywords: carnosic acid, lipopolysaccharide challenge, broiler, intestinal health, mitochondrial antioxidant enzymes

110 Not Presented

111 Broiler chicken responses to incremental levels of dietary yeast-based nucleotides reared under high-stocking density

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High stocking densities (HSD) are commonly employed by farmers to maximize profit. However, they lead to oxidative stress in poultry, which is normally mitigated by antibiotic growth promoters (AGP). The routine use of AGP in food-producing animals has been linked to the development of antibiotic-resistant bacterial strains, resulting in significant public health concerns. This study, therefore, investigated the effect of using graded levels of yeast-based nucleotides (NCL) as a dietary substitution for antibiotic growth promoters (AGP) in densely stocked Ross 308 broilers. The North-West University Animal Production Research Ethics Committee approved (NWU-00816-24-A5) the protocols used in this study. A total of 900, one-week-old male Ross 308 broiler chicks were randomly allotted into 36 pens (25 birds/1.2m2). The birds were raised on standard broiler diets formulated to contain 0% yeast-based nucleotides and no AGP (NCON); 0% yeast-based nucleotides and 0.05% AGP (PCON); 0.025% yeast-based nucleotides and no AGP (NCL1); 0.05% yeast-based nucleotides and no AGP (NCL2); 0.075% yeast-based nucleotides and no AGP (NCL3); and 0.10% yeast-based nucleotides and no AGP (NCL4), each treatment was replicated 6 times. Feed intake and body weights were measured after each production phase. At day 42, the birds were slaughtered to measure visceral organs and carcass and meat quality parameters. The data was analyzed using the general linear model procedure of SAS (2013) for analysis of variance, with diet as the only main factor. The relationship between diets and response variables for all measured parameters was determined using response surface regression analysis (PROC RSREG; SAS, 2013). For all statistical tests, the significance was declared at $P < 0.05$. Positive quadratic responses were observed for feed intake ($P < 0.05$) in the starter phase, as well as the average body weight gain ($P < 0.05$) and feed conversion ratio ($P < 0.05$) in the finisher phase as the NCL levels increased. Neither linear nor quadratic trends ($P > 0.05$) were observed on shear force, cooking loss and drip loss. However, differences were observed in thigh colour, where diet NCL3 promoted higher thigh-yellowness ($P < 0.05$) than all other treatment groups except for NCL1. No treatment differences were observed ($P > 0.05$) for thigh-redness and thigh-lightness. Dietary supplementation with yeast-based nucleotides at 0.05% promoted efficient feed utilization, however, at higher rates nucleotides negatively affected bird performance metrics.

Keywords: Antibiotic resistance, Meat quality, Nucleotides, Performance, High stocking density stress

112 Combined antioxidants are more effective than single antioxidants in alleviating oxidative stress in the gut-liver-muscle axis of broilers by modulating the TLR-NF-κb pathway

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Oxidative stress caused by dietary oxidized lipids impairs poultry health. This study evaluated the effects of antioxidant combinations in protecting gut, liver, and muscle health. In vitro analyses first quantified lipid peroxidation in oxidized vs fresh oils. Subsequently, 144 broilers received oxidized/fresh oil diets to evaluate growth performance and oxidative stress responses. Finally, 360 one-day-old male broilers were randomly allocated to five dietary treatments (6 replicates, 12 birds each): 1) Control (oxidized diet as basal diet), 2) basal diet + 54 g/ton BHT, 3) basal diet + 90 g/ton EQ, 4) basal diet + 18 g/ton BHT + 15 g/ton EQ (BE), and 5) basal diet + 18 g/ton BHT + 15 g/ton EQ + 9 g/ton citric acid (BEC), to systematically compare their protective effects. The result indicated that oxidized oils exhibited severe lipid peroxidation, indicated by elevated MDA, acid value, peroxide value, and TOTOX versus fresh oils. Feeding oxidized oils impaired growth performance and caused oxidative damage in intestinal, hepatic, and muscular tissues, evidenced by increased ROS, reduced SOD/CAT/GSH-Px activities, and NF-κB activation. Among antioxidants, BEC showed superior efficacy: compared to BE and single antioxidants, it significantly lowered feed conversion ratio ($P<0.05$), increased breast muscle ratio, and enlarged muscle fiber area ($P<0.05$). Mechanistically, BEC most effectively reduced ROS ($P<0.001$) and upregulated GPX7/SOD/CAT in intestine/liver/serum ($P<0.001$), while enhancing gut barrier integrity via MUC-2/ZO-1/Occludin/Claudin-1, reducing hepatotoxicity markers (AST/ALT/ALP/ADase), promoting muscle development through MyoG/MyoD/MyHC, and suppressing proteolysis markers (TRIM63/Mafbx/Caspase-3). Notably, BEC maximally inhibited NF-κB-mediated inflammation by suppressing p65 phosphorylation ($P<0.001$). The study identifies the BEC ternary antioxidant system as superior to individual/binary formulations in broilers, showing enhanced multi-tissue protection against oxidized oil-induced stress

through synergistic gut-liver-muscle axis regulation, offering an optimized strategy for sustainable poultry production under oxidative stress.

Keywords: Oil oxidation, Combined Antioxidants, Gut-liver-muscle axis, Inflammation, Broilers

113 Not Presented

114 Impact of different dosages of yeast-based nucleotides on growth performance, meat quality, and physiological responses of densely-stocked Jumbo *Coturninx* quail

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Yeast-based nucleotides (YbN) are safe alternatives to antibiotic growth promoters (AGP) that can potentially ameliorate high stocking density-induced stress and enhance product quality in quail. However, the most effective supplementation level of dietary YbN in place of AGP is unknown for the Jumbo quail. This study, therefore, evaluated the inclusion of incremental levels of YbN in densely-stocked Jumbo *Coturnix* quail diet on growth performance, carcass characteristics, meat quality, and haematological and serum biochemical parameters. A total of 450, 7-day-old unsexed quail chicks (20.1 ± 0.12 g live-weight) were distributed into 30 multitier cages based on body weight and randomly assigned with 5 dietary treatments, each with 6 replicate cages holding 15 birds. The cages measured 100 cm long × 60 cm wide, with a floor space of 0.6 m^2 including the space occupied by the oval feeder and water-fountain tube. The treatments were a standard quail diet containing zinc bacitracin AGP (PosCon), a standard quail diet without zinc bacitracin and YbN (NegCon), and NegCon containing with 0.025 (YbN25), 0.05 (YbN50) and 0.075% YbN (YbN75). Growth performance metrics were recorded until day 35 of age and all the birds were slaughtered for post-mortem measurements. Data were analysed using PROC GLM in SAS version 9.4 with the cage designated as the experimental unit, and significance declared at $P < 0.05$ using Tukey HSD test for LSmeans. Polynomial regression analysis was also used to evaluate the data for linear and non-linear responses ($P < 0.05$). Results showed no dietary YbN influence ($P>0.05$) on feed intake, weight gain, gain-feed ratio, mortality, carcass characteristics and breast meat quality attributes. The highest YbN level (0.075%) caused heavier relative spleen weights ($P < 0.05$) than the other treatment groups. Birds fed diet YbN50 had higher ($P < 0.05$) serum calcium levels than birds on the other treatments. Glucose levels were higher in birds fed diet YbN25 than the PosCon group. It was concluded that supplementation of YbN as an alternative to zinc bacitracin AGP does not enhance the performance, carcass and meat quality of stressed Jumbo quail. Furthermore, dietary inclusion of YbN could compromise blood filtering and some serum biochemical indices in quail.

Keywords: Antibiotic growth promoters, Performance, Jumbo quail, Nucleotides, High stocking density stress

Metabolism and Nutrition: General Nutrition II

115 Developments and challenges in poultry nutrition

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Poultry production continues to be one of the most efficient and sustainable animal protein sectors. Nutrition has been pivotal to achieving results due to genetic advances in growth rate, meat yields, and egg production under variable environmental conditions. However, there is room for improvement. This presentation will discuss the latest developments in energy systems, methods to quantify amino acid (AA) content, digestibility, and mineral bioavailability. Relations between apparent metabolizable energy (AME), AME corrected to zero nitrogen retention (AMEn), and the proposed net (NE) and productive energy (PE) systems will be discussed. Most NE predictions require accurate measurement of the AME value of the feed, which needs to be better standardized among researchers. However, NE also needs accurate measurement of total and fasting heat production using nutritionally balanced diets that should include the same ingredients under evaluation. This data has been more challenging to obtain due to methodological difficulties. The controversy surrounding the potential minor improvements in efficiency or prediction ability when using NE or PE in poultry will be discussed. A short. overview of methods to determine nutrient requirements will lead to modeling advances, status, and future perspectives. Difficulties in broiler breeder nutrition will be discussed. Variability of nutrient and antinutrient content, quality post-processing, and digestibility in feedstuffs is a constant challenge in daily feed formulation. Current options to obtain, manage, and apply that information will be presented. Near-infrared spectroscopy (NIRS) has been employed as the primary rapid method for feedstuff and feed analysis. Prediction of proximate values, AA, fiber components, and phytate content has received more attention. Effects of heat during processing on feedstuffs are also considered critical for feedstuff quality valuation. All this information is critical for effectively reducing dietary nitrogen content, strategically adding enzymes, and enhancing phosphorus and energy utilization. Proximate analysis is becoming obsolete due to the availability of updated chemical information. Therefore, equations to predict energy values and AA content based on proximate values are less accurate than the direct predictions based on specific chemical components present in feed and feces. The available information should be applied in feed formulation; however, the least-cost formulation based on linear programming has limitations. Non-linear and stochastic programming for feed formulation has been considered and developed, but it remains underutilized. The difficulties in applying this dynamic information will also be discussed.

Keywords: energy, nutrient variability, amino acids, food formulation, NIRS

116 Not Presented

117 Dietary fiber modulates gut microbiota to mitigate antibiotic resistance gene dissemination in broiler chickens

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This study investigated the effects of dietary inulin supplementation on cecal microbiota, fecal antibiotic resistance genes (ARGs), and growth performance in yellow-feathered broilers, aiming to provide a nutritional strategy for improving gut health and attenuating ARGs transmission. A total of 144 one-day-old male yellow-feathered broilers were randomly divided into 4 groups (6 replicates per group; 6 birds per replicate): a control group (CON), a florfenicol group (FFC, 25 mg/kg BW florfenicol), a 3% dietary fiber group (LFD, 3% inulin), and a 6% dietary fiber group (HFD, 6% inulin). The trial period was 56 days. The results showed that the feed-to-gain ratio (F/G) in the HFD was significantly lower than that in the CON and FFC. Meanwhile, the F/G in the LFD was significantly lower than in the FFC and extremely significantly lower than in the CON. Jejunal index was significantly higher in LFD and HFD than in CON, and significantly higher in HFD than in FFC. Cecal index was extremely significantly higher in HFD than in CON and FFC. The results of cecal microbial analysis showed that HFD had an extremely significantly lower relative abundance of *Lawsonibacter* than CON. *Petroclostridium* and *Lawsonibacter* abundance in LFD was significantly lower than in CON. *Massilioclostridium*, *Eisenbergiella*, and *Papillibacter* abundance in HFD was significantly lower than in CON. Compared to FFC, LFD had significantly lower *Petroclostridium* and *Ruminococcus*, *Enterocloster*, *Massilioclostridium,* and *Lawsonibacter.* HFD had significantly *Enterocloster*, *Massilioclostridium*, *Petroclostridium*, *Lawsonibacter*, *Papillibacter*, and *Ruminococcus*. Metagenomic analysis of feces revealed dietary fiber intervention significantly reduced the relative abundance of the *LAP-2* gene. Decreasing trends were observed for *aadA2*, *arr-3*, *cmlA1*, *dfrA12*, *mphA*, *sul3*, *oqxA*, *ogxB*, and *gacH*. Among individual mobile genetic elements (MGEs): *IS26,* Tn916-orf13, Tn916-orf15,Tn916-orf16, and Tn916-orf19, Tn916-orf17 and *IstA2* abundance were extremely significantly lower in LFD than in CON and FFC. Dietary inulin supplementation improves gut health in yellow-feathered broilers and reduces the relative abundances of specific fecal ARGs. This study preliminarily clarifies the effects of dietary inulin on gut health and ARG dynamics in broilers, thereby providing a promising nutritional strategy to mitigate ARG dissemination in poultry production.

Keywords: broilers, dietary fiber, nutritional intervention, gut microbiota, antibiotic resistance genes

118 Not Presented

119 Effects of metabolic energy on performance and hepatic lipid metabolism in laying hens fed low-protein diet with sustained-release amino acids

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The objective of this study was to evaluate whether reducing the metabolic energy of the diet could mitigate hepatic lipid accumulation and maintain performance in laying hens fed low-protein diets supplemented with sustained-release amino acids. A total of 480 50-week-old Hy-Line Brown laying hens were fed low-protein diets with sustained-release amino acids for eight weeks. The birds were randomly assigned to five treatments (eight replicates each): a control diet (CON; CP 16.5%, ME 2,700 kcal/kg); low-protein diet groups (LP; CP 13.5%, ME 2,700 kcal/kg), supplemented with either crystalline amino acids (LP-CAA) or microencapsulated amino acids (LP-MAA); and low-protein, low-energy diets (ME reduced by 100 kcal/kg), supplemented similarly (LPLE-CAA and LPLE-MAA). The crystalline amino acids included Lys, Met, Thr, and Arg, while the sustained-release form consisted of 45% amino acids, 5% polyethylene glycol, and 50% lipid-based coating (hydrogenated palm oil and glycerol monostearate). A one-way ANOVA followed by a Duncan test was used to evaluate the effects of dietary treatments on production performance, egg quality, and lipid metabolism parameters. A two-way ANOVA followed by a Tukey test was used to evaluate the main and interaction effects of amino acid form and ME level on these parameters. Results showed that reducing ME significantly decreased serum ALT ($P < 0.05$) and tended to reduce serum TG and LDL-C ($P = 0.07$; $P = 0.08$). ME reduction also lowered liver and abdominal fat indices, as well as hepatic TG ($P < 0.05$). ME reduction attenuated the hepatic lipid vacuolation and droplets observed in the LP groups. A significant interaction between the level of ME and the form of amino acids was observed for TCHO ($P < 0.05$), with LPLE-MAA showing the lowest hepatic TCHO. Gene expression analysis revealed that LP-CAA increased hepatic *mTORC1* and *ACC* expression ($P < 0.05$) and tended to increase *SREBP-1c* expression ($P = 0.07$). Conversely, ME reduction decreased the expression of these lipogenesis-related genes ($P < 0.05$). In conclusion, reducing dietary crude protein by three percentage points did not significantly impact production performance, but it did exacerbate hepatic lipid metabolism disorders. Low-protein diets with reduced ME alleviated hepatic lipid deposition. This was potentially achieved through the *AMPK-mTORC1* pathway by regulating the expression of *mTORC1*, *SREBP-1c*, and *ACC* to suppress hepatic lipogenesis and enhance lipolysis.

Keywords: Amino acid, Low-protein diet, Laying hen, Lipid metabolism, Metabolic energy

120 Fructose enhances intestinal development and barrier function while driving hepatic lipogenesis and inflammation in broilers

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To test the effects of fructose on the intestinal health, nutrient metabolism, and production performance of broilers, both in vitro and in vivo studies were conducted. In vitro, treatment with 5 mM fructose increased the size of intestinal organoids from broilers ($P < 0.05$). For in vivo trial, 96 one-day-old male AA broilers with similar body weights were randomly divided into 2 treatment groups (control and fructose) with 6 replicates of 8 birds each. All birds received the same commercial diet, with the control group receiving tap water and the fructose group receiving 1% fructose in water for 42 days. Fructose supplementation had no effects on body weight, feed intake or FCR throughout the trial ($P > 0.05$). It increased the dressed percentage of the broilers ($P < 0.05$), without affecting other carcass characteristics or abdominal fat index. Apparent digestibility of dietary crude fat was reduced by fructose ($P < 0.05$), while crude protein digestibility remained unchanged ($P > 0.05$). However, fructose enhanced hepatic lipids synthesis, as evidenced by upregulated expression of *ACC1* and *Srebf1* ($P < 0.05$) and suppressed fatty acids oxidation through reduced *CPT1* expression and lower serum palmitoylcarnitine levels ($P < 0.05$). Liver inflammation was also increased, indicated by elevated expression of *IL-1β* and higher serum concentrations of pro-inflammatory mediators such as 5-HETE ($P < 0.05$). Fructose improved intestinal morphology, including increased villus height to crypt depth ratio and lengthening of the duodenum and ileum ($P < 0.05$). Tight junction gene expression was also upregulated, indicating enhanced barrier integrity. Fructose influenced the intestinal microbiota and serum metabolites of broilers.Fructose reduced the relative abundance of Firmicutes while increasing Bacteroidota, resulting in a lower Firmicutes-to-Bacteroidota ratio ($P < 0.05$). Enrichment analysis showed fructose-associated bacteria were positively correlated with intestinal morphology, while control-associated taxa were linked to fat metabolism. Serum metabolomics analysis revealed distinct clustering between groups, with differentially abundant metabolites (VIP > 1, $P < 0.05$) enriched in lipid metabolic pathways. In conclusion, supplementation with 1% fructose via drinking water enhanced intestinal development and microbial composition in broilers without affecting growth performance. However, it reduced dietary fat digestibility and induced hepatic lipogenesis and inflammation. These findings suggesting the need for further research to optimize its use for promoting gut health without compromising liver function or nutrient utilization.

Keywords: Fructose, Broilers, Intestine Growth, Fat Metabolism, Microbiota

121 Prediction of net energy requirement in Chinese domestic broilers by factorial method

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The objective was to develop prediction model for net energy requirement (NEr, kcal/d) in Chinese domestic broilers (Mahuang Chicken) according to factorial method. A total of 600 female birds were allotted to 5 dietary treatments with 6 replicates per treatment and 20 birds per replicate pen. The trial implemented 3-phase feeding program (starter, grower, finisher). Diets were formulated with 5 decremental levels of net energy by 65 kcal/kg per gradient. The daily ME intake (ME_i) and rentetion energy (RE) were estimated based on total excreta collection and comparative slaughter method. Daily maintenance NE (NE_m) was extrapolated based on the linear regression ($RE=a+b\times ME_i$) between ME_i and RE. Thus, NEr was estimated by $NEr=RE + NE_m$. Body protein (BP) and body fat (BF) were analyzed to partition RE into energy associated with BP or BF. The leg muscle (LMGr, g/d) or pectoral muscle growth rate (PMGr, g/d) was used to predict BP (g/d), while abdominal fat deposition rate (AFDr, g/d) was used to predict BF (g/d). Simple or multiple linear regression was performed by using “lm” function in R program (4.4.1). Results indicated that linear regression between ME_i and RE for starter, grower and finisher were as

follows:
ME_i=0.7363×RE − 26.09 (R^2 = 0.827, $P <$ 0.01); ME_i=0.7428×RE − 73.34 (R^2 = 0.756, $P <$ 0.01); ME_i=0.7504×RE − 100.73 (R^2 = 0.726, $P <$ 0.01). Thus, NE_m and efficiency were 26.09 kcal/d and 73.5% for starter; 73.34 kcal/d and 74.3% for grower; 100.73 kcal/d and 75.0% for finisher. The correlation between RE, BP, and BF were RE (kcal)=7.02×BP(g)+9.79×BF(g) for starter; RE (kcal) =5.31×BP(g)+8.97×BF(g) for grower; and RE (kcal)=6.70×BP(g)+9.00×BF(g) for finisher. Prediction of BP or BF for starter were BP deposition rate = 0.78 ×LMGr + 1.39 ×PMGr (R^2=0.740, P<0.001) BF deposition rate = 4.05× AFDr (R^2=0.710, P<0.001) Prediction of BP or BF for grower were BP deposition rate = 0.25×LMGr + 3.61×PMGr (R^2=0.757, P<0.001) BF deposition rate = 3.32× AFDr (R^2=0.751, P<0.001) Prediction of BP or BF for finisher were BP deposition rate = 0.45×LMGr + 2.76×PMGr (R^2=0.761, P<0.001) BF deposition rate = 1.67×AFDr (R^2=0.780, P<0.001) To conclude, NEr based on this trial was 60.52, 175.89, 245.83 kcal/d for starter, grower and finisher broilers, respectively. The NEr prediction for starter was NEr = 7.02×(0.78 ×LMGr + 1.39 ×PMGr) + 9.79× (4.05× AFDr) + 26.09; The NEr prediction for grower was NEr = 5.31×(0.25 ×LMGr + 3.61 ×PMGr) +8.97× (3.32× AFDr) + 73.34 The NEr prediction for finshier was NEr = 6.70×(0.45 ×LMGr +2.76 ×PMGr) + 9.00× (1.67×AFDr) + 100.73.

Keywords: net energy requirement, prediction, retention energy, factorial method, Chinese domestic broiler

122 Effects of feed pellet diameter and length on production performance, organ development, blood biochemical indicators digestive enzyme activity, and nutrient utilization efficiency in Cherry Valley ducks

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This study investigated the effects of pellet diameter and length on growth performance, organ development, digestive enzyme activity, and nutrient digestibility in Cherry Valley ducks during the grower (8-21 d) and finisher (22-42 d) phases. A total of 288 one-day-old Cherry Valley ducks were randomly assigned to one of three dietary treatments: a control group (CON) fed a standard pellet diet, a diameter-increased group (DI), and a length-increased group (LI). Each treatment included eight replicate pens with twelve ducks per pen. The trial lasted for 42 days. During the grower phase, ducks in the LI group exhibited significantly lower average daily gain (ADG) and higher feed conversion ratio (FCR) compared to the CON group (P<0.05). The DI group showed significantly reduced plasma concentrations of glucose, albumin, total cholesterol concentrations, as well as decreased apparent ether extract (EE) digestibility (P<0.05). Both DI and LI groups had significantly reduced body slope length, back width, keel length, and chest width (P<0.05). During the finisher phase and the overall period (1-42 d), ducks in the DI group demonstrated significantly higher average daily feed intake (ADFI) and ADG compared to those in the CON and LI groups (P<0.05). In the finisher phase, the LI group had significantly lower body weight and oblique body length (P<0.05), but significantly improved EE digestibility (P<0.05). Apparent digestibility of dry matter (DM) and crude protein (CP) also showed a tendency to increase. Both DI and LI groups exhibited significantly elevated intestinal lipase activity (P<0.05). Additionally, the muscular stomach index was significantly higher in the DI group at both 21 d and 42 d (P<0.05). In conclusion, the effects of pellet geometry were phase-specific. Increased pellet diameter or length during the grower phase impaired growth performance, body development, and nutrient utilization. However, increasing pellet diameter during the finisher phase enhanced feed intake, growth performance, gizzard development, and lipid digestion. Adjusting pellet diameter during the finisher phase may therefore optimize growth efficiency and nutrient utilization in Cherry Valley ducks.

Keywords: feed particle size, growth performance, organ index, digestive enzymes, nutrient utilization efficiency

123 Molecular mechanism of intestinal DNA methylation regulating the function of absorptive enterocytes in fatty liver layer chickens

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Early studies have identified an association between DNA methylation in chickens and the expression of genes involved in fat metabolism (such as PPARγ), while gut microbiota and their metabolites can affect epigenetic modifications DNA methylation). Absorptive enterocytes exhibit selective nutrient absorption through markers like CD36 and FATP4. This study aims to explore whether intestinal DNA methylation regulates absorptive enterocytes and whether gut microbiota influences DNA methylation via metabolites. Here, we grouped laying hens based on serum triglyceride levels (Con: TG < 7.5 mmol/L; FL: TG > 15 mmol/L), collected intestinal tissues, detected lipid levels and organ indices in the liver and intestine, and analyzed DNA methylation of differentially expressed genes in the intestine using MassArray technology to quantitatively characterize intestinal DNA methylation in fatty liver laying hens. Additionally, 16S rRNA sequencing was performed on gut microbiota of FL and Con hens to screen for differential microbes and analyze metabolites. Intestinal organoids were treated with differential metabolites to detect DNA methylation levels and gene/protein expression of absorptive enterocyte markers, exploring the effect of intestinal DNA methylation on absorptive enterocytes. Finally, a DNA methylation site mutation model was constructed to treat intestinal stem cells, with detection of absorptive enterocyte markers and gene/protein expression related to Wnt and Notch signaling pathways to reveal the mechanism by which intestinal DNA methylation regulates absorptive enterocytes. This study is expected to clarify the association between intestinal DNA methylation and absorptive enterocyte function in the fatty liver state and reveal the role of Wnt and Notch pathways in methylation regulation. Innovatively integrating intestinal DNA methylation, microbiota metabolism, and absorptive enterocyte function, this research provides a new perspective for health regulation of laying hens with fatty liver.

Keywords: Layer, DNA methylation, Absorptive enterocytes, Organoids

124 Net energy requirement of layer-type chicks reared at different temperatures

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Precision feeding of poultry depends on the dynamic estimation of nutrient requirement, which is related to the physiological stage and rearing temperature. The objective of this study is to determine the effect of temperature on the net energy (NE) requirement of layer-type chicks in the periods from 1 to 14 d of age. In trail 1, NE requirement for maintenance of chicks at different temperatures was measured. A total of 160 one-day old Jingfen layer-type chicks were divided into five groups with four replicates of eight chicks. The chicks were randomly subjected to one of five temperature treatments: 30, 32, 34, 36, and 38 C. After 4-days adaptation, the oxygen consumption and CO_2 production were measured at 6-day-old for 72 h with open-circuit negative pressure respiratory thermometry device. In trial 2, the growth performance of chicks reared at different temperatures was determined to evaluate the NE requirement for body weight (BW) gain in the periods from 1 to 14 days of age. A total of 480 1-day-old chicks were divided into five groups and each group had 12 replicates of eight chicks. The temperatures were 32-30-29 C, 33.5-32-30.5 C, 35-34-32 C, 36.5-36-34 C, and 38-38-35 C in the periods from D 1 to 3, D 4 to 7, and D 8 to 14, respectively. The body weight and feed intake were measured weekly. Body composition and energy content were determined at beginning and end of the trial. Data were analyzed by one-way ANOVA model. Differences between the treatments were evaluated using Duncan's multiple comparisons tests. The orthogonal comparisons were applied for linear and quadratic effects of temperature treatments. In trial 1, the result showed that the fasting heat production (**FHP**) was affected influenced by rearing temperature ($P < 0.001$). The FHP of chicks at 30, 32, 34, 36, and 38 C was 519.7, 710.8, 493.5, 361.7, and 437.9 kJ/kg $W^{0.75}$/day, respectively. In trial 2, the result showed that BW gain and feed intake were decreased ($P < 0.001$) when temperature increased, whereas the feed conversion ratio was changed by temperature only in the period of D 1 to D 7 ($P < 0.01$). The organ index of heart ($P < 0.05$), liver ($P < 0.001$), and breast muscles ($P < 0.001$) were decreased by high temperature. The NE requirement was not altered ($P > 0.05$) by temperature. The NE requirement of Jingfen layer-type chicks in the periods from 1- to14-days of age is: **NE** (kJ/bird/day) = $(51.232T^3 - 471.47T^2 + 1219.9T - 273.06)\ W^{0.75}$ (kg) + 7.16 BW gain (g/d).

Keywords: Fasting heat production, Energy requirement, Net energy, Temperature, Layer-type pullet

125 Effect of pellet feed with different ring die compression ratios on reproductive performance of breeding pigeons, growth performance, meat quality and intestinal function of squab pigeons

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Effect of pellet feed with different ring die compression ratios on reproductive performance of breeding pigeons, growth performance, meat quality and intestinal health of squab pigeons were investigated. 150 pairs of White King breeding pigeons aged between 16 and 18 months old with similar performance were randomly assigned to 5 groups with 6 replications of 5 pairs of parental pigeons each, and were fed a pellet feed with a ring die compression ratio of 1:6 (control group), 1:6.5, 1:7, 1:7.5 or 1:8 for 46 days, respectively. The pre-feeding period was 7 days. Data were analyzed by one-way analysis of variance using SPSS statistical software. Orthogonal polynomial contrasts were used to assess the linear and quadratic effects of ring die compression ratio of pellet feed. Differences among groups were tested using Turkey's multiple range test and statistical significance level was set at $P < 0.05$. Ring die compression ratios linearly increased hardness and durability of pellet feed, and these effects were more pronounced at a ratio of 1:8. A ring die compression ratio of 1:7.5 linearly increased feed intake of breeding pigeons during 1 to 46 days. Ring die compression ratios of 1:7.5 and 1:8 linearly and quadratically increased serum glucose level of squabs. Ring die compression ratios of 1:7 to 1:8 linearly reduced serum creatinine level of squabs. A ring die compression ratio of 1:7 quadratically increased serum aspartate aminotransferase activity of squabs. A ring die compression ratio of 1:6.5 linearly and quadratically increased serum alanine aminotransferase activity of squabs. Ring die compression ratios of 1:7 and 1:7.5 quadratically decreased pH_{24h} and linearly and quadratically decreased L^*_{48h} in pectorales, linearly and quadratically increased jejunal villus height. A ring die compression ratio of 1:7 quadratically decreased pH_{48h} in pectorales. Ring die compression ratios of 1:7.5 and 1:8 linearly increased a^*_{48h} in pectorales. Ring die compression ratios of 1:7 to 1:8 quadratically decreased drip loss in pectorales, and linearly increased ileal villus height and villus height to crypt depth ratio. A ring die compression ratio of 1:8 linearly increased b^*_{48h} in pectorales, linearly decreased ileal crypt depth, and linearly and quadratically increased jejunal *ZO1* and ileal *ZO2* mRNA expression. Results indicate that ring die compression ratio exerts certain effects on pellet feed processing quality, feed intake of breeding pigeons, meat quality, and intestinal health of squabs. The recommended compression ratio for best effect is 1:8.

Keywords: ring die compression ratio, reproductive performance, meat quality, intestinal health, pigeons

126 Study on the correlation between nutritional indicators and feed conversion ratio as well as meat production cost of broilers

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This experiment was conducted to study the effects of different crude protein (CP:21-23.5 %) and metabolic energy (ME:2730-3262 kcal/kg) levels on the feed conversion ratio (FCR) and meat production cost. The 15-day-old broiler chickens were selected with an average weight of approximately 515 grams. Different CP and ME levels were set up in each batch, with 12 replicates per group, and 40-50 broilers per replicate, the experiment lasted for 24 days. The results showed that a strong correlation between feed cost and meat production cost (r=0.839, N=20). The ME and CP levels in the diet showed significant correlations with feed

cost (r=0.949, r=0.658, respectively), and there was also a certain correlation between FCR and meat production cost (r=0.408). Based on the FCR corresponding to different CP and ME diets, combined with feed cost, the meat production cost was calculated. Prediction equations of CP, ME, FCR, and meat production cost were established as followed: Meat production cost = 0.0379 * CP+ 0.0030 * ME + 3.8271 * FCR - 9.6486 (R-squared=0.9290). In conclusion, while meeting the nutritional requirements of broilers, appropriately reducing CP and ME levels can achieve the effects of meat production cost saving. Pursuing a low FCR by increasing CP and ME levels is uneconomical and may even lead to wastage. Therefore, the optimal nutritional level should be established by integrating both the farming costs and the expected returns in order to achieve the best economical benefit.

Keywords: CP, ME, FCR, Cost

Poultry Management, Waste, and Well-Being II

127 Visual laser enrichment for the broiler: What are the birds telling us?

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Modern poultry organizations have become increasingly interested in providing diverse enrichment options for the meat bird during their lifespan. Understanding enrichment options for broilers is important for both the bird and the people that raise them. One enrichment option that has been explored in both research and commercial settings is a patented laser enrichment device that projects 5 randomly moving red dots on the floor of the poultry house. Recent work has emphasized determining optimal length and number of exposures per day, with a range of 4 to 8 minutes of exposure in research settings and 2-6 exposures per day during the light cycle. Broiler chicks in this series of studies were housed in 1.22m x 2.44m floor pens for 7 weeks and assigned to either control or laser enrichment, with enrichment blocked by room. General analyses include feed intake and weight compiled by growth phase, ethograms related to behavior and movement for a select group of focal birds, leg lameness, bone quality, and other welfare indicators. Video was recorded on days 0 to 9, 16, 23, 30, 37, and 42. Welfare assessments including gait score, breast blister presence, and footpad dermatitis were assessed on all focal birds weekly. Home pen behavior and walking distance data were analyzed daily during laser enrichment periods for days 0-9 and analyzed by week thereafter (d2, 9, 16, 23, 30, 37, and 42). All data were analyzed using the GLIMMIX procedure with fixed effects of treatment, day or week, and their interaction, while performance data were analyzed with Proc Mixed procedure (SAS 9.4, significance denoted at $P \leq 0.05$). Over the course of working with this enrichment option, outcomes generally support equal or significantly improved performance outcomes (FCR, gain, feed intake, etc.) as compared to non-enriched controls, and improved bone quality due to increased exercise. While lasers were activated, laser enrichment increased bird activity and walking distance during the first 9 days of life and during select later weeks and overall did not negatively impact broiler welfare measures. Across several studies, blood corticosterone was found to be equal or significantly reduced due to enrichment ($P<0.05$). Optimized settings indicate 8 minutes per exposure may result in birds less interested in interacting with the laser near the end of the period. Over the course of several experiments, no negative impacts were observed in gait score, breast blister presence, and footpad dermatitis due to laser enrichment ($P > 0.05$). We continue to optimize duration and exposures across research and commercial settings and have found this to be a viable option for producers to enrich broiler environments.

Keywords: broiler, behavior ethogram, environmental enrichment, welfare, walking distance

128 Quantifying broiler activity in commercial housing

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Artificial intelligence has the potential to impact broiler production by improving management efficiency and accuracy. While tracking broiler bird activity using such technology is not commonly practiced in commercial settings, it can be a valuable tool for evaluating animal welfare and assessing the effectiveness of personnel management within poultry production facilities. The objective of this study was to evaluate the use of EthoVision XT tracking software in measuring broiler activity in a commercial house, specifically in response to variations in walkthrough assessments typical of broiler house management protocols. Over a 42-day growout cycle of a 20,000-head house, broilers were exposed to three conditions (no walkthrough control, slow-paced walkthroughs, and fast-paced walkthroughs) three days per week. The control recordings started 15 minutes before human presence in the facility in four pens throughout the house. The fast and slow conditions were replicated across two pens, with fast-paced walkthroughs performed in pens 1 and 3 and slow-paced walkthroughs in pens 2 and 4. Videos (n=96) were processed using EthoVision to quantify mean activity (%), velocity (cm/s), and distance moved (cm). Tracking was restricted to a defined arena between feed and water lines (middle zone) to isolate responses excluding human movement. All data were analyzed using JMP Pro v.17 software. ANOVA was used to compare control and walkthrough conditions, and t-tests were used to assess fast vs. slow walkthrough effects. Parameters were significantly different amongst pens within the control and walkthrough pens, respectively ($P<0.0001$). Pen-specific differences suggest environmental factors, such as tunnel fan placement, may further influence broiler activity. Yet, when pens were pooled, broiler activity was significantly higher during walkthroughs of any speed compared to control (2.35% ± 0.19 vs. 1.65% ± 0.17; $P = 0.0037$). Mean activity % and velocity were significantly greater in fast-paced walkthroughs compared to slow-paced (3.36 ± 0.35% vs. 1.34 ± 0.08%, and 0.0672 ± 0.007 cm vs. 0.0076 ± 0.043 cm; $P < 0.0001$). Inactive states were reduced during fast walkthroughs (5.72 ± 1.20 s vs. 20.57 ± 3.21 s; $P < 0.0001$). Increased activity in response to fast-paced walkthroughs may indicate heightened arousal or distress, reinforcing the need for standardized assessment protocols to avoid inducing stress-related behaviors such as piling.

Keywords: broiler, welfare, commercial, tracking

129 Not Presented

130 Semi-open water sources minimally affect positive behaviors, but may influence negative behaviors in Pekin ducks depending upon the number of ducks per source

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Environmental enrichment plays an important role in shaping welfare, behavior and health of commercially raised Pekin ducks. Specifically, open-water sources as environmental enrichment for ducks have been shown to promote positive behaviors. We set out to investigate whether preening cups and Pekinos, 2 forms of semi-open water sources used for commercial Pekin ducks, would promote natural behaviors (preening, dunking head) and reduce unwanted behaviors (feather pecking). Our first experiment used control pens (CON, N=6, 65 ducks/pen) which had only water lines (8 ducks/nipple) and experimental pens (N = 6, 65 ducks/pen) had the same water line plus one preening cup (PC). Behavior data was collected using scan sampling with video being recorded for 72 continuous hours at 4 different ages: 25 d, 30 d, 36 d, and 40 d. The first 15 min/hr in each pen for AM (3:30, 4:30, and 5:30) and PM (14:30, 15:30, 16:30) were analyzed. Behavior was analyzed using GLIMMIX procedure (SAS 9.4) to examine treatment differences in the proportion of ducks performing dry preening, wet preening, feather picking and feather pecking. More ducks housed with the PC performed wet preening behaviors compared to the control ducks (at 25 days of age: P=0.0143; and also at 30,36 and 40 days of age, P<0.0001). Though not significant, we saw an increase in feather pecking within PC pens. Our second experiment included the CON and PC treatment groups adapted from the first experiment, with the addition of a Pekino (PEK) group (water line plus 2 PEK per pen). Final N=4 pens/treatment with 30 ducks/pen. Behavior data was collected and analyzed the same as experiment 1, using the same interval and times, but for 3 weeks after enrichment placement. There were no significant differences found among groups for wet preening, but more ducks in the PEK group dry preened than ducks in the CON group (P=0.0005). There were also no significant differences found among groups for feather pecking. The results of these two experiments suggest that semi-open water sources do promote natural behaviors, but it is still unclear if it decreases unwanted behaviors like feather pecking. It is unclear if semi-open water sources provide an outlet for positive behaviors and reduce unwanted behaviors. More research is needed to determine the appropriate ratio of ducks to enrichment devices as there may be an optimal balance.

Keywords: open water, welfare, resource guarding

131 Effects of positive versus negative pressure ventilation systems on thermal environment uniformity in yellow-feathered broiler houses in Northwest China

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The optimization of ventilation modes in poultry housing is crucial for thermal environment regulation, particularly in Xinjiang's arid climate with significant diurnal temperature variations. This study conducted comparative thermal environment assessments in two adjacent yellow-feathered broiler houses (positive-pressure vs. negative-pressure ventilation systems) during summer, utilizing identical boiler heating systems but differing ventilation strategies. Continuous environmental monitoring was performed on 67-day-old broilers from the same batch, with systematic adjustments of ventilation protocols. Results demonstrated that the positive-pressure house achieved superior thermal control precision, exhibiting 1.2±0.35°C (summer) lower daily mean temperatures compared to the negative-pressure system ($P<0.05$). Three-dimensional thermal analysis revealed significantly enhanced environmental uniformity in the positive-pressure house across longitudinal, lateral, and vertical dimensions ($P<0.05$). The coefficient of variation (CV) in negative-pressure poultry houses correlates positively with ambient temperature, increasing as it rises, whereas the CV in positive-pressure houses shows no significant($P>0.05$) change with environmental temperature; when ambient temperature exceeds 30°C, the CV in negative-pressure houses is more than 3% higher than in positive-pressure houses. And the concentration of carbon dioxide in the positive pressure chamber is lower($P<0.05$). The findings validate that positive-pressure ventilation effectively improves environmental stability and spatial homogeneity under Xinjiang's extreme climate conditions, providing critical technical support for precision environmental management in arid poultry farming regions.

Keywords: Yellow-feathered broilers, positive pressure ventilation, negative pressure ventilation, thermal environment, the coefficient of variation

132 Not Presented

133 New concept incubation: strategies to approach 100% hatchability

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Machine incubation of poultry eggs is widely utilized for hatching chicks in modern production operations for efficient handling of huge numbers of eggs, reducing labor costs, and to save the breeder hens from incubation to lay more eggs. Tremendous research has been undertaken that allows fine adjustment of incubation conditions such as temperature, humidity, ventilation and egg turning, so to support maximally embryo development and to achieve the highest possible egg hatchability. Though the current commercial incubation machines have utilized sophisticated intelligent controlling systems that can well regulate incubation atmosphere, embryo mortality is still common with the hatchability achieved at approximately 95%, which is still in short of the 100% achieved by the natural incubation by the hen itself. Such deficiencies in machine incubation can be attributed to the embryo mortalities that occur at different incubation stages. With the current designed and operated incubator, the early stage embryo mortality within the first 7 days of incubation is approximately 5%, and the late stage mortality that mostly occurred after full development of chorio-allantoic membrane in the range of 5% to 10%. The latest development in incubation technology that mimicked the hen natural incubation manner indicated both early and late mortalities can be substantially reduced and the hatchability accordingly improved. For example an extra-long time gradual rise of temperature from 30°C to 38°C over 6 days at the start of incubation reduces the early mortality by 3 percentages. While the wide angle egg turning at 75° during incubation reduced the late mortality by 4%, an angle alteration from 75° to 60° after chorio-allantoic membrane enclosure at the small end further reduced late mortality by 1.5%. On the basis of these progresses, a new concept of egg incubation technology can be proposed that can improve fertile egg hatchability to approach 100%. This new approach should combine the extra-long time temperature rise at the start of incubation and the wider angle egg turning at 75°, which should be reduced to 60° at the later stage of incubation.

Keywords: incubation, temperature, egg turning angle, embryo mortality, maximal hatchability

134 Not Presented

135 Impact of different intensities of in-ovo Green-Red dichromatic light on embryonic growth and subsequent post-hatch performance in chickens

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Light is an important environmental factor that can help to improve the performance of the hatchery industry; however, commercially, eggs are incubated in a dark environment. Therefore, this study was planned to evaluate the impact of various light intensities of dichromatic Green-Red (**GR**) during the incubation period on the hatching and post-hatch performance of broiler and layer chickens. For that purpose, 0, 150, 250, and 350 lux of dichromatic light were provided for 12 hours per day during the incubation period [Genotype (2) × Lighting intensity (4) × n (125) = 1000 eggs], and 125 eggs (Ross & Bovans) were assigned to each of 8 treatments in a Randomized Complete Block Design. Results indicated that embryo index was significantly improved in broilers under 250 lux GR light ($p<0.05$). Similarly, hatching time was also significantly decreased in 250 and 350 lux light intensities, and interaction showed that layer-hatched chicks' percentage was significantly higher at 23, 13, and 6 hours before hatch pull under 250 lux GR light. Moreover, hatchability and hatch of fertile were significantly improved in broilers and layers under 250 lux, followed by 350 lux ($p=0.002$). Significantly lower dead-in-shell and dead-germ percentages were noted in 150 and 350 lux lighting treatments, respectively ($p>0.05$). In growth performance, birds that were incubated under 350 lux dichromatic light showed higher body weight, while feed intake, FCR, and livability percentage were not affected by light intensities ($p>0.05$). Whereas, interaction results showed that significantly better body weight was observed in broilers that were incubated at 350 lux. Similarly, improved FCR was noted in the broiler chicks that were given lighting intensities during incubation. It was concluded that 250 lux light can improve the embryonic growth and hatch window along with decreasing late embryonic mortality, while 350 lux light during embryogenesis can help to improve post-hatch growth performance.

Keywords: Incubation, Poultry, Light Intensity, Embryogenesis, Dichromatic Light

136 Improving hatchability, chick quality and post-hatch performance of broiler chickens: the role of incubation temperature and early in ovo feeding

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The experiment investigated the relationship between incubation temperature and early in-ovo feeding with eggshell temperature and post-hatch performance of broiler chickens. A total of 1,269 Arbor Acres broiler breeder eggs were randomly assigned to 3 incubation temperatures (36.5, 37.0 and 37.5°C) in separate incubators at 423 eggs each. Eggs per each temperature group were further randomised into glucose, vitamin D3 and control groups with 141 eggs per group. Data were analysed using analysis of variance with the Generalised Linear Model Procedure in Minitab 19. After the in ovo feed application, eggshell temperature was significantly highest in eggs incubated at 37.0°C and lowest in eggs incubated at 36.5°C across the incubation days. Eggshell temperature was significantly lowest in the glucose-fed eggs and highest in eggs supplemented with vitamin D3 across the incubation days. Hatchability was significantly highest (81.92%) in eggs incubated at 37.5°C and in the glucose-injected eggs incubated at 37.5°C (89.86%). Chick weight was significantly highest (51.72g) in vitamin D3-fed eggs incubated at 37.5°C. Body weight (1283.52g) and body weight gain (1234.80g) were significantly highest in eggs incubated at 37.0°C. In ovo feeding can be used to optimise eggshell temperature and improve hatchability and post-hatch performance of broilers, but feeding glucose and incubating eggs at 37.5°C is recommended.

Keywords: Eggshell temperature, hatchability, incubation temperature, in ovo feeding, post-hatch performance

137 Research on the evaluation and optimization of thermal environment in broiler houses based on computational fluid dynamics

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This experiment conducted steady-state simulations using tunnel ventilation and small window ventilation in chicken houses. The on-site measurement data were used as boundary conditions for the steady-state simulation calculations, and the simulated results were the chicken house breeding environment. The angles of the diversion plates at different positions were adjusted, and simulations were carried out for three different positions of the diversion plates with different opening angles to explore the impact on the chicken house thermal environment. In spring and autumn, the wet curtain deflector plates at the side walls and gable walls are opened at 5°, 10°, and 15° for the upper layer, middle-upper layer, middle-lower layer, and lower layer respectively. In the small window ventilation mode, the deflector plates at the small window, 1/3 of the way from the gable wall, 1/2, and the last 2/3 are set at 60°, 45°, and 30° respectively. The results show that when the deflector plates at the gable wall are changed, the wind speed above 1m of the chicken house increases by 0.1m; when the deflector plate angle of the side wall is changed, the wind speed above 1m of the chicken house increases by 0.2m, and the unevenness index of the air flow inside the house decreases by 2%; when the deflector plate angles of the gable wall and side wall are changed, the wind speed above 1m of the chicken house increases by 0.3m, but the unevenness index of the air flow inside the house increases by 3%. In the small window ventilation mode, different deflector plate angles are set at different positions of the chicken house, and the influence of the unevenness index inside the house is not significant, and the effect on the air flow velocity in the living area of the chickens inside the house is relatively small.

Keywords: computational fluid dynamics, Chicken coop thermal environment, Window ventilation, Angle of the diversion plate

Genetics and Genomics

138 Decoding the chicken genome's regulatory landscape for sustainable poultry production

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Advances in nextgeneration sequencing and functional genomics are transforming how we understand the genetic and epigenetic architecture of economically important traits in poultry. Chickens, as both a cornerstone of global food production and a model organism for biological research, present a unique opportunity to bridge basic science and applied breeding. Over the past several years, our team has generated one of the most comprehensive regulatory atlases in chickens, integrating multiple omics layers—genomics, epigenomics, and transcriptomics—across dozens of tissues and breeds. This atlas maps millions of regulatory elements, including promoters, enhancers, silencers, and chromatin states, and links them to gene expression and target genes. By combining DNaseseq, ChIPseq, ATACseq, and RNAseq datasets, we have been able to systematically annotate regions of the genome that control transcription and identify how genetic variants alter regulatory activity. These resources provide unprecedented insights into the noncoding genome of the chicken, an area long overlooked but critical for understanding complex traits. Using this framework, we have begun to dissect the molecular basis of traits vital to poultry production and resilience, including growth efficiency, egg production, and resistance to major infectious diseases such as Newcastle disease and avian influenza. Our analyses reveal tissuespecific and conserved regulatory modules, uncover enhancer networks linked to stress response pathways, and identify key genetic variants that influence how birds adapt to environmental and pathogenic challenges. By presenting this integrated view of the chicken genome's functional architecture, this talk will illustrate how the convergence of genetics, epigenetics, and computational biology is reshaping poultry science. The resulting regulatory atlas is not only a foundation for future research but also a resource for advancing sustainable production, improving animal health, and supporting global food security.

Keywords: Regulatory atlas, Epigenomics, Functional genomics, Sustainable poultry production, Genomic selection

139 Genetic determinants for age-related decline in female reproductive performance through multi-omics analysis in chicken

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Reproductive aging, marked by reducing fertility, often precedes general aging despite normal somatic function. It is influenced by genetic, environmental, and physiological factors, with multiple organs involved, though inter-tissue coordination remains unclear. Laying hens, with regular ovulation and distinct ovarian structures, serve as ideal models. In this study, we analyzed 100 week laying hens via multi-omics to elucidate the molecular mechanism of genetic regulation, offering insights into reproductive aging in animals and humans. We selected 254 Rhode Island Red hens of 100 week from a population multi-omics analysis. Genomic DNA was extracted from blood, and RNA-seq was performed on 10 tissues (hypothalamus, pituitary, liver, pancreas, duodenum, cecum, ileum, ovary, magnum, and uterus, n = 2475). Ovaries, magnums, and uteri from 72- and 100-week-old hens were used for scRNA-seq. SNP array and WGS data were processed by mixed linear model association testing. RNA-seq data were analyzed with FastQC, fastp, Hisat2, StringTie, and WGCNA; key genes were identified with GeneSelectR. cis-eQTL mapping used TensorQTL, TWAS and SMR identified trait-associated genes. BayesPrism, scPagwas, and Scissor pinpointed relevant cell types. Candidate gene functions were validated in DF-1 and granulosa cells via overexpression or siRNA knockdown, with senescence induced by H2O2. qRT-PCR, β-gal staining, immunofluorescence, and ELISA (E2, PROG) were used to assess gene function, with all assays in biological triplicates. GWAS, eQTL, and TWAS identified variants and genes involved in hormone signaling and cell cycle regulation. scRNA-seq revealed key roles for ovarian epithelial cells and fibroblasts in late-stage decline. And single-cell eQTLs uncovered regulators of oocyte meiosis. Integrated analyses identified candidate genes (*CCDC47*, *DPH2*, *ACSL3*) associated with ovulation and aging. Cross-species comparisons showed conserved roles for fibroblasts and epithelial cells, linking to menopause and ovarian reserve. In total, 93 functional genes were mapped to networks regulating hormone signaling and metabolism. Validation in granulosa cells confirmed roles for *CCDC47*, *CCND1*, *CDK6*, *DPH2*, and *MMP2* in promoting steroidogenesis and follicle development, supporting their importance in maintaining fertility with age. In summary, this study identified key genetic variants and functional genes linked to reproductive capacity across aging tissues and highlighted ovarian epithelial cells and fibroblasts as central to reproductive decline, laying a foundation for strategies to extend reproductive lifespan and improve ovarian health in aging females.

Keywords: multi-tissues, reproductive capacity, multi-omics, genetic regulation mechanism, laying hen

140 Microscopic morphology and single-cell level evaluation of day-old chick ovary vitrification and orthotopic transplantation for breed restoration

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With the expansion of industrial farming, numerous poultry breeds worldwide are facing extinction. Traditional live conservation, along with cryopreservation of semen, oocytes, and stem cells, all exhibit significant drawbacks or technical challenges. Therefore, this study focused on developing a low-cost, efficient cryopreservation and recovery technique for avian female genetic resources (ovarian tissue). Ovaries were collected from day-old Barred Plymouth Rock chicks (donors), underwent vitrification cryopreservation for over two weeks, and were transplanted orthotopically into day-old White Leghorn recipient chicks. The recipient hens upon reaching sexual maturity were artificially inseminated with semen from donor strain roosters, and recovery efficacy was assessed based on offspring feather color. Concurrently, ovaries from Beijing You chicken chicks were used to evaluate cryodamage mechanisms. Fresh ovaries served as controls, while vitrified-cryopreserved ovaries were used as the experimental group for evaluating multiple parameters. and, for the first time, We first applied single-cell RNA sequencing (scRNA-seq) to analyze cell types and cryopreservation-related differential gene expression, aiming to elucidate cryodamage mechanisms at the single-cell level. Results showed that one out of six recipient hens successfully produced offspring exhibiting the donor feather color phenotype. This hen produced 62 donor-line offspring before 40 weeks of age, attesting successful live recovery via cryopreserved ovary transplantation. Cryopreservation efficacy assessment revealed: ovarian cell viability and tissue morphology remained well-preserved after cryopreservation. Cryopreserved ovaries retained the ability to colonize the embryonic chorioallantoic membrane (CAM) and establish vasculature. ScRNA-seq identified 12 distinct cell types. Comparative differential gene expression analysis between germ cells and granulosa cells of the two groups showed no significant overall alteration in gene expression patterns. The identified differentially expressed genes were primarily enriched in functional pathways related to cellular assembly, hormone synthesis, and secretion. In conclusion, this study confirms that vitrification cryopreservation of chick ovaries combined with transplantation constitutes a feasible strategy for avian germplasm resource conservation, with multiple indicators demonstrating minimal cryodamage. Subsequent research will integrate multi-omics technologies to comprehensively investigate the effects of cryopreservation and transplantation on ovarian function and the underlying mechanisms, providing crucial technological and data support for advancing avian genetic resource conservation.

Keywords: Germplasm resource conservation, Vitrification, Chicken ovarian transplantation, Live restoration

141 Not Presented

142 Effects of low protein amino acid balanced diets and astragalus polysaccharides on antioxidant status, lipid metabolism, and related gene expression in heat stressed laying hens

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This study aims to evaluate the impact of heat stress on laying hens' production, egg quality, follicle development, antioxidant status, lipid metabolism, and gene expression, and to explore the role of Astragalus polysaccharides (AP) and low protein amino acid balanced diets in mitigating heat stress. 768 Hy-Line brown chickens(52-week-old) were randomly divided into 4 experimental groups, with 8 replicates in each group and 24 hens in each replicate. Control group was maintained at 24 celsius with a basal diet (CON), while all experimental groups were exposed to 32 celsius, fed basal diet at (HB), low protein diet (HL), low protein diet and AP (HLA), respectively. The egg production rate of HB and HLA were lower than those of CON ($P<0.05$). The feed conversion ratio of HB was higher than that of CON ($P<0.05$). HLA's albumen height was significantly higher than that in HL and CON ($P<0.05$). Egg yolk color in HB, HL and HLA were higher than that in CON($P<0.05$). The ratio of egg yolk in HL was lower than that in CON ($P<0.05$). The superoxide dismutase (SOD) activity in HL was higher than that in CON. The malondialdehyde (MDA) content in HL and HLA was lower than that in CON. The glutathione peroxidase (GPx) activity in HL and HLA were higher than that in CON. The number of follicles 2-4 mm in diameter in HL and HLA was more than that in CON. Heat stress leads to elevated levels of triglycerides and cholesterol in the liver and serum. The effect of HL on restoring triglyceride levels was limited, but the addition of AP significantly improves the recovery of cholesterol levels ($P<0.05$). *GPx* mRNA expression in liver was down-regulated in the HB group compared to the other groups ($P< 0.05$), while *SOD* mRNA expression was highest in the HL group than in the other groups ($P<0.05$). The mRNA expression of *low-density lipoprotein receptor* and *3-hydroxy-3-methylglutaryl-coenzyme a reductase* in the HL and HLA was higher than that in the HB ($P<0.05$), with no significant difference compared to the CON. The study found that the adverse effects of high temperatures are directly related to oxidative stress. Supplementing protein levels and Astragalus polysaccharides relatively alleviates these adverse effects, indicating tissue specificity. Therefore, the importance of feeding strategies such as Astragalus polysaccharides and low protein amino acid balanced diets for laying hens under heat stress conditions has been identified.

Keywords: Heat Stress, Low Protein Amino Acid Balanced Diet, Antioxidant Status, Lipid Metabolism, Astragalus Polysaccharides

143 Studies on evolutionarily conserved functional features of chicken NK cell subsets revealed by single-cell transcriptomics

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While the functional dichotomy of natural killer (NK) cells is well established in mammals, the identity and functional characteristics of chicken NK cell subsets remain largely unexplored. We hypothesized that recombination-activating gene 1-deficient (*RAG1-/-*) chickens lacking adaptive lymphocytes would enable the identification and characterization of NK cell subsets, allowing for functional cross-species comparison with humans and mice. We utilized *RAG1-/-* chickens to eliminate adaptive lymphocyte interference and performed single-cell RNA sequencing (scRNA-seq) of splenic immune cells to identify potential NK cell heterogeneity and compare them with mammalian counterparts. Spleens from 1-week-old *RAG1-/-* chickens were processed for scRNA-seq using the 10x genomics platform. Subsequent data integration and comparative analysis with existing human and murine NK scRNA-seq datasets were conducted using Seurat and harmony method. The hypothesis testing for each condition was performed using the MAST method, and FDR-adjusted p-values less than 0.05 were deemed as significant. GO and KEGG pathway analyses were performed to infer subset-specific functions. Two distinct NK cell subsets (NK-1 and NK-2) were identified in *RAG1-/-* chicken spleens. NK-1 cells expressed genes associated with cytotoxicity (e.g., *PRF1, GZMA*), actin remodeling, and phosphatidylinositol signaling, indicating effector functions.

NK-2 cells showed expression of immunoregulatory genes (e.g., *IL22, IL2RA*) and transcription factors for early maturation (e.g., *TCF7, TOX*). Despite transcriptomic divergence, functional conservation across chicken, mouse, and human NK cell subsets was evident, implying that NK cell mediated immune function was conserved among chickens, humans, and mice. This study defines evolutionarily conserved functional dichotomy among NK cell subsets in chickens, mirroring those in humans and mice. Our findings validate chickens as a relevant avian model for studying innate immunity and zoonotic disease mechanisms.

Keywords: Chicken immunology, Cross-species analysis, NK cells, RAG1 knockout, Single cell RNA seq

144 Generating genome-edited chickens through ovarian injection *in situ*

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Chickens, the most widely farmed avian species and a major source of global meat production, pose significant challenges for genetic manipulation due to the inaccessibility of ova and zygotes. Currently, genome editing in chickens relies primarily on primordial germ cell (PGC)-mediated approaches, which are technically demanding and require multiple generations to obtain homozygous individuals. To overcome these limitations, we developed ovarian injection *in situ* (OVIS) method to generate genome-edited chickens, aiming to provide a rapid, efficient, and scalable platform for avian genome editing. We chose 16-week-old hens before they had their first egg to perform OVIS operation. The hens were anesthetized and an incision was made between the second-to-last and third-to-last ribs to expose the ovary with developing follicles. Then, Cas9 RNPs targeting *IHH* gene were injected into the medulla of hen ovary at multiple sites. After injection, the hens were kept in cages and reared normally. When the first egg was laid, the hens were artificially inseminated, and the fertilized eggs were collected daily for 6~8 weeks and incubated every week. On ED12.5, we removed chicken embryos from eggs and examined phenotypic changes and gene editing events. We observed that more embryos died in the early stage of embryonic development (ED1~ED7) or were smaller in size with subcutaneous fluid accumulation than the wild-type chicken embryos. The genomic DNA of these abnormal embryos was subsequently extracted to detect gene editing events. After PCR amplification of gRNA sites, heteroduplex bands were detected using heteroduplex mobility assay capillary electrophoresis. TA clone sequencing revealed that single nucleotide mutations and 3-bp deletions occurred upstream of the gRNA-R3 site and deletions of different sizes from 1 to 122 bp and base substitutions occurred at the gRNA-R1 site. The whole-genome sequencing (WGS) also revealed the large deletion and small indels in these abnormal embryos. Some fertilized eggs were incubated until hatching and were reared to the adult stage. The hen #34496 with short shanks presented a similar phenotype as Xingyi Creeper. The WGS revealed that the same 3-bp deletion located in the signal peptide of IHH, which could affect the secretion of IHH protein and resulted in the creeper phenotype. We also found that the semen of cockerel #7077 underwent gene editing events, which indicated that chickens have the potential for germline transmission. In conclusion, we successfully generated G0 mosaic chickens that displayed both embryonic defects and adult Creeper phenotype. This strategy holds significant promise for developmental biology, biomedicine, synthetic biology, and poultry breeding.

Keywords: genome-editing, chicken, ovarian injection, Cas9 RNPs, IHH

145 Detection of *Myoglobin* gene polymorphism associated with pigeons growth traits

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Myoglobin (Mb), a mobile carrier of oxygen, is without a doubt an important player central to the growth of skeletal muscle. To the best of our knowledge, although Mb is one of the most studied of all proteins, much of the research has focused on its protein chemistry rather than its function under physiological conditions. This study aimed to investigate the single nucleotide polymorphisms (SNPs) of *Mb* gene and analyze their associations with pigeons growth traits. A total of 260 female pigeons from Tianxiang 1 strain, aged in 28 months, were randomly selected to collect blood samples. The pigeons were measured for the following growth traits: shank length, shank circumference, keel length, chest depth, chest width and body length. To compare the sequencing results with the *Mb* gene sequence using SnapGene version 6.0.2 software, the peak map of the sequencing results were observed and the genotypes of polymorphic sites were recorded. A one-way ANOVA was conducted using IBM SPSS 27.0 to examine the relationship between various genotypes and haplotype combinations of SNP loci in the *Mb* gene with respect to growth traits in pigeons. Six SNPs were detected, 1 located in the exon 2 (g.174230233 C > T), 3 SNPs (g.174230157 G > C, g.174230158 T > C and g.174230175 T > G) located in intron 2, 2 SNPs (g.174231334 T > C and g.174231495 T > C) located in 3'-untranslated region (3'-UTR). To assess the impact of SNPs on mRNA secondary structure, the RNAfold online software was used. The results showed that the free energy of the mRNA secondary structure was minimally affected by the g.174230233 C > T and g.174231334 T > C, whereas the g.174231495 T > C mutation increased the free energy. Analysis of allelic information and genetic indexes for SNPs of the *Mb* gene indicates the 6 SNPs were in accordance with the Hardy-Weinberg equilibrium, the H_O exceeded the He for all SNPs and 3 SNPs exhibited moderate polymorphism with PIC (0.25 < PIC < 0.5). Association analysis indicated that two SNPs were significantly associated with shank circumference, while the other four showed no significant associations with growth traits. However, haplotypes analysis did not show significant associations with the growth traits.In a nutshell, 2 SNPs of the *Mb* gene were found to be significantly associated with shank circumference. These 2 SNPs were located in the 3'-UTR. These results provide new insights into the genetic mechanisms underlying phenotypic variations of growth traits in pigeons.

Keywords: Pigeon, Myoglobin gene, Single nucleotide polymorphisms (SNPs), Growth traits, Association analysis

146 Abnormal liver protein acetylation directly regulates fatty acid metabolism in laying hens with fatty liver hemorrhagic syndrome

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Dysregulated lipid metabolism is an indispensable factor contributing to the sudden decline in egg production observed in high-yielding laying hens. Fat metabolism in animals is co-regulated at various levels (e.g., genetic, proteomic) by diverse modifying factors, including post-translational modifications such as acetylation. While numerous studies have elucidated that alterations in protein acetylation levels directly or indirectly affect lipid metabolism in mice and humans, relevant research in chickens remain limited. In this study, Hy-Line Brown laying hens with normal (control, Con) and fatty liver (FL) were selected as the experimental subjects, and the acetylated proteome of the liver was detected and analyzed by the Label-free method. A total of 1863 acetylation sites of 711 proteins were identified in liver tissues, and 1787 acetylation sites of 678 proteins among them were quantified. Eleven proteins exhibited multi-site acetylation, harboring over 10 acetylated lysine residues. Compared with Con, 103 peptide segments in the FL group underwent significant changes, including 58 up-regulation and 45 down-regulation. Annotation and enrichment analysis revealed that proteins harboring these differential acetylation sites are primarily enriched in small molecule metabolic process, have catalytic activity, are located in the cytoplasm and mitochondria, and are highly associated with lipid metabolic pathways. Statistical analysis of the distribution characteristics of acetylated lysines within protein domains indicated that the majority were located within NAD(P)-binding domain superfamily. Protein-protein interaction (PPI) analysis showed significant clustering within fatty acid synthesis (11 proteins) and fatty acid degradation pathways (22 proteins). Furthermore, the acetylation levels of key lipid metabolic enzymes exhibited a significant correlation with the degree of pathological fat deposition. These findings collectively indicate that acetylation modification can directly influence hepatic lipid metabolism in laying hens by modulating the acetylation status of key metabolic enzymes. To verify this hypothesis, Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha (HADHA, a key enzyme in β-oxidation) was selected for mechanistic investigation in a chicken hepatocyte cell line. Results demonstrated that HADHA acetylation impacts its enzymatic activity, thereby contributing to abnormal hepatic fat deposition. In conclusion, this study provides a theoretical foundation for addressing fatty liver syndrome in laying hens through the lens of protein acetylation.

Keywords: Fat metabolism, Acetylation Proteomics, Acetylation, HADHA, Fatty Liver

147 Assessing the impact of cryopreservation on *Lueang Hang Khao* chicken primordial germ cells for sustainable genetic management

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Chicken primordial germ cells (cPGCs) are a valuable resource for preserving chicken genetics due to their diploid stem cell nature and ability to produce germline-derived progeny. However, cryopreservation, essential for long-term storage, may impact cell quality. To ensure sustainable genetic management, this study aimed to determine the effects of cryopreservation on key biological characteristics of cPGCs isolated from Lueang Hang Khao (LK) chickens, a Thai native breed. Five cPGC lines (3 male, 2 female) from LK chickens were evaluated to compare fresh cells with those 10- and 20-days post-thawing. Characterization included morphology, proliferation, viability, gene expression (RT-qPCR), immunocytochemistry, and global DNA methylation (dot blot). Statistical analyses included one-way ANOVA with Tukey's post-hoc test, and Kruskal-Wallis H test with Dunn's post-hoc for non-parametric data. Significance was set at $p<0.05$. Morphological analysis revealed cPGCs with an approximate diameter of 9–18 µm, a spherical shape, and cytoplasmic granules. Critically, a visibly higher number of dead cells was observed immediately post-thawing compared to fresh cells, and cryopreservation initially significantly affected viability during the first 2 days post-thawing. Proliferation was significantly lower in cryopreserved cPGCs for both sexes compared to fresh controls during days 6 to 8 post-thawing; however, no significant difference was found at day 10. The average doubling time (DT) during days 2 to 10 post-thawing showed no significant difference among groups. Relative gene expression analysis revealed that only *NANOG* gene was significantly affected by cryopreservation in male cPGCs. In contrast, female cPGCs showed significantly decreased expression of *SOX2*, *NANOG*, and *TERT* genes for the first 20 days post-thawing. For DNA methylation-related genes, cryopreservation significantly affected only *DNMT3A* in male cPGCs. Female cPGCs showed significantly lower expression of *DNMT1* at day 20 post-thawing and significantly decreased *DNMT3A* and *DNMT3B* at day 10 post-thawing compared to fresh cPGCs. Immunocytochemistry confirmed the cytoplasmic expression of *DAZL*, and the cell surface expression of EMA-1 and SSEA-1. Global DNA methylation (5-mC and 5-hmC levels) was not affected by cryopreservation, though female cPGCs displayed significantly higher 5-hmC levels compared with male cPGCs. Our findings suggest that cryopreservation is a robust method for maintaining the critical characteristics of LK cPGCs, thereby supporting their long-term sustainability for genetic resource management. This work highlights the potential of cryopreserved cPGCs as a valuable tool for conserving indigenous chicken breeds.

Keywords: Primordial germ cells, Cryopreservation, Thai native breed, DNA methylation

148 Culture and chimera production of chicken Primordial Germ Cells (PGCs)

Kexin Wu*[1], Zheyi Jiang[1], Xiqiong Wang[1], Yuzhou Gu[1], Ning Yang[1] [1]*China Agricultural University, State Key Laboratory of Animal Biotech Breeding and Frontier Science Center for Molecular Design Breeding, Beijing, China*

Chickens serve as both a key avian model and an economically vital agricultural species. Recent advances in avian gene editing propose that targeted trait development through genetic engineering can accelerate elite chicken line establishment. Researchers have developed several approaches to generate gene-edited chickens, among which the PGC-mediated method has gained broad application. Therefore, our study compares PGC culture establishment efficiency across chicken breeds and successfully established a GFP-overexpressing White Leghorn (WL) PGC line via lipofectamine-based transfection, ultimately producing GFP-expressing chimeric chickens. PGCs were isolated from White Plymouth Rock, White Cornish, and Rhode Island Red embryos (Beijing Huadu Yukou Poultry Co., Ltd.) and White Leghorn embryos (Poultry Genetic Conservation and Breeding Experimental Station of China Agricultural University) at 5.5-6 days post-incubation. Following enzymatic dissociation, neutralization, centrifugation, and plating, PGCs were expanded in culture medium containing avian KO-DMEM (equivalent to 250 mOsmkg-1 and calcium chloride free) supplemented with 1× GlutaMAX, NEAA, nucleosides,1.2 mM pyruvate, 0.1 mM β-mercaptoethanol, 0.2% ovalbumin, 0.01% sodium heparin, 4 ng/mL FGF2, and 25 ng/ml activin A. Either 5 µg/mL OT or 0.2% chicken serum was added to the final culture medium. The proliferation rate of PGCs varies significantly across chicken breeds. WL PGCs showed significantly accelerated proliferation vs. Rhode Island Red ($p<0.05$). To validate gonadal colonization, WL-P35 PGCs were transfected with piggyBac plasmids and selected with puromycin. Fertilized eggs were incubated to 55-60 h, at which GFP cells were injected into the embryo vasculature, and incubation was continued. Colonization of PGCs was observed at 6 d, 8 d, and sexual maturity in the gonads. Furthermore, immunofluorescence was performed on the gonads of sexually mature roosters, and mature sperm were observed. In summary, this study assessed PGC establishment efficiency in four chicken breeds and generated GFP chimeras using WL PGCs. These findings establish the basis for PGC culture in additional breeds and enhance trait research in specific breeds. Future comparisons with local breeds aim to uncover new insights.

Keywords: Commercial Breeds, PGCs, Cell Proliferation, PiggyBac, GFP Chimeric Chickens

149 Multi-omics reveals key cell types and gene families regulating eggshell strength in chicken uteri

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Eggs are an affordable and nutritious source of high-quality animal protein. Over the past several decades, the production levels and reproductive performance of laying hens have been significantly improved. However, improving eggshell quality, particularly eggshell strength, while maintaining high productivity poses a new challenge for breeders. In this study, we analyzed the eggshell strength phenome of Rhode Island Red chickens and applied multi-omics technologies to high and low eggshell strength individuals, including single-cell transcriptomes of the uterus, multi-tissue transcriptomes, and the proteome of the uterine fluid, to explore the regulatory mechanisms influencing eggshell strength. There were no significant differences in the concentrations of serum calcium and phosphorus between the high and low eggshell strength groups. We successfully constructed the first single cell transcriptome atlas of the uterus, identifying nine cell types in this atlas. These cell types can be divided into four categories: smooth muscle cells, epithelial cells, endothelial cells, and immune cells. Through integrating multi-omics results, we clarified the roles of collagen family genes (*COL4A1/2*, *COL1A1/2*, *COL5A1*, and *COL6A1/2/3*), solute carrier family genes (*SLC4A4/7*, *SLC6A4*, *SLC9A2/9*, and *SLC38A2*), ATPase family genes (*ATP1A1*, *ATP1B1*, *ATP2B1/2*, *ATP2A2/3*, and *ATP2C1*), calcium voltage-gated channel family genes (*CACNB2*, *CACNA1C*, and *CACNA2D1*), annexin family genes (*ANXA5* and *ANXA6*), and integrin subunit family genes (*ITGB1* and *ITGA9*) in determining eggshell strength. These genes associated with eggshell strength are mainly closely related to focal adhesion, regulation of actin cytoskeleton, ECM-receptor interaction, and the calcium signaling pathway. Integrated transcriptomic and proteomic analyses revealed that collagen genes (*COL6A1*, *COL6A2*, *COL6A3*, *COL4A2*, and *COL1A1*) were enriched in the focal adhesion pathway and showed higher expression levels in the high eggshell strength group. In addition, we found that collagen family genes are mainly expressed in smooth muscle cells. This may be related to the inversion that occurs in the uterine region during eggshell calcification, which enables a closer combination of calcium ions and matrix proteins. The insights derived from this study provide a theoretical basis for understanding the regulatory mechanisms underlying the potential for enhancing eggshell strength. Moreover, they lay a theoretical foundation for achieving an ultra-long laying period in laying hens in the future.

Keywords: eggshell strength, multi-omics, single cell RNA sequencing, uterus

POSTER PRESENTATIONS

Animal Health

150P Not Presented

151P Innovative natural compounds for effective coccidiosis control in poultry

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Coccidiosis, caused by *Eimeria spp.*, is a parasitic disease frequently affecting poultry and livestock, leading to significant economic losses estimated at USD 14.4 billion annually for the global poultry industry. The disease reduces feed efficiency, weight gain, and immunity, while increasing mortality and biosecurity costs, with an infection rate exceeding 75% in poultry. Although synthetic anticoccidial agents (e.g., diclazuril, amprolium, salinomycin) and vaccines are commonly used, resistance issues and restricted feeding periods have heightened the demand for natural anticoccidial alternatives. This study aimed to develop a natural anticoccidial agent suitable for shuttle programs to complement existing synthetic agents. The study included both *in vitro* and *in vivo* experiments. For in vitro evaluation, three *Eimeria* species (*Eimeria acervulina*, *E. maxima*, and *E. tenella*) were tested against natural compounds following the USDA protocol. For the *in vivo* trial, 288 seven-day-old broiler chicks were allocated into four groups (n=72 per group): uninfected control, infected control (challenged at day 14), salinomycin-treated, and natural compound-treated and reared for 25 days. The most promising candidates underwent further *in vitro* evaluation for inhibition of parasite invasion and asexual reproduction in MDBK cell lines, as well as assessment of anti-inflammatory and antioxidant properties. Subsequently, a mixture of two candidates was tested *in vivo*, productivity and the Anticoccidial Index (ACI) were assessed to evaluate efficacy. Data were analyzed using one-way ANOVA, and differences among treatment groups were assessed for statistical significance ($p<0.05$). Nine natural compounds exhibiting sporozoite lethality rates exceeding 50% were initially screened for their direct efficacy against *Eimeria* spp. The combination of the two selected natural compounds demonstrated significant inhibition of *Eimeria* spp. invasion and asexual reproduction *in vitro*, along with notable anti-inflammatory and antioxidant effects. *In vivo*, the natural compound mixture significantly improved productivity and anticoccidial index (ACI) compared to the infected control group, with efficacy comparable to salinomycin ($p<0.05$). The selected natural compound mixture exhibited strong anticoccidial activity, supporting its potential as an effective alternative to synthetic anticoccidials in poultry. Further long-term feeding trials and field evaluations are warranted to confirm sustained performance and monitor resistance development.

Keywords: Coccidiosis, Eimeria spp., Natural anticoccidials, Poultry health, Anticoccidial Index

152P Not Presented

153P Not Presented

154P Not Presented

155P Not Presented

Genetics and Genomics

156P Not Presented

157P Investigation of *ELOVL5*'s role in duck cell proliferation and adipogenesis

Dandan Sun*[21], Zhuocheng Hou[21] [1]*China Agricultural University, Beijing, China;* [2]*National Engineering Laboratory for Animal Breeding, Beijing, China*

Elongase *ELOVL5* is a key enzyme regulating very long-chain fatty acid biosynthesis, yet its functions in avian adipocyte biology remain poorly characterized. Given the agricultural importance of lipid metabolism in poultry, elucidating *ELOVL5*-mediated mechanisms in duck adipogenesis is physiologically and economically relevant. This study investigated the role of *ELOVL5* in duck embryo fibroblast cell line CCL-141, which can be induced to undergo adipogenic differentiation, focusing on proliferation, adipogenesis, and fatty acid (FA) synthesis. Knockout (*ELOVL5*-KO) and overexpression (*ELOVL5*-OE) cellular models were established in CCL-141 cells using CRISPR-Cas9 (sgRNA targeting exon 2, 55.7% editing efficiency) and plasmid transfection, validated by genomic analysis, qPCR, and Western Blot. Proliferation was assessed via CCK-8 assay (2,800 cells/well, measurements at days 0–4). Adipogenic differentiation was induced in *ELOVL5*-KO, *ELOVL5*-OE, and empty vector control (EVC) cells, with lipid accumulation quantified by Oil Red O staining (510 nm absorbance). FA composition (SFA, MUFA, PUFA including N-3/N-6 series) was analyzed using GC-MS. Results showed significant proliferation inhibition in both *ELOVL5*-KO and *ELOVL5*-OE cells versus EVC ($P < 0.05$). Both groups exhibited significantly higher relative lipid content post-differentiation ($P < 0.001$). GC-MS revealed significantly lower SFA, MUFA, and PUFA (including N-3 and N-6) levels in *ELOVL5*-KO cells versus control ($P < 0.05$). Conversely, *ELOVL5*-OE cells displayed significantly elevated levels of all FA classes ($P < 0.05$).We conclude *ELOVL5* crucially regulates duck embryo fibroblast proliferation (inhibited by KO/OE) and adipogenesis (enhanced lipid deposition by KO/OE), while directly promoting synthesis of SFA, MUFA, PUFA, and N-3/N-6 series FA. These findings establish *ELOVL5* as a regulator coordinating proliferation-adipogenesis balance in avian fibroblast-to-adipocyte transition, offering novel targets for poultry breeding strategies. *ELOVL5*-mediated FA composition modulation provides a potential avenue for improving nutritional value in poultry products through genetic approaches.

Keywords: ELOVL5, Duck Adipocytes, Cell Proliferation, Adipogenesis, Fatty Acid Composition

158P Not Presented

159P Not Presented

160P Not Presented

161P Not Presented

162P Not Presented

163P Not Presented

164P Not Presented

165P Mitochondrial DNA D-loop analysis on population genetic of native chickens in Kalimantan Indonesia

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Kalimantan (referred to as Borneo) is the third-largest tropical island in the world. It is surrounded by the ocean, also a home for three existing countries (Indonesia, Malaysia, Brunei Darussalam) and has become a strategic route for trade and the spread of religion since the pre-colonial era, with chicken as one of the commodities, along with the process of human migration to Indonesia. While this fact might explain the diversity of native chickens on the island, their population genetic diversity remains underexplored. Here, we analyzed the D-loop, the fastest-mutating region in mitochondrial DNA, of 38 native chickens from various sampling locations in two cities (Balikpapan and Samarinda) in East Kalimantan to elucidate the population genetic diversity and phylogenetic relationship with other chickens in Indonesia and Asia. Polymorphism analysis determined 16 haplotypes derived from 46 segregating sites and estimated a high haplotype diversity at 0.927±0.028 and nucleotide diversity at 0.00645±0.00082 in overall East Kalimantan population. Phylogenetic tree and median-joining network analysis inferred the sample distribution across major matrilinear haplogroups B, C, D, and E, as well as a new haplogroup V. The Balikpapan population appeared to have higher diversity compared to the Samarinda population. AMOVA analysis also showed the highest variation (66.20%) at the population within-group level, indicating a significant difference between the populations. These evidences suggest a high diversity status of native chicken populations in East Kalimantan.

Keywords: Genetic diversity, Haplogroup, Kalimantan, mtDNA D-loop, Phylogenetic tree

166P Not Presented

167P Proteomic analysis of ubiquitination-mediated regulatory mechanisms in chicken primordial germ cell differentiation and proliferation

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Against the backdrop of global poultry breed endangerment and inapplicable mammalian germplasm preservation techniques, this study aimed to elucidate cPGC differentiation/proliferation regulatory mechanisms via proteomic analysis for optimized in vitro poultry germplasm conservation culture. Label-free proteomics of ESCs and cPGCs identified 1,050 DEPs (CV < 0.1, FC > 1.5 or < 0.67). cPGCs showed upregulated spermatogenesis/oogenesis/meiosis proteins (e.g., DAZL, VASA), indicating early gamete formation preparation. GO/KEGG analyses revealed DEP enrichment in energy metabolism, epigenetic/post-translational modification, and autophagy. Glycolysis proteins (HK1, PGK1) were downregulated, while TCA cycle (ACOX1, ACOX2) and respiratory chain proteins were upregulated. In vivo/in vitro experiments showed glycolysis activation/oxidative phosphorylation inhibition reduced genital ridge cPGC formation. cPGCs upregulated autophagy initiators (PIK3C2A, BECN1), with TEM confirming higher autophagy; rapamycin increased cPGC numbers, while 3-MA decreased them. During cPGC formation, chromatin remodelers (SMARCA5, SMARCAD1) suppressed DNA methylation/histone acetylation. DNMT3A/TET1 interference showed low DNA methylation was essential. Upregulated CRLs, proteasome, and Neddylation proteins indicated activated ubiquitination. Western blot showed higher NEDD8-Cullins in cPGCs, and C2EIP's Cullin domain deletion inhibited cPGC development. Activated MAPK, PPAR, Wnt, and JAK-STAT pathways promoted cPGC formation via intravascular injection, with PPI analysis showing coordinated regulation via shared genes (e.g., EP300). Modulating Neddylation identified 447 ubiquitinated targets. SQSTM1 promoted cPGC formation via autophagy/Nrf2 signaling, while PLK1 was critical for proliferation. Validation showed ubiquitination degraded SQSTM1 to enhance cPGC formation and downregulated PLK1 for self-renewal. In conclusion, ubiquitination coordinates cPGC differentiation/proliferation by regulating multiple processes. Targets SQSTM1 and PLK1 optimize in vitro cPGC culture, advancing poultry germplasm conservation and precision breeding.

Keywords: Chicken primordial germ cells, Label-free proteomics, Ubiquitination, Germplasm conservation, Cell proliferation

Immunology

168P Not Presented

169P Generation and characterization of polyclonal antibodies against Dengue, Zika, and Chikungunya virus antigens for multiplex diagnostic applications in chicken egg yolk

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The increasing global incidence of Dengue, Zika, and Chikungunya virus infections underscores the urgent need for rapid, reliable, and cost-effective diagnostic tools to support effective epidemic control. In this study, we explored the potential of generating polyclonal antibodies in chickens targeting specific antigens from these viruses for diagnostic applications. To achieve this, immunogenic viral protein epitopes were first selected through analysis using Immune Epitope Database (IEDB), and the highly immunogenic regions of each antigen were further determined by 3D modeling with AlphaFold. Recombinant antigens-including the NS1 domains of Dengue and Zika viruses and the E2 domain of Chikungunya virus-were expressed in *Escherichia coli* and subsequently purified using Ni2+-NTA affinity chromatography. The successful expression and purification of each recombinant protein were confirmed by SDS-PAGE, which revealed bands at approximately 46 kDa, consistent with their predicted molecular weights. The identities of these proteins were further validated by immunoblotting using anti-His and anti-FLAG antibodies. Purified NS1 and E2 antigens were used to immunize hens, and the production of antigen-specific antibodies in post-immunization sera was confirmed by Western blot analysis. These results demonstrate the strong immunogenicity of the NS1 and E2 domains and the effective induction of polyclonal antibodies in chickens. Notably, due to the high sequence homology and structural similarity of the NS1 antigens from Dengue and Zika viruses, cross-reactivity was observed. Collectively, our findings indicate that chickens can serve as a reliable source of polyclonal antibodies against Dengue, Zika, and Chikungunya virus antigens, supporting the potential application of these antibodies in the development of diagnostic kits.

Keywords: Polyclonal antibody, Chicken, Dengue virus, Chikungunya virus, Zika virus

170P Not Presented

171P Not Presented

172P Effects of Quercetin and Rutin on meat quality and cecal microbiota in broiler chickens under high stocking density

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This experiment was conducted to explore the effects of quercetin and rutin (glycoside form of quercetin) on growth performance, gut morphology, meat quality and cecal microbiome of broiler chickens under high stocking density. A total of 480 one-day-old male Cobb500 broiler chickens were divided into six treatments: one normal stocking density (10 birds/m^2, NSD) and five high stocking density (20 birds/m^2, HSD), with six replicates for each group for 40 days. For five HSD groups, one group was fed the basal diet (H), and the other four groups were fed the basal diet supplemented with 400 ppm quercetin (LQ), 800 ppm quercetin (HQ), 400 ppm rutin (LR) and 800 ppm rutin (HR), respectively. The results showed that HQ can alleviate the decline in feed intake and body weight gain of broilers caused by high stocking density. Jejunal villi height and crypt depth were reduced in the H, however, HQ and HR alleviated this attenuation. Besides, LQ, HQ, LR and HR could significantly reduce the decrease in pH of the pectoral muscle 24 hours after death and significantly improve the meat color brightness at 45 min after slaughter ($P < 0.05$). In addition, HR significantly reduced the drip loss at 24 hours after slaughter. Furthermore, LQ, HQ, LR and HR significantly increased the chao index and PD index ($P < 0.05$). And compared with H, LQ, HQ, LR and HR significantly increased and decreased the abundance of *Bacteroidota* and *Firmicutes* respectively ($P < 0.05$), they also could reduce the proliferation of pathogenic *Streptococcus* and increase the proliferation of functional *Faecalibacterium*, thereby enhancing intestinal health. In conclusion, the results of this study indicate that quercetin and rutin can improve the growth performance, meat quality and intestinal health of broilers under the influence of high stocking density by increasing the diversity of microorganisms and regulating the intestinal flora. Therefore, quercetin and rutin can be used as feed additives in poultry production to improve meat quality and protect the intestinal health of poultry.

Keywords: Quercetin, Rutin, High stocking density, Meat quality, Intestinal flora

173P Effects of Quercetin and Rutin on growth performance, intestinal barrier function and cecal microbiota in broiler chickens challenged with *Salmonella* Enteritidis

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Salmonella is one of the leading bacterial foodborne pathogens and is commonly found in poultry. In this experiment, 360 one-day-old male Cobb500 broilers were selected and randomly divided into six treatments including the control group (Con), *Salmonella* challenged group (S), dietary supplement with 400 or 800 mg/kg quercetin under *Salmonella* challenge (LQ or HQ separately), dietary rutin (a glycoside of quercetin) with 400 or 1600 mg/kg under *Salmonella* challenge (LR or HR separately). Broiler chickens were orally gavaged with 1 ml of 1×10^9 CFU/mL S*almonella* on day 3. On d 11, broilers/treatment were euthanized and eviscerated to collect the test data for analysis. In this study, there were no significant differences in the growth performance of broilers among each group. Compared with the Con, *Salmonella* challenge significantly decreased the villus height-to-crypt depth ratio of the jejunum and ileum, however LQ, HQ, LR and HR significantly alleviated the decreased caused by salmonella challenge. In addition, compared with the other several groups, S would cause a significant upregulation of the ileal genes *ZO-1*, *TLR4* and *IL22* in broilers ($P < 0.05$). Resides, compared with the S, HQ, LR and HR would cause a upregulation of the ileal genes *Mucin2*, *CAT*, *GPX1*, *GPX2*, *GPX3* and jejunal in broilers. Compared with the S, LR and HR could significantly increase the relative abundance of *Lactobacillus*. In conclusion, adding appropriate doses of quercetin and rutin can improve oxidative stress in broilers by regulating gene expression, enhance the antioxidant capacity of broilers, and enrich the lactic acid bacteria in the cecum to strengthen the intestinal barrier, protecting intestinal health and the physiological functions of the host. Therefore, quercetin and rutin can be used as potential feed additives in poultry production to improve the intestinal barrier function and cecal flora of broilers challenged by *Salmonella* enteritidis and protect the intestinal health of broilers.

Keywords: Salmonella, Quercetin, Rutin, Intestinal barrier

174P Not Presented

Meat and Egg Quality

175P Maternal low-protein diets influence sex-specific growth performance, meat quality, and intestinal morphology of broiler offspring

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This study was conducted to elucidate the effects of maternal low-protein on growth performance, meat quality, intestinal morphology, and nitrogen retention rate in broilers offspring with different sex. A total of 300 Tianfu broiler breeders (10-week-old) were randomly allotted to 2 dietary treatments with either normal diets (**NP**) or low crude protein (**CP**) diets **(LP)**. During three phases, diets were formulated to be isocaloric and calculated CP content of the LP diets was 30g/kg lower than the NP diets [phase 1 (10 to18 wk): 150 vs, 120 g/kg, phase 2 (19 to 21 wk): 160 vs 130 g/kg, and phase 3 (22 to 33wk): 160 vs, 130 g/kg]. Using 2×2 experimental design (maternal dietary CP level and sex of offspring), at 34-week-old of breeder, 91 female and 91 male 1-day-old chicks at each maternal treatment were randomly divided into seven replicates for feed a periods of 84 days with normal diets. Results showed that maternal low-protein dietary intervention exerted no significant effects ($P > 0.05$) on offspring growth performance (final body weight, average daily gain, feed conversion ratio) and immune organ indices. However, the male broilers exhibited greater growth performance and intestinal villus height-to-crypt depth ratio than the female. ($P < 0.05$). The maternal fed low-protein diet reduced the initial body weight and leg muscle rate of all broiler offspring, increased the semi-eviscerated and eviscerated rates in female broilers. ($P < 0.05$). It is also impaired meat quality, exhibiting increased drip loss in broilers and reduced the cross-sectional area of breast muscle fiber as well as diameter in males. ($P < 0.05$). Maternal fed low-protein reduced jejunal crypt depth in male broilers and jejunal villi height in female broilers, but not changed the villus height-to-crypt depth ratio. Furthermore, it reduced nitrogen intake by 21.97%, nitrogen excretion by 15.56% compared to the NP. In conclusion, the experimental findings demonstrated that maternal low-protein diets exerted sex-specific effects on broiler physiology without compromising overall growth performance.

Keywords: Meat quality

176P Bright-field automated identification method for eggshell moist spots detection

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Eggshell moist spots are a common eggshell defect and accurate phenotyping is essential to address this issue. Current detection methods typically use single-sided dark-field imaging to assess the ratio of sum of spot areas to sum of shell area (**RSS**). However, questions remain regarding the representativeness of single-sided image measurements and the potential overestimation of RSS by dark-field images (**RSSd**). This study aimed to verify the representativeness of single-side imaging and to develop and validate a bright-field automated identification method to ensure the accuracy of RSS. Five hundred and ten pink-shell eggs were detected. First, the symmetry of moist spots on eggshell was assessed. The results showed that the distribution of moist spots on both sides of the image bounded by the long axis of the egg was significantly symmetrical for both under dark-field ($r = 0.979$, $P < 0.001$) and bright-field ($r = 0.952$, $P < 0.001$), confirming that single-side imaging was representative. Second, a bright-field automated method with specific image processing adjustments was established to represent the true RSS, including optimized threshold, background subtraction, feature filters of moist spots based on the RSS by bright-field images **(RSSb)**. Comparing of RSSb and RSSd of 510 eggs revealed significant difference was found ($P < 0.001$). This indicates that dark-field imaging could not accurately reflect the true RSS under bright-field conditions. The limitations of RSSd were further analyzed using segmented linear regression. The results showed that when the severity of eggshell moist spots was high, RSSd was greater than 7.12%, which was significantly correlated with the RSSb ($r = 0.969$, $P < 0.001$). However, when the severity of eggshell moist spots was low, RSSd was less than 7.12%, the correlation became smaller ($r = 0.498$, $P < 0.001$), and RSSd could not evaluate the true RSS. This meant that the old method based on dark-field images could not accurately reflect the degree of eggshell moist spots and bright-field image method should be used in future.

Keywords: egg quality, eggshell moist spots, bright-field imaging, automated identification, segmented regression

177P Comparative glycoproteomics reveals adaptive divergence in avian eggshell mammillary layer

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The eggshell is vital for the normal embryonic development of birds. It provides protection against microbial invasion and serves as a key source of calcium for embryonic skeletal development. The mammillary layer is a major structural component of the eggshell, determining both its mechanical properties and the embryo's ability to utilize calcium. Different bird species show distinct morphological features in their mammillary layers. During the formation of the mammillary layer, eggshell matrix proteins play a primary regulatory role. Some non-specific matrix proteins are widely expressed in various parts of the egg but perform different functions in different egg regions. This functional divergence is likely caused by differences in their prevalent post-translational modifications, particularly glycosylation. It influences protein folding patterns and thereby affects their biological functions. Based on the above hypothesis, we generated 3D eggshell models from 4 species (chicken, duck, pigeon, parrot) using micro-CT. Mammillary features (knob density, columnar volume) were quantified with 3D Slicer. Matrix proteins from 3 eggs per species underwent proteomics/glycoproteomics. Key proteins were modeled and in reglycosylated (Glycoshape), with surface potential/SASA analyzed. Oligomeric proteins were docked (HDOCK) and ion channels characterized (Caver). Statistical comparisons used ANOVA. The mammillary knobs transitioned from conical (chicken) to flattened (pigeon), with parrots displaying a reticulated structure. Chickens had higher knob density but smaller columnar units than ducks/pigeons. Proteomics identified 817 shared proteins, primarily calcium-binding. Pigeons lacked Ovocleidin-17, a protein associated with amorphous calcium carbonate (ACC) crystal morphology. And parrots showed deficiency in Lysozyme C, which is involved in ACC. Glycoproteomics showed chicken ovalbumin glycosylated only at 293N while ducks/pigeons had additional 97N modification. Chicken ovalbumin exhibited reduced negative charge and SASA, lowering calcium binding capacity. Ions channels in chickens showed higher throughput, lower energy cost, shorter length, and reduced curvature-enhancing calcium acquisition. Mechanistically, uterine fluid triggers ovalbumin unfolding to initiate mineralization. Chicken ovalbumin's superior calcium uptake and lower unfolding threshold accelerate knob formation.

Keywords: Eggshell, Mammillary layer, Mineralization, Glycosylation, Ovalbumin

178P Effects of uterine microbiota and metabolites on the formation of speckled eggs in laying hens

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This study aims to elucidate the potential mechanisms involved in speckle formation, offering a scientific basis and practical strategies to mitigate the incidence of speckled eggs in commercial egg production. A total of 1,200 White Leghorn and 2,000 Red Leghorn purebred hens were used to produce an experimental population, which was raised to 60 weeks of age. Uterine samples were collected from six individuals each in the speckled and normal eggshell groups. Untargeted metabolomics was applied to comprehensively characterize the metabolic profiles of uterine tissues. Untargeted metabolomics data were analyzed by PCA and OPLS-DA using SIMCA software, with metabolites showing VIP > 1 and P < 0.05 considered significant. Microbial community differences were visualized via PCoA and tested by PERMANOVA. Significant shifts in microbial composition were further confirmed by Kruskal-Wallis tests. The LEfSe analysis was employed to identify characteristic microbial biomarkers differentiating the two groups. A total of four microbial taxa were identified as significant discriminators between the groups, including *Pseudomonas* (genus), *Pseudomonadaceae* (family), *p_Actinobacteria* (phylum level), and *c_Actinobacteria* (class level). Among them, *Pseudomonas* and *Pseudomonadaceae* showed significantly higher relative abundance in the speckled egg group, whereas *p_Actinobacteria* and *c_Actinobacteria* were markedly enriched in the normal egg group. Untargeted LC-MS/MS-based metabolomics analysis of uterine samples identified a total of 283 differential metabolites, among which 143 were significantly upregulated and 140 were significantly downregulated in the speckled egg group compared to the normal group. **Discussion:** In this study, 16S rRNA sequencing and untargeted metabolomics analyses were performed on uterine mucosal tissues from speckled and normal eggshell-producing hens. Microbiome analysis revealed that *Pseudomonas* (genus) and *p_Actinobacteria* (phylum) were the key differential microbial taxa between the two groups. These microbes may influence uterine mucosal immune function by modulating the local microenvironment. Metabolomics analysis identified a total of 283 differentially abundant metabolites, which were primarily enriched in glycerophospholipid metabolism, sphingolipid metabolism, the TCA cycle, pyruvate metabolism, and porphyrin metabolism pathways. Notably, metabolic pathways closely associated with ATP synthesis — namely pyruvate metabolism and the TCA cycle — were relatively more active in the speckled group during the 1–2 hours prior to oviposition. This increased activity may enhance the efficiency of protoporphyrin IX extracellular transport.

Keywords: Speckled egg, Uterus, Microbiota, Metabolites

179P Green and sustainable freshness indicator: Utilizing pitaya peel anthocyanin films for monitoring meat freshness

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This research introduces a green and sustainable method for monitoring the freshness of meat products using anthocyanin films derived from pitaya peels. Traditional freshness detection methods for meat products often rely on chemical reagents, which are not only time-consuming and labor-intensive but also pose environmental concerns due to chemical waste. In contrast, our method leverages the natural pH-responsive properties of anthocyanins extracted from pitaya peels, an agricultural waste product, to create a biodegradable and edible film that changes color in response to the volatile compounds released by deteriorating meat. The film's production process is energy-efficient, with a significant reduction in raw material consumption and waste generation compared to conventional plastic films. In applications involving pork, chicken wings, and shrimp, the pitaya peel anthocyanin film exhibited a color change from red to purple, which was positively correlated with the freshness changes of the meat and the TVBN (Total Volatile Basic Nitrogen) content variations. Specifically, as the meat deteriorated, the pH of the surrounding environment increased, causing the anthocyanin molecules in the film to undergo structural changes that resulted in a visible color shift. This color change provided a clear and immediate indication of the meat's freshness status, allowing for early detection of spoilage. For instance, in a study involving chicken wings stored at 4°C, the anthocyanin film changed from red to purple on the fifth day when the TVBN content exceeded 15 mg/100g, indicating spoilage. Increasing the storage temperature accelerated the color change, demonstrating that the anthocyanin film is suitable for freshness detection under different storage temperature conditions. This innovation aligns with the global trend of sustainable development, offering a green, cost-effective, and efficient solution for the meat industry, with potential for broad application in food packaging. By utilizing an agricultural waste product as the raw material, this method not only reduces environmental pollution but also transforms waste into a high-value resource, contributing to a circular economy. The anthocyanin films have shown high sensitivity and specificity in freshness detection, making them a promising alternative to traditional chemical reagents. Future work will focus on optimizing the film formulation and scaling up the production process to meet industrial demands, further promoting the adoption of this sustainable technology in the food industry.

Keywords: Anthocyanin film, Meat freshness monitoring, Pitaya peel waste valorization, pH-responsive biosensor, TVBN correlation

180P Eco-friendly synthesis of Ag-Cu nanoparticles via mannitol reduction for meat freshness monitoring

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This study presents an eco-friendly synthesis of bimetallic silver-copper nanoparticles (Ag-Cu NPs) using food-grade D-mannitol as a reducing agent, demonstrating their dual-mode sensing mechanism for detecting sulfides and biogenic amines in meat freshness monitoring. The Ag-Cu NPs exhibit distinct optical responses: for sulfides (e.g., Na_2S), low concentrations (0-130 μM) enhance Ag/Cu plasmonic peaks via sulfur-metal coordination (Ag-S/Cu-S), while higher concentrations induce oxidative etching, leading to structural collapse and signal quenching. For biogenic amines (e.g., dimethylamine), selective silver enrichment (via amine-mediated reduction) and copper dissolution occur, forming stable Ag-N surface complexes. The NPs show high sensitivity ($\Delta A450 = 0.00435C$ for amines; $\Delta A450 = 0.78114C$ for sulfides, $R^2 > 0.998$) and selectivity against interferents (VOCs, ions). Validation tests on whiteleg shrimp, pork, and chicken demonstrated precise detection of freshness thresholds, particularly when total volatile basic nitrogen (TVB-N) exceeded safety limits (15 mg/100g). For instance, in chicken samples stored at 4°C, the Ag-Cu NPs detected TVB-N levels exceeding 15 mg/100g within 72 hours, indicating spoilage. The sensing platform showed consistent performance across different storage temperatures (4-15°C), with accelerated responses under abusive conditions (e.g., 15°C). The synthesis process is environmentally benign, using D-mannitol as both a reducing and stabilizing agent, replacing hazardous chemicals commonly used in nanoparticle synthesis. The hydrothermal method employed ensures rapid and efficient nanoparticle formation under mild conditions (130°C, 25 minutes), aligning with green chemistry principles. The Ag-Cu NPs not only provide a sensitive and selective detection method for meat freshness but also offer antimicrobial protection, enhancing food safety. This work establishes a sustainable nanotechnology solution for intelligent food packaging, combining green synthesis, dual functionality (sensing and antimicrobial protection), and practical applicability in the meat industry. Future work will focus on optimizing the nanoparticle formulation and scaling up the production process to meet industrial demands, further promoting the adoption of this sustainable technology in the food industry.

Keywords: Bimetallic silver-copper nanoparticles, Sulfide and biogenic amine detection, Meat freshness monitoring, Green synthesis, Dual-mode sensing mechanism

181P Molecular characterization of soft-shelled egg formation in laying hens: Integrated genomic and transcriptomic analysis

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To elucidate the molecular mechanisms underlying persistent soft-shelled egg production in laying hens, focusing on genetic regulation, uterine/liver transcriptomic alterations, and calcium transport pathways. We conducted a case-control study comparing hens persistently laying soft-shelled eggs (cases, n=30) with normal-egg-laying hens (controls, n=30) and the transcriptomic expression profile between soft-shelled and normal groups from a commercial flock of 5,000 39-week-old layers. Blood samples were collected from all 60 hens. Uterine and liver tissues were harvested from 5 hens per group post-slaughter. Whole-genome sequencing (WGS) was performed on all 60 hens. Uterine and liver transcriptomes were sequenced from the 10 slaughtered hens. GWAS identified genetic variants associated with the trait. Differential gene expression analysis (DESeq2, $|\log_2 FC|>1$, FDR<0.05) and functional enrichment (KEGG, GO; Fisher's exact test with FDR correction) were performed on transcriptomic data. **Results Uterine Transcriptome:** Significant downregulation ($P<0.05$) of *EDIL3*, *TMTC1*, *GRIK1*, *ADGRD1*, *ATP2C2*, and *ATP2B4*, and upregulation of *TF*, *SAA*, *NFKBIE*, *PIGR*, and *CCL20*. Upregulated genes enriched immune pathways (Cytokine-cytokine receptor interaction, Phagosome; $P<0.05$). Downregulated genes enriched calcium signaling ($P<0.05$), focal adhesion, and ECM-receptor interaction. GO terms implicated cell adhesion and transmembrane transport ($P<0.05$). **Liver Transcriptome:** Significant downregulation of *SLC22A23* and *MOCOS* ($P<0.05$), and upregulation of *THRSP* and *NFIA,* enriched in protein processing (ER), oxidative phosphorylation, and transmembrane transport ($P<0.05$). **GWAS:** Identified significant association ($P<5\times10^{-8}$) near the *7SK* locus on chromosome 3. We identified a suite of differentially expressed genes and pathways related to calcium transport, energy metabolism, endoplasmic reticulum function, and ion transmembrane transport. The formation of soft-shelled egg may result from impaired uterine cellular uptake or efflux of calcium ions, consequently disrupting calcium carbonate deposition. Additionally, we pinpointed the *7SK* gene as a potential genomic regulator of this trait. These results provide a foundation for understanding the molecular mechanisms underlying eggshell mineralization.

Keywords: Transcriptome, Genome, Soft-shelled eggs, Uterus, Calcium transport

182P Not Presented

183P Not Presented

184P Not Presented

Metabolism and Nutrition: Amino Acids

185P Not Presented

186P Alanyl-glutamine dipeptide mitigates *E. coli*-induced diarrhea in hy-line chicks: mechanistic insights into microbiome modulation, antioxidant response, and intestinal integrity

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Escherichia coli (*E. coli*) infection in poultry triggers severe diarrhea and intestinal damage ultimately disrupting gut function. Alanyl-glutamine (Aln-Gln) dipeptide not only exhibits potent gut-repairing efficacy but also restories barrier integrity and counters *E. coli* pathology through mucosal healing and immune modulation. In this study, the effects of Aln-Gln on microbiome modulation and intestinal integrity in growing hens challenged with *E. coli* were evaluated. The 250 healthy, *Hy-line* brown pullets (42 day old) were randomly divided into 5 experimental groups each having five replicates of 10 chicks for a 14 day trial. Five dietary groups include a basal diet without added Aln-Gln and no *E.coli* challenge (NC), a basal diet without added Aln-Gln and orally administered 2.0×10^4 CFU/mL *E.coli* suspension (C), a basal diet added 0.1% Aln-Gln and orally administered 2.0×10^4 CFU/mL *E.coli* suspension (G1), a basal diet added 0.2% Aln-Gln and orally administered 2.0×10^4 CFU/mL *E.coli* suspension (G2) and a basal diet added 0.3% Aln-Gln and orally administered 2.0×10^4 CFU/mL *E.coli* suspension (G3). The results had shown that Aln-Gln supplementation (0.2–0.3%) reduced diarrhea severity, restored ileal morphology (villus height, crypt depth), improved antioxidant enzymes activity (GSH-Px, SOD) and reduced oxidative markers (MDA) ($p < 0.05$). Aln-Gln decreased pro-inflammatory (IL-1β, IL-6, NO) and apoptotic (Bax) responses and enhanced barrier integrity. It rebalanced gut microbiota by suppressing pathogenic *Clostridium* (75% in C vs. 29.9% in G3) and increasing beneficial *Lactobacillus* in G2/G3 compared with C. These findings make Aln-Gln as a beneficial dietary source combating *E. coli* pathogenic impact by preserving gut health and microbial equilibrium in poultry birds.

Keywords: E. coli, gut, diarrhea, poultry, ileal

187P Not Presented

Metabolism and Nutrition: Enzymes and Feed Additives

188P *Glycyrrhiza uralensis* as a phytogenic approach on modulating early-life muscular and intestinal development via *in-ovo* injection in commercial broiler embryo

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Muscle growth and gut health play significant roles in promoting production of broiler chickens. Both muscle and gastrointestinal tract intensively develop during embryo stage, especially muscle fiber hyperplasia. Moreover, the embryo stage is responsible for one of third lifespan in commercial broilers. Therefore, early nutritional administration has been found to enhance tissues development. However, an effective agent for promoting both muscular and intestinal development is rarely discussed. Phytogenic represents a suitable approach due to complex ingredients that potentially benefit in many aspects. Previous studies showed supplement with *Glycyrrhiza Uralensis* (GU) in diet increased muscle growth and gut health in hatched broilers. However, the effect of GU on muscular and intestinal development in broiler embryo remains unknown. We hypothesize that ethanol extract GU (GUE) promotes both muscular and intestinal development via *in-ovo* administration in yolk-sac of different ages broiler embryo. A total sixty eggs of Arbor Acre broiler were randomly divided into four groups after balance of egg weight. The eggs were injected with 0.2 mL of either PBS or 5 mg/egg GUE on day E10 (GUE-E10) or day E18 (GUE-E18). Embryo chicks were euthanized on E20 and E21 for GUE-10 and GUE-E18, respectively. The total thigh muscle samples were weighted and the gene expression, protein expression, muscle fiber density, and relative cross-sectional area were measured for quantification of muscular development. The intestinal length, cecal length, intestinal morphology and gene expression were measured. Further, the intestinal permeability, glucose absorption, and anti-inflammation ability were detected via apical-out enteroids model for quantification of intestinal development. Student's t-test was used for statistical analysis. In results, GUE increased total thigh muscle weight, muscle fiber density, and relative cross-sectional area in both GUE-E10 and GUE-E18. GUE treatment enhanced intestinal length in GUE-E10 while increased cecal length in GUE-E18. Further, GUE altered intestinal morphogenesis in both GUE-E10 and GUE-E18. Intestinal permeability was lower in enteroids derived from GUE-E10 compared to its control group, but no change on intestinal permeability was found in enteroids derived from GUE-E18 compared to its control group. Overall, the results indicate *in-ovo* administration of GUE on both GUE-E10 and GUE-E18 stimulate muscle growth and gut health in different levels. Understanding the programming effects of early nutrition supplement with phytogenic would shed light on developing novel feeding strategies for improving muscle growth and gut health in broilers.

Keywords: In-ovo, Glycyrrhiza Uralensis, Enteroids, muscle growth, gut health

189P Not Presented

190P Not Presented

191P Not Presented

192P Effects of compound extracts of Chinese herbal medicines for liver and kidney protection on the production performance, liver and kidney tissues and liver gene expression of laying hens in the late laying period

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This experiment aims to explore the effects of compound extracts of Chinese herbal medicines for liver and kidney protection on the production performance, liver and kidney tissues, and liver gene expression of laying hens in the late laying period. In this study, 504 351-day-old Hailan grey laying hens were selected and randomly divided into a blank control group (CON) and three 12-day treatment group with drinking water and feeding drugs (AD), with 6 replicates in each treatment, each repeated 12 were slaughtered and sampled for testing on 12 days.The drinking water administration group was respectively fed with low (LT), medium (MT) and high (HT) doses of compound extracts of Chinese herbal medicines through drinking water. The results showed that, compared with CON, water administration treatment could increase the egg-laying rate of laying hens in the later laying stage, and the HT group could significantly reduce the feed-to-egg ratio ($P < 0.05$). However, compared with CON, there was no significant change in the egg quality of the administration group. In the results of pathological tissue sections, the number of fat vacuoles in the administration group was significantly reduced compared with CON, and the effect of MT was the best. In addition, compared with CON, the administration group significantly upregulated the expressions of liver genes *FASN*, *ACACA*, *SCD* and *SREBF1* ($P < 0.05$), affecting lipid synthesis. In conclusion, the results of this study indicate that the compound extract of Chinese herbal medicines for liver and kidney protection has shown good effects on the production performance and liver and kidney tissues of laying hens in the later laying period. However, its impact on liver gene expression requires in-depth research to explore its influence mechanism. Therefore, the compound extract of Chinese herbal medicines for liver and kidney protection can be used as a health care drug in poultry production to improve production performance and the liver and kidney health of laying hens in the later laying stage, and to increase economic benefits.

Keywords: Chinese herbal medicines, Production performance, Liver and kidney tissues, Liver gene expression, Fat vacuoles

193P Advanced multi-step poultry *in vitro* evaluation platform: Assessing the characteristics and efficacy of poultry feed additives

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The development of reliable evaluation models for predicting nutritive value and functionality is crucial in the pursuit of precision nutrition. To address this need, an innovative *in vitro* model has been established as part of the CJ BIO ANH Application Platform. This model evaluates feed processing stability and predicts poultry growth performance by assessing digesta viscosity, dry matter digestibility (DMD), oligosaccharides(OS) profiles, and intestinal microbiota changes. Non-starch polysaccharides (NSP) in feed are known to reduce digestibility and nutrient absorption. Using NSP-degrading enzymes (NSPase) effectively mitigates these issues, and the Application Platform enables the selection of high-performance enzymes. In the Feed Application, three different products are compared for feed processing stability. In the gastrointestinal tract(GIT) and gut microbiota-metabolites(GMM) Application, we assessed the efficacy of Xylanase and complex enzymes to determine their relative effectiveness. All experiments were conducted in triplicate to allow robust statistical analysis. Xylanase from different manufacturers was added to the feed at 100 U/g and pelleted at various temperatures(75~95 °C, 30 sec) in the Feed Application (pilot feed plant) to assess pelletizing stability. Xylanase efficacy was evaluated by analyzing DMD, viscosity, and Xylo-OS profiles using the GIT Application; moreover, changes in intestinal microbiota were analyzed using next-generation sequencing after anaerobic culture by the GMM Application. Data obtained in this study were analyzed using the ANOVA procedure of SAS 9.4 (SAS Institute, Cary, NC, USA). Xylanases showed varying residual activity across pelletizing conditions at higher temperatures. The GIT Application could screen the superior enzyme product by the reduction of viscosity and improvement of DMD based on the equal enzyme activity across various products ($P < 0.05$). Furthermore, analysis of cultured-gut microbiota in the anaerobic culture system that mimics the gut environment could evaluate enhanced microbial diversity, increased beneficial bacteria such as *Lactobacillus* spp., and inhibition of harmful bacteria in the CJ-treated group ($P < 0.05$). This *in vitro* model can be used to pre-evaluate efficacy before application in feed, while ensuring statistical validity with a minimal number of experimental repetitions. Recent advancements in control technologies and sensor systems have significantly enhanced the precision and consistency of *in vitro* research models, leading to their increasing utilization in research as a cost-effective alternative to conventional animal experiments.

Keywords: Application Platform, pilot feed plant, monogastric GIT system, microbiota, in vitro evaluation model

194P Effects of mixed polyphenols or herbal complexes on production performance and oviduct health in laying hens

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In the late laying period, salpingitis in laying hens severely impacts the economic benefits of the poultry industry. This study conducted to investigate the effects of mixed polyphenols (MP, mangosteen polyphenol: rosmarinic acid=1: 1) or herbal complexes (HC, dandelion: motherwort: codonopsis: caulis spatholobi=4: 2: 3: 1) on production performance, anti-inflammatory and antioxidant capacity, and oviduct health in laying hens, providing experimental evidence for improving production performance and ensuring oviduct health in late-phase laying hens. The experiment was designed as a 2×3 factorial layout, involving two challenge types [phosphate buffered saline (PBS), lipopolysaccharide (LPS)+organic chemical reagent (OCR)] and diet (0, 400 mg/kg MP, 2,000 mg/kg HC). A total of 288 Hy-Line Grey laying hens (55 weeks old) were selected and randomly allocated into three groups: CON group (basal diet), MP group (400 mg/kg MP), and HC group (2,000 mg/kg HC), with eight replicates per group. After an 8-week feeding period, 16 hens from each group were chosen and further divided into non-challenged groups (2 mL PBS per hen) and challenged groups (2 mL LPS + OCR per hen), forming 6 treatment groups (4 replicates/treatment, 2 hens/replicate) for a 48 h trial. Results: (1) challenge effect:

LPS+OCR challenge reduced production performance and antioxidant indices in the oviduct , while increasing pro-inflammatory cytokines and mRNA expression of TLR-4, MyD88, and NF-κB in the ovary and oviduct($P \le 0.05$). (2) dietary treatment effects: MP and HC both reduced pro-inflammatory factor levels in the serum, oviduct, etc., and downregulated TLR-4 and MyD88 mRNA expression in the oviduct ($P < 0.05$). They also increased anti-inflammatory factor content and mRNA expression in the oviduct ($P < 0.05$). HC outperformed MP in improving inflammatory cytokine levels in the jejunal mucosa and uterus ($P < 0.01$). (3) interaction effects ($P \le 0.05$): under challenge, MP and HC can both reduced pro-inflammatory cytokines in the oviduct, downregulated TLR-4, NF-κB, and MyD88 mRNA expression, enhanced anti-inflammatory cytokines and antioxidant indices in the oviduct. HC exhibited stronger suppression of TLR-4 and NF-κB inflammatory gene expression compared to MP under challenge. In summary, supplementation with either additive alleviates the expression of pro-inflammatory factors in the oviduct following LPS and OCR challenge, which is associated with the TLR-4/MyD88/NF-κB signaling pathway. Furthermore, 2,000 mg/kg herbal complexes demonstrate superior anti-inflammatory efficacy compared to 400 mg/kg mixed polyphenols.

Keywords: Salpingitis, Mixed polyphenols, Herbal complexes, Laying hens

195P Bile acids improve the laying performance of aged hens by promoting yolk precursor synthesis and deposition in hierarchical follicles

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The liver-ovary axis dysfunction in aged hens is an important reason for the decline in egg production and quality, which leads to major economic losses in the poultry industry. This study focuses on exploring whether dietary bile acids supplementation could delay the decline in laying performance of aged hens through promoting yolk precursor synthesis and deposition in hierarchical follicles. A total of 320 health 63-wk-old Hy-Line Grey hens were randomly assigned into four groups, with 8 replicates per group and 10 hens per replicate. The control group hens were fed a basal diet, and the experimental group hens were fed basal diets with 200, 500, and 800 mg/kg of bile acids (BA200, BA500, and BA800, respectively; contain hyocholic acid 7.88%, hyodeoxycholic acid 70.23%, and CDCA 18.01%.) for 6 weeks. Compared with the control group, dietary supplementation with BAs significantly increased the laying rate, total egg production and total egg weight, while decreased average egg weight and FCR in the entire trial period. Post hoc multiple comparison analysis showed that supplementation with 200 and 500 mg/kg BAs were significantly increased the laying rate, total egg production. Additionally, it was observed that the number of hierarchical follicles and the amount of yolk deposition in F3 - F5 follicles in BA500 group compared with the control group. The real-time PCR assay results indicated that dietary supplementation with 500 mg/kg BAs was significantly increased VLDLR mRNA levels but decreased ERβ mRNA levels. Moreover, dietary supplementation with BAs significantly enhanced the TG contents both in the liver and blood, especially in BA500 group compared with the control group. Meanwhile, dietary supplementation with BAs significantly reduced plasma VLDL particle size in a dose-dependent manner ranging from 25 to 44 nm. Furthermore, a gene set enrichment analysis method was used to analyze the transcriptome data. It was shown that VLDL particle package pathway was significantly enriched in the liver of BA500 group compared with the control group. The western blot results also demonstrated that dietary supplementation with 500 mg/kg BAs significantly enhanced the VTG2 protein contents in the liver. These results indicated that BAs could promote the laying performance of aged hens by enhancing yolk precursor synthesis and accelerating lipid deposition in small hierarchical follicles. The optimal supplementation level of BAs in aged hens was 500 mg/kg.

Keywords: Aged laying hens, Bile acids, Hierarchical follicles, Liver-ovary axis, Yolk precursor

196P Effect of amla and chili supplementation on production performance, stress markers, and cytokine response in breeder hens

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Currently, heat stress increasingly affects poultry health and productivity in both evaporative and open-house systems, prompting alternative feed additives. This study investigated the effects of amla and chili supplementation in breeder hens raised under different housing systems. A total of 320 SUT breeder hens (34 weeks old) and 40 Leung Hang Khao roosters were used. The study followed a 2 × 4 factorial arrangement in a completely randomized design, with two housing systems (evaporative and open-house) and four dietary treatments: control (CON), amla (AMP), chili (CAP), and a combination (MIX). Eight treatment combinations were tested, each with five replicates of eight hens, housed with 1 rooster per 8 hens. The experiment lasted for a period of 12 weeks. Production performance, stress, and immune responses were evaluated. Statistical analysis was performed using ANOVA followed by Tukey's multiple comparison test. No significant interactions ($P > 0.05$) were observed for egg production, egg weight, hatching rate, egg mass, feed conversion ratio (FCR), and IL-6. The evaporative house showed better egg production, egg weight, egg mass, and lower FCR. AMP supplementation yielded the highest egg production, while CAP led to the highest feed intake. MIX supplementation reduced IL-6 level, while CON had the poorest results. Significant interactions ($P < 0.05$) were found for egg fertility, feed intake, SOD, MDA, glutathione, and TNF-α. Hens in the evaporative house with MIX had the highest fertility. Feed intake was higher across diets in the evaporative house, while in the open house, only CAP improved intake, suggesting it may counteract heat-induced anorexia. The evaporative + MIX group showed the highest SOD activity and lowest MDA levels. In the open house, only AMP, CAP, and MIX reduced

MDA, with CON showing the highest. Glutathione was elevated in the evaporative + AMP or MIX groups, and the open-house + MIX group. TNF-α was lowest in MIX groups across both systems, highest in CON. These results show that while evaporative housing supports baseline health, dietary supplements, especially MIX, enhance reproductive performance, antioxidant status, and immune function. Combining housing and dietary strategies can improve productivity and resilience under heat stress.

Keywords: amla, chili, housing system, breeder hen, stress biomarker

197P Targeted delivery of butyric acid: Performance and survivability outcomes in broilers fed protected butyrate

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Butyric acid (BA) is widely acknowledged for its role in improving gut health and nutrient utilization in broilers, but its free form is volatile and rapidly absorbed in the upper gastrointestinal tract. A total of 600 one-day-old Hubbard broilers (43.76 ± 0.11 g) were randomly allocated to four dietary treatments, with 10 replicate pens per treatment. The treatments consisted of a negative control diet (NC) without any acidified products, organic acids, or probiotics; the NC diet supplemented with 70% sodium butyrate protected by sodium salts of medium-chain fatty acids (DS); the NC diet with 60% di- and triglycerides of butyric acid (PR); and the NC diet with 54% sodium butyrate protected by buffer salts (BB). All butyrate groups were included to provide the same level of BA (54%). The study was conducted over 35 days, with performance parameters including body weight (BW), feed intake (FI), feed conversion ratio (FCR), European Production Efficiency Factor (EPEF), and mortality (MR%). Diets were pelleted to meet FEDNA (2021) specifications for starter (0–14 d), grower (15–27 d), and finisher (28–35 d) phases. Data of productive parameters were analyzed using linear mixed model, with one way ANOVA and Tukey's HSD in JMP Pro 17; significance was set at $P \leq 0.05$. In case of MR%, generalized mixed model was used assuming beta distribution. Throughout the trial, all butyrate groups demonstrated significant improvements in performance compared to the NC group ($P \leq 0.05$). Over 35-day period, DS birds achieved the highest BW gain (2364g) and best FCR (1.369), followed by BB (2300g; FCR 1.381), PR (2310 g; FCR 1.400) and NC (2264g; FCR 1.446; all $P<0.001$). FI was greatest in NC (3341g) versus DS and PR (3296 g) and lowest in BB (3238g; $P=0.0226$). MR was reduced in butyrate groups with an ODDs RATIO from 0.39 to 0.41 in all butyrate groups vs NC ($P<0.01$), with estimated marginal means of MR from 10% to 4.3%, driving improvements in EPEF (DS 485 > BB 468 > PR 461 > NC 416; $P<0.001$). Weekly trends mirrored these findings: in the starter phase, DS significantly decreased FCR and increased ADG versus NC ($P<0.05$), with PR and BB showing intermediate gains; during the grower phase, DS maintained superior BW gain and FCR; in the finisher phase, all protected butyrate forms sustained lower mortality and improved FCR relative to NC, with DS exhibiting the greatest impact. In conclusion, all forms of butyric acid improved performance, with MCFA-protected sodium butyrate showing the greatest effect on growth and efficiency. MCFA likely enhanced SCFA action through synergistic antimicrobial and gut-modulating effects.

Keywords: Broilers, Butyric Acid, Productive Performance, Mortalities, Economic Evaluation

198P Impact of phased yeast-based nucleotide supplementation on growth performance, and physiological and meat quality parameters in densely-stocked broilers

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Supplemental nucleotides (NTs) have emerged as a promising alternative to antibiotic growth promoters (AGPs) in rapidly growing or physiologically stressed broilers. In such birds, the demand for NTs often exceeds the capacity of both *de novo* synthesis and the salvage pathway, necessitating exogenous supplementation. However, continuous NT supplementation can elevate production costs and may not always be essential. This study compared the effect of continuous versus phased yeast-based NT supplementation on performance metrics, and physiological and meat quality responses in densely-stocked broiler chickens. A total of 900, one-week-old, male Ross 308 broiler chicks were randomly allotted to 36 pens (experimental unit with 25 birds/1.2 m^2) and reared on six isonitrogenous and isocaloric diets. The diets were: 1) a standard diet containing AGP, fed from starter phase through to finisher phase (POSCON); 2) a standard diet with neither AGP nor NTs, fed from starter phase through to finisher phase (NEGCON); 3) a standard diet containing 0.05% NTs in place of AGP, fed from starter phase through to finisher phase (NCC); 4) a standard diet containing 0.05% NTs in place of AGP, fed in starter phase only for a week (NCS); 5) a standard diet containing 0.05% NTs in place of AGP, fed in grower phase only for two weeks (NCG); and 6) a standard diet containing 0.05% NTs in place of AGP, fed in finisher phase only for two weeks. Growth performance were estimated weekly then at day 42 of age, 10 birds per experimental unit were slaughtered for post-mortem measurements. Data were analyzed using PROC GLM in SAS version 9.4, mean comparisons were done using Tukey's HSD test, and statistical significance was declared at $P < 0.05$. Diet NCS promoted the lowest ($P < 0.05$) feed intake (FI) similar to NCG, NCF and NCC, while POSCON promoted the highest FI. In addition, POSCON promoted the highest ($P < 0.05$) body weight gains followed by NCG. Diet NCC promoted the highest ($P < 0.05$) feed conversion ratio (FCR), whereas POSCON promoted the least FCR. Diets NCC and NCS promoted the highest proventriculus size, and NCF promoted the highest ileum lengths. Drip loss and shear force were the only meat quality traits affected ($P < 0.05$) by diets. Blood urea nitrogen (BUN), albumin (ALB), alanine transaminase (ALT), and total bilirubin (TBIL) were the only biochemical parameters affected ($P < 0.05$) by diets, with NCG promoting the highest BUN, ALB, ALT, and TBIL, while POSCON promoted the lowest values. Yeast-based NTs were inferior to AGPs in terms of growth performance of densely-stocked broilers. Supplementing NTs in the grower phase resulted in better BWG, FCR, and shear force outcomes compared to the starter and finisher phases.

Keywords: Antibiotic growth promoters, Broilers, Exogenous nucleotides, Stocking density, Phased supplementation

199P Not Presented

Metabolism and Nutrition: General Nutrition

200P Not Presented

201P Effect of the use of microencapsulated amino acids supplementation on the utilization advantage and intestinal barrier function of low-protein diets in laying hens during late laying period

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This study investigated the effects of microencapsulated amino acids added to low-protein diets on the performance and intestinal health of laying hens during late laying period. The aim was to provide insights for the improved application and application of low-protein diet technology in laying hens' production, and mitigate environmental pressures associated with poultry farming. One hundred and eighty 59-week-old Beijing Powder 6 laying hens with similar body weight and egg production rate were divided into three treatment groups: the control group (**CON**) was fed with a crude protein level of 16%, the low-protein diet crystal amino acid supplementation group (**LCP-CAA**) and the microencapsulated amino acid supplementation group (**LCP-MAA**) with a crude protein level of 13%. The standardized ileal digestible amino acid levels in the LCP-CAA and LCP-MAA diets were maintained equivalent to those in the CON diet. The experimental period was 13 weeks, including a 1-week pretest period and a 12-week positive test period. The results showed that average daily feed intake (**ADFI**), laying rate (**EP**), egg weight (**EW**), and egg mass (**EM**) were significantly reduced ($P < 0.05$) after lowering the crude protein level, but no significant difference was found in feed conversion ratio (**FCR**) ($P > 0.05$). After 12 weeks of dietary treatment, both Chicken diamine oxidase (**DAO**) and D-Lactic acid (**D-LA**) levels were significantly lower in the LCP-MAA group than in the LCP-CAA group ($P < 0.05$), and jejunal and ileal trypsin and chymotrypsin activities were significantly higher in the LCP-MAA group than in the CON group and the LCP-CAA group ($P < 0.05$) ,and the expression of genes related to the amino acid transporter showed an upward The expression of amino acid transporter-related genes also showed an upward trend. Moreover, the expression of *CAT-1*, y^+LAT1, $b^{0,+}AT$, B^0AT, *rBAT*, *EAAT3* and *PepT1* in the duodenum, jejunum and ileum was not affected by dietary treatments, suggesting microencapsulation did not alter amino acid transport. Fecal nitrogen content was significantly reduced ($P < 0.01$) in the low-protein diet groups. The addition of microencapsulated amino acids to low-protein diets enhanced intestinal barrier function and amino acid absorption balance of laying hens, without significantly affecting on intestinal amino acid transport, thereby supporting the performance and intestinal health of laying hens during late laying period.

Keywords: Laying Hens, Late Laying Period, Low-protein Diets, Microencapsulated Amino Acids, Intestinal Barrier Function

202P Not Presented

203P Metabolic characteristics of the uterus in the eggshell mineralization cycle of laying hens and their association with the eggshell quality during the late laying period

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Uterine metabolism of laying hens directly regulates the mineralization process and provides a suitable mineralized environment for eggshells. This study aimed to characterize the uterine metabolism of laying hens in the eggshell mineralization cycle and to reveal its association with the decline in eggshell quality during the late laying period. In the experiment, a total of 100 healthy laying hens were selected from each of the peak laying period (40-week-old) and late laying period (72-week-old). Then, the uterine tissue samples were collected at 7 h and 17 h post-oviposition (PO). The eggshell quality, ultrastructure, and calcium transport in uterine tissues were detected, and the metabolites in the uterus were assayed using UHPLC-MS/MS. Statistical significance between the two groups was evaluated using a two-tailed Student's t-test. The results showed that the thickness, strength, hardness, toughness, thickness and proportion of the effective layer, and calcium content of eggshell in aged laying hens were significantly lower than those of eggshell in young laying hens ($P < 0.05$). The expression of *TRPV6*, *CALB1*, *ATP2A2*, *ATP2A3*, *ITPR1*, *ITPR3*, *SLC8A1*, and *SLC8A3* was significantly higher at 17 h PO than at 7 h PO in young laying hens ($P < 0.05$), but no such difference was observed in aged laying hens ($P > 0.05$). There were significant differences in the uterine metabolism of laying hens at 7 h PO and 17 h PO, with 175 and 178 differential metabolites identified in peak and late laying period, respectively (VIP > 1, $P > 0.05$). Moreover, significant differences were also observed in the uterine metabolism of peak and late laying hens, in which 197 and 212 differential metabolites were identified at 7 PO and 17 h PO, respectively (VIP > 1, $P > 0.05$). Notably, the metabolism of phospholipids in the uterus of laying hens was enhanced in aged laying hens relative to the young laying hens, and the levels of phosphatidylglycerol and phosphatidylserine were significantly higher in the aged laying hens at the 7 h PO ($P > 0.05$). Meanwhile, the level of lysophosphatidylcholine in the aged laying hens was significantly higher than that in the young laying hens at the 17 h PO ($P > 0.05$). Therefore, the disordered lipid metabolism of the uterus may disrupt calcium transport in the eggshell mineralization cycle, resulting in decreased eggshell calcium deposition and poor eggshell quality during the late laying period. These findings contribute to the precise regulation of eggshell quality and extended laying cycle in laying hens.

Keywords: laying hen, uterine metabolism, eggshell quality, eggshell mineralization, calcium transport

204P Effects of dietary protein restriction and excess on production performance, egg quality, organ indices and serum parameters in late-phase laying hens

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This was conducted to evaluate the effects of dietary protein restriction and excess on production performance, egg quality, organ indices and serum parameters late-phase laying hens. A total of 270 (88-week-old) Lohmann pink hens were randomly assigned to three dietary treatments: control group (CON, basal diet CP 15.94%), HP group (CP 18.88%) and LP group (CP 12.92%), Each treatment consisted of 10 replicates, with 9 hens per replicate. The ratios of digestible EAAs were kept consistent across all groups, while no additional amino acid supplementation provided to the LP group. The formal experimental trial was conducted for 12 weeks. The results showed that neither dietary protein restriction nor excess significantly affected the egg-laying rate during the late laying period ($P > 0.05$). The LP group had significantly decreased egg weight (wk 1 to 4, 5 to 8, 9 to 12, and overall 1 to 12) ($P \leq 0.01$), and the HP group had significantly decreased FCR (wk 9 to 12, and 1 to 12) ($P \leq 0.05$). The LP group exhibited significant increases in albumen height, yolk weight ratio, yolk color and haugh unit ($P \leq 0.05$), along with a significant decrease in albumen weight ratio ($P \leq 0.05$). The HP group had significant increases albumen weight ratio and eggshell color (a* and b*) ($P \leq 0.05$). Protein restriction and excess does not affect the body weight, hepatic index, abdominal fat percentage and ovarian weight percentage in laying hens ($P > 0.05$). Additionally, liver *S6k* gene expression was significantly upregulated in the LP group, whereas *TSC2* expression was downregulated ($P < 0.05$). Conversely, SLC7A5 expression was significantly downregulated in the HP group ($P < 0.05$). Serum biochemical parameters were altered in response to dietary protein levels, UA was significantly increased in HP group ($P < 0.05$), whereas ALT was significantly reduced ($P \leq 0.01$). TP and ALB exhibited a tendency toward reduction in the LP group ($P = 0.07$, $P = 0.08$). The HP group exhibited elevated serum levels of Val and Cys, reduced levels of Ser, Ala, Met, Trp and Lys ($P < 0.05$). The LP group showed decreased serum levels of Thr, Val, Met, Trp, Lys, Arg, and with increase in Cys ($P < 0.05$). In conclusion, the dietary protein restriction without negatively affecting in laying rate, egg quality and organ indices. Instead, it significantly reduced serum ALT levels, changed the content of free amino acids, and the expression of mTOR-related genes in the liver. These findings suggest that laying hens mount an adaptive response to protein restriction during the late laying period, potentially enhancing amino acid utilization efficiency. Further research is warranted to elucidate the underlying molecular mechanisms.

Keywords: Protein restriction, Laying rate, Egg quality, Organ indices, Serum parameters

205P Black soldier fly larvae oil ameliorates gut barrier integrity in broiler chickens fed a low-protein diet

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Feeding low crude protein (**LCP**) diets supplemented with crystalline amino acids provides benefits for production, environmental sustainability, and animal welfare. However, LCP diets can adversely affect intestinal growth and barrier function in broiler chickens. Black soldier fly larvae oil (**BSFLO**), consist high lauric acid, has potential for improving gut heath. A 3 x 2 factorial was conducted to investigate the effect of BSFLO supplementation on jejunum morphology, barrier integrity, and inflammation responses in broiler chickens fed LCP diets. A total 288 broilers were divided into 6 treatments: based on three levels of crude protein (**CP**): (200, 185, or 170 g/kg; high [**HCP**], medium [**MCP**] or low [**LCP**]) and two oil sources (BSFLO and crude palm oil; CPO), with 6 replicates and 8 birds each. **Results** showed that reducing CP levels significantly decreased villus height (**VH**; $P = 0.001$) but had no effect on villus width (**VW**), crypt depth (**CD**), and VH:CD ratio ($P > 0.050$). Oil supplementation had no significant impact on jejunum histomorphology parameters ($P > 0.050$). The expression of tight junction-related genes (*CLDN-1, JAM-2, and ZO-1*) was significantly downregulated ($P < 0.001$) in broilers fed LCP diets, particularly at a 30 g/kg CP reduction. However, supplementation with BSFLO significantly upregulated ($P < 0.001$) the expression of tight junction-related genes (*JAM-2* and *ZO-1*) despite CP reduction. Moreover, dietary LCP diets increased ($P < 0.001$) the expression of pro-inflammatory genes (*IL-6, IL-18, and TNF-α*) and decreased ($P = 0.033$) the expression of the anti-inflammatory gene (*IL-13*). Notably, BSFLO supplementation mitigated ($P < 0.001$) the upregulation of pro-inflammatory gene expression (*IL-6 and IL-18*) and significantly increased the expression of anti-inflammatory genes (*IL-13*). This study demonstrates that BSFLO improved gut barrier integrity in broiler chickens fed LCP diets by upregulating the expression of tight junction-related genes (*JAM-2* and *ZO-1*) and anti-inflammatory cytokines (*IL-13*), while downregulating pro-inflammatory cytokines (*IL-6 and IL-18).*

Keywords: Poultry, Protein, Insect, Intestinal, barrier function

Metabolism and Nutrition: Vitamins and Minerals

206P Mechanism of action of organic zinc in regulating the growth performance and meat quality of yellow-feathered broilers

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To investigate the effects of different levels of organic zinc (OZ) replacing inorganic zinc on biochemical variables in plasma, amino acids and metabolites of muscle, etc., thus to

analyze the mechanism of its role in regulating the growth performance and meat quality of yellow-feathered broilers. A total of 1,200 1-d yellow-feathered broilers were randomly allocated to 4 treatments, with 10 replicates per treatment, and 30 birds per replicate. The trial lasted for 75 days. Zinc sulfate was used in the control diets (CON), and OZ was used to replace zinc sulfate in the ratio of ‘1/3’, ‘2/3’ and ‘1’ (OZ1, OZ2, OZ3). The effects of treatments were examined by ANOVA and Duncan's multiple range tests. The results showed that, compared with birds in CON, 1) OZ reduced the ADFI of birds from 61 to 75 days of age (OZ2) and F/G during the whole phase (OZ3) ($P < 0.05$). 2) the relative expression of *MT*, *MT3*, *MT4* and *DMT* in ileum was enhanced ($P < 0.05$) in birds from OZ2. 3) for meat quality, OZ decreased ($P < 0.05$) the shear force (OZ1), increased ($P < 0.05$) the a* value 45 min post-mortem and the pH value 72 h post-mortem (OZ2), and decreased ($P < 0.05$) the drip loss (OZ3). Moreover, the levels of TVB-N 72 h post-mortem were also decreased in birds from OZ2 and OZ3 ($P < 0.05$). 4) All treatments enhanced ($P < 0.05$) the muscle fiber density. 5) for biochemical variables in plasma, OZ decreased ($P < 0.05$) the triglyceride (OZ1), and the total cholesterol (OZ2, OZ3), and enhanced ($P < 0.05$) the high-density lipoprotein cholesterol (OZ2, OZ3). 6) Compared with birds in CON, all treatments enhance ($P < 0.05$) the levels of 13 free amino acids of muscles, including isoleucine, threonine and lysine. 7) Liver transcriptomics and muscle metabolomics analyses of birds from CON and OZ2 further revealed that hepatic differential genes were enriched in the PPAR signal pathway, steroid hormone biosynthesis, and adipocytokine signaling pathway when treated with OZ ($P < 0.05$); Differential metabolites in muscle were enriched in nicotinic acid and nicotinamide metabolism, cofactor biosynthesis, TCA cycle, linoleic acid metabolism, and other metabolic pathways ($P < 0.05$). In conclusion, the dietary supplementation with OZ replacing inorganic zinc enhanced the relative expression of intestinal zinc transporter proteins and improved the growth performance of broiler chickens; OZ improved muscle fiber density, increased the amino acid content of muscle, thus improved the muscle quality and flavor; and its action may be related to the regulation of lipid metabolism through the PPAR signaling pathway. Under the conditions of this experiment, the optimal ratio of OZ replacing inorganic zinc is 2/3.

Keywords: organic zinc, yellow feather broiler, meat quality, zinc transporter protein, PPAR signaling pathway

207P Chromium propionate as a dietary strategy for heat stress mitigation in broilers

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This study was conducted to evaluate the effects of chromium propionate (Cr-Pro) levels in broiler diets under heat stress (HS) on performance, digestibility, immunity, carcass characteristics, and mineral retention. One hundred and sixty 1-day-old Arbor Acres broiler chickens (initial body weight of 37.98 ± 0.22 g) were used in this experiment for d 35. They were assigned to four treatment groups (eight replications, four birds each per cage): PC, birds fed a basal diet under thermoneutral temperature; NC, birds fed a basal diet and were exposed to cyclic HS at d 14 of age; CP2, NC with supplementing chromium (Cr) 200 µg/kg as Cr-Pro; CP4, NC with supplementing Cr 400 µg/kg as Cr-Pro. The HS conditions exposed the birds to cyclic periods of elevated temperature (32 ± 2°C) for 8 h daily, from 10:00 A.M. to 6:00 P.M., with the temperature gradually increasing and decreasing over 30 min, to mimic the summer season in the Republic of Korea. The HS treatment was applied from d 15 to 35. Data on all analysis items were statistically analyzed by one-way ANOVA using JMP (JMP Pro version 16.0.0, SAS Institute Inc., Cary, NC, USA). Differences between treatment means were determined using Tukey’s multiple-range test. A probability level of $P \le 0.05$ was indicated to be statistically significant, and a level of $0.05 < P \le 0.10$ was considered to have such a tendency. In this study, the NC exhibited decreased ($P < 0.05$) performance and nutrient digestibility compared to the PC, while showing increased ($P < 0.05$) mineral excretion and abdominal fat. Additionally, HS increased ($P < 0.05$) cortisol, norepinephrine, and immunoglobulin A and G levels, while decreasing ($P < 0.05$) glucose levels in serum. However, the CP4 showed higher ($P < 0.05$) body weight gain, crude protein digestibility, and retention of copper and Cr than the NC. Also, Cr-supplemented groups showed lower ($P < 0.05$) abdominal fat than the NC. In the serum profiles, Cr supplementation decreased serum norepinephrine levels under HS. Serum glucose levels were significantly higher ($P < 0.05$) in the CP4 compared to the NC. Serum IgG levels were significantly lower ($P < 0.05$) in the CP2 and CP4 compared to the NC. The CP4 showed a significant decrease ($P < 0.05$) in serum cortisol and H:L ratio compared to the NC. In conclusion, Cr-Pro is an additive that can mitigate the effects of HS, supplementation with 400 µg/kg Cr is suggested as a more effective alternative than 200 µg/kg.

Keywords: Heat stress, Broiler, Mineral retention, Blood Profile, Chromium propionate

Microbiology and Physiology

208P Not Presented

209P Not Presented

210P Not Presented

211P Not Presented

212P Effect of dielectric barrier discharge cold plasma combined with lactic acid on the microbial community structure of yellow-feather chicken carcasses

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The objective was to investigate the impact of dielectric barrier discharge (DBD) cold plasma combined with lactic acid (LA) on the microbial community structure of yellow-feather chicken carcasses during cold storage at 4 °C. Chicken carcasses were randomly assigned to four groups: control (CK, untreated), DBD (150 kV, 115 s), LA (2.0% lactic acid), and DBD+LA (sequential DBD after LA treatment). Samples were collected on days 1, 3, 5, 7, and 9 post-treatment for microbial analysis, with each group containing

4 replicates to ensure statistical reliability. For microbial characterization, surface microbes were eluted using 0.85% saline, and total DNA was extracted using the E.Z.N.A.® Soil DNA Kit. The V3-V4 region of the 16S rDNA gene was amplified with primers 341F and 806R, followed by Illumina NovaSeq PE250 sequencing. Bioinformatics analysis included OTU clustering at 97% similarity using UPARSE, Alpha diversity indices (Richness, Chao1, Shannon, Simpson), and taxonomic annotation against the Silva 138 database. Principal component analysis (PCA) and heatmap clustering were performed to visualize community dynamics, while one-way ANOVA with Duncan's multiple range test ($P < 0.05$) was used to assess significant differences among groups. The results demonstrated that DBD+LA treatment significantly modified the microbial community structure of yellow-feather broiler carcasses during cold storage. At the early stage (days 1–3), DBD+LA reduced microbial richness indices (Richness, Chao1, ACE) and increased Shannon and Gini-Simpson diversity indices compared to the control. At the phylum level, *Proteobacteria* was the dominant phylum in all groups, with DBD+LA significantly inhibiting its abundance while increasing *Bacteroidetes*. At the genus level, DBD+LA reduced the relative abundance of *Pseudomonas* by 26.00% and increased *Vibrionimonas* by 15.13% on day 1. Principal component analysis revealed that the control group shifted to a *Pseudomonas*-dominated community by day 7, whereas DBD+LA maintained a more stable microbial composition. Throughout storage, DBD+LA effectively suppressed the growth of dominant spoilage bacteria and altered the microbial community structure, as confirmed by statistical analysis (ANOVA, $P < 0.05$). In conclusion, DBD+LA synergistically modulates the microbial community of yellow-feather chicken carcasses by inhibiting dominant spoilage bacteria such as *Pseudomonas* and preserving microbial diversity, thereby extending shelf-life by 4 days compared to CK. This non-thermal technology offers a promising approach for industrial poultry preservation.

Keywords: cold plasma technology, lactic acid treatment, microbial community structure, Yellow-feather chicken, poultry preservation

213P Not Presented

214P Single-cell sequencing reveals an imbalance of cellular homeostasis in the uterus of laying hens during the late laying period

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Characterizing changes in the uterus at the cellular level and its mechanisms is essential for improving uterine function deterioration and eggshell quality in laying hens during the late laying period. Here, A total of 40 healthy Hy-Line Brown were selected, including peak egg-laying period (38-week-old) and late egg-laying period (77-week-old). The characteristics of uterine homeostatic imbalance were detected by transmission electron microscopy and RT-qPCR. Then, a single-cell transcriptomic landscape reveals changes in cell-type specificity and mechanisms in the uterus of laying hens. Statistical significance between the two groups was evaluated using a two-tailed Student's t-test. The results showed that membrane damage, intramembrane matrix lysis, and cristae disintegration of mitochondria were observed in the uterine cell structure of aged laying hens. Notably, mRNA expression levels of *IL-1β*, *PARP1*, *P21*, *P16*, *ATR*, and *ATM* genes were significantly higher in the uterus during the late egg-laying period compared to the peak egg-laying period ($P < 0.05$), suggesting an imbalance in uterine cellular homeostasis. Furthermore, a total of 13 distinct cell types were identified, including fibroblasts, smooth muscle cells, epithelial cells, ciliated epithelial cells, non-ciliated epithelial secretory cells, epithelial progenitor cells, endothelial cells, CD4+ T cells, CD8+ T cells, B cells, plasma cells, erythroblasts, and lymphatic endothelium. Epithelial and endothelial cells were increased in the aged group compared to the young group, which was accompanied by a decrease in the number of CD4+ T cells, CD8+ T cells, and B cells ($P < 0.05$). The analysis of CellPhoneDB revealed that increased cell-cell communication and key regulators involved in regulating cell proliferation, inflammation, and apoptosis were identified in the aged uterus ($P < 0.05$). Meanwhile, senescence scores of epithelial cells and immune cells were significantly higher in the aged uterus compared to the young uterus ($P < 0.05$). The analysis of pseudotime trajectory indicated that the DNA damage response is an essential biological pathway driving the process of cellular senescence in the uterus. Therefore, cellular damage-driven cellular senescence results in an imbalance of homeostasis in the uterus of aging laying hens. These findings enhance the comprehension of the biological mechanisms associated with uterine aging and function deterioration, thereby facilitating the investigation of regulatory strategies for oviducal health and extended laying cycle in laying hens.

Keywords: laying hen, cellular homeostasis, uterine aging, single-cell transcriptomic

215P Not Presented

Poultry Management and Well-Being

216P Not Presented

217P Not Presented

218P Not Presented

Processing and Products

219P Protein differences reveal the key mechanism for the high-value utilization of broiler breast fillet myopathy

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Broiler breast fillet myopathy, including pale, soft, and exudative meat, white striping, and wooden breast, are significant economic and safety issues in the global poultry industry. To elucidate the intrinsic relationship between myofibrillar protein (MP) structure of different types of breast fillets and the quality of further-processed products, and to address the problem of high-value utilization of breast fillets. 100 fillets (n=20/group) were selected and categorized into *pectoralis major* muscle of normal (NORM-M) and wooden (WB-M), *pectoralis minor* muscle of normal (NORM-m), wooden (WB-m), and tender feathering (TF-m). The study focused on the structure, gel properties, water distribution, and proteomic differences of MPs in different categories of breast fillet. The results showed that the α-helix content and myosin heavy chain levels were significantly reduced in the WB-M, WB-m, and TF-m groups ($p < 0.05$). After heat induction, the gel strength, elasticity, and rheological properties of WB-M, WB-m, and TF-m were significantly lower than those of NORM-M and NORM-m ($p < 0.05$). Proteomics further discovered that the protein composition and content of breast fillets with different myopathy condition were significantly different from both NORM-M and NORM-m, revealing the abnormal protein composition in WB-M, WB-m and TF-m. Additionally, the surface thiol content of MPs in WB-m and TF-m was significantly reduced, with increased hydrophobicity and decreased free radical scavenging capacity ($p < 0.05$), accelerating the deterioration of flavor and texture in meat products. Histopathological sections and low-field nuclear magnetic resonance imaging showed that, compared to NORM-m, WB-m exhibited more complex myofiber disorganization and intermuscular water loss than TF-m. The study results indicate that differences in protein composition and gel properties between NORM, WB, and TF are key factors causing product quality differences, and improving protein structure and enhancing gel properties are fundamental strategies for improving the quality of further-processed products from WB and TF.

Keywords: breast fillet myopathy, protein structure, meat quality, myofibrillar protein, gel properties

220P Antioxidative and neuroprotective effects of phosvitin phosphopeptides against H_2O_2-induced oxidative stress in SH-SY5Y cells

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Phosvitin is a highly phosphorylated protein in egg yolk and is an excellent source of phosphopeptides production. Phosphopeptides are among the most interesting bioactive peptides with various biological functions, including metal-chelating, anti-cancer, anti-inflammatory, tissue whitening, and antimicrobial activities. The objective of this study was to determine the antioxidant activities of phosvitin phosphopeptides on the H_2O_2-induced cell damage in human neuroblastoma SH-SY5Y cells. Phosvitin from chicken egg yolk was pretreated using high-temperature mild pressure (HTMP) conditions and then hydrolyzed using trypsin (T), Multifect 14L (M), and trypsin + Multifect 14L (TM) to prepare 4 phosvitin phosphopeptides (PPPs) treatments (HTMP, HTMP-T, HTMP-M, and HTMP-TM). The antioxidant and protective properties of PPPs were assessed using chemical analyses and human neuroblastoma (SH-SY-5Y) cells. The one-way ANOVA was used for multiple groups and the Duncan's multiple range test was used to evaluate the significance level ($p < 0.05$). All PPPs exhibited reducing power and radical scavenging, metal-chelating, b-carotene bleaching inhibitory, and lipid peroxidation inhibitory activities, but HTMP-TM had the highest antioxidant activity ($P < 0.05$). HTMP-TM increased cell viability and reduced ROS production and apoptosis in stressed (H_2O_2-treated) SH-SY-5Y cells ($P < 0.05$). Additionally, HTMP-TM downregulated Bax/Bcl-2 and caspase-3 expression but upregulated catalase expression in oxidatively stressed SH-SY-5Y cells. It was concluded that HTMP-TM exerted protective effects through their antioxidative and anti-apoptotic properties in oxidatively stressed SH-SY-5Y cells.

Keywords: Phosvitin phosphopeptides, Antioxidant activity, Neuroblastoma cell, Protective effect, Anti-apoptotic properties

221P Not Presented

Small-Holder Farming

222P Not Presented

SYMPOSIUM PRESENTATIONS

WPSA Lecture

223S Poultry Diversity: From genetics to products

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No abstract submitted.

Myopathies in fast growing broilers: Challenges to growers and the meat industry

224S Multi-Omic Insights: Comprehensive views of biological processes associated with growth-related myopathies

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Growth-related myopathies significantly compromise poultry meat quality and industry economic viability. These issues are hypothesized to stem from a complex, multifactorial metabolic and physiological imbalance. This presentation aims to underline the distinct yet complementary findings among omics approaches in identifying the etiologies of the myopathies. Transcriptomics have pinpointed biological alterations. Theses include metabolic pathways of macronutrients, extracellular matrix, collagen organization, protein oxidation, focal adhesion, cytoskeleton regulation, and MAPK signaling. Spatial transcriptomics identified reprogramming of lipid metabolism in perivascular macrophages during onset of the myopathies. At the proteomic level, aberrant post-translational modifications (e.g., methylation, acetylation, oxidation) on key muscle proteins indicate disrupted signaling pathways and profound redox imbalance. The protein-level findings support biological alteration at the gene expression level. They also provide a direct link to phenotypic changes, confirming widespread protein damage and dysfunction. Metabolomics complements these insights by identifying energy-stress signatures. In white striping, perturbations in the tricarboxylic acid cycle were observed. The results showed energy production under hypoxia. However, β-oxidation was limited and long-chain fatty acids accumulated in the abnormal birds. Markers of hypoxia and oxidative stress were elevated. Specifically, arginine-to-citrulline conversion for nitric oxide increased, and osmolytes (i.e., taurine, sorbitol, alanine) rose in severe cases. Collectively, multi-omics findings implicate hypoxia-induced muscle damage, redox imbalance, metabolic reprograming, and altered cell-matrix adhesion and structural remodeling. Based on the biological understanding, feeding interventions have been established. Reducing lysine by 15% or altering lysine/arginine ratio during mid-growth period significantly decreased white striping and wooden breast rates without harming performance. Similarly, supplementing all-plant diets with guanidinoacetic acid reduced lesion severity. To minimize oxidative stress and muscle hypoxia during rearing, optimal ventilation, temperature and early growth are emphasized, hence protecting muscle health during rapid growth. Modern broiler breeding now integrates meat-quality markers with growth traits, reflecting holistic insights from genomics. Overall, integrating multi-omic datasets provide comprehensive understanding for developing targeted mitigation strategies, from nutritional modulation to selective breeding, paving the way for more resilient poultry production.

Keywords: Myopathies, Fast-Growing, Broilers, multi-omic, biological pathway

225S Meat quality: A primary breeder's approach

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In order to ensure the supply of high quality animal protein to the consumer, meat quality traits along with breast yield are a key focus for primary breeders. When issues with meat quality arise, such as breast myopathies, it is important that they are understood and characterised so that a strategy can be developed to reduce their incidence. In the first instance the genetic basis must be ascertained by estimating the genetic basis (i.e., heritability) of the individual myopathies and the genetic correlation with other associated traits in the breeding goal. The successful estimation of the genetic parameters and implementation of a selection strategy relies upon the accurate definition and subsequent detection of the trait. In the case of breast myopathies, their heritabilities are low to moderate ranging from 0.04 for spaghetti breast, 0.024-0.097 for wooden breast and 0.185-0.338 for white striping. The limited heritabilities indicate that while there is a genetic component to the myopathies there is a major influence from the non-genetic factors. In terms of selection strategy, it is possible to select against the genetic propensity to the expression of breast myopathies however it should be considered more of a long term strategy. The more influential non-genetic factors should be a key focus as part of a short term strategy as they can likely be altered more immediately and have a greater impact on the incidence of breast myopathies. It is important to identify which non-genetic factors are linked with meat quality and the development of the myopathies; these can be environmental, nutritional, or factors in the processing plant. Once identified these factors can be adjusted or implemented accordingly as part of a strategy for reducing the incidence of the myopathies. Another key aspect of the selection strategies and understanding the aetiology of the myopathies is estimating their genetic correlations with important traits such as breast yield and body weight. These have been found to be low to moderate, thus indicating that there is limited shared genetic background between the myopathies and the performance traits. This is important as it indicates that selection for improved breast yield and broiler performance, and reduced incidence of myopathies is possible through balanced breeding.

Keywords: Broiler, Myopathy, genetics, meat quality, selection

226S Addressing myopathies in a broiler breeding program

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Genetic improvement of broilers have accelerated thanks the use of genomic selection and advanced phenotyping technologies. These technologies target key traits such as growth, meat yield, feed efficiency, reproduction and end product quality. To ensure balance, selection for performance traits is combined with fitness-related traits (e.g., reproduction and livability), along with animal welfare and health traits. This is accomplished through the use of carefully designed profit index approaches. However, rapid genetic selection for growth rate, breast muscle yield and feed conversion ratio in modern broilers has led to unintended consequences, such as increased incidence of muscle myopathies. The most significant of these myopathies are Wooden Breast (**WB**), White Striping (**WS**), and Spaghetti Meat (**SM**). These conditions involve muscle fiber degeneration, inflammation, fat infiltration, oxidative stress, and impaired blood circulation. These factors lead to changes in meat texture, water-holding capacity, and both physical and sensory qualities of broiler meat. Each of the mentioned myopathies negatively impacts the quality of chicken meat, affecting customer experience and acceptance. Additionally, they reduce efficiency in processing facilities and result in significant economic losses throughout the poultry supply chain. This presentation explores how myopathies are measured and recorded in pedigree populations. This is done directly through visual scoring during bird dissections, and indirectly using biomarkers predicted through Near-Infrared technology. It also discusses data analysis to evaluate the genetic background (heritability) of these conditions, anticipated outcomes of genetic selection to reduce their incidence, and genetic correlations to understand potential selection tradeoffs with other economic traits in Cobb's breeding program. This is achieved by incorporating the trait(s) into the selection index. Such approach has been successfully implemented for WB, which is the most prevalent and economically impactful meat quality problem. This approach is expected to help decrease the occurrence of myopathies down the multiplication pipeline, leading to the production of commercial broilers that are less likely to exhibit this condition at harvest. Besides genetic selection, improvements in management practices, nutrition, and processing plant approaches can be implemented as part of a comprehensive strategy to control muscle pathologies in broilers. Understanding and addressing broiler myopathies is essential for ensuring the sustainability and profitability of broiler production, especially in light of changing consumer expectations and market demands.

Keywords: broilers, meat quality, myopathies, wooden breast, white stripping

227S Myopathies in fast growing broilers: Nutritional aspects

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With continued genetic selection for growth rate, major increases in broiler chicken growth rate, feed efficiency and breast meat yield have been achieved. As a complex biological system, however, genetic selection focusing on few traits can lead to unintended consequences for other traits. Past experience has shown that focusing too heavily on production traits, with little regard for physiological systems, health and well-being can lead to skeletal, cardiovascular, pulmonary and immune system problems. These problems have been drastically reduced in modern broilers in part through management and nutrition, but mainly through the use of more balanced genetic selection programs that include physiology, health and well-being, as well as performance criteria in breeding decisions. The broiler industry is currently facing challenges due to a variety of myopathies primarily affecting the breast meat of chickens with rapid growth, grown to heavy body weights and high breast meat yields. Woody breast describes a very hard, wood-like consistency of regions or the entire breast. White striping describes the presence of abnormal striations of fat and connective tissue parallel to the muscle fibres of the breast. Spaghetti meat describes the post-mortem splitting of the muscle fiber bundles into smaller fibers resembling spaghetti pasta. Other myopathies include deep muscle myopathy ('green muscle disease") and pale, soft, exudative (PSE) meat. Most of these myopathies are directly related to rapid growth and heavy final body weights at processing. Birds grown to lower processing weights, and birds grown at slower rates of gain tend to have much lower incidence of these myopathies. Although some nutritional interventions show promise in reducing the incidence of these pathologies, the current approach of industry has been to balance growth rate with the use of affected portions for further processing purposes where whole breast appearance and textures will not reduce consumer acceptance. Although genetic selection may reduce the incidence of these myopathies in the future, broiler processors must make economic decisions based on the reduced value of affected meat relative to the lost economic efficiencies associated with slower growth rates and lighter body weights.

Keywords: broiler chicken, muscle myopathy, woody breast, white striping, spaghetti meat

228S Global perspectives of myopathies in fast growing broilers

Shai Barbut*[1] [1]*University of Guelph, Food Science, Guelph, Ontario, Canada*

Myopathies in broiler chickens started to become a concern about a decade ago when the so-called White Striping (WS) and Woody Breast (WB) conditions appeared on the market. Myopathies refer to muscle disorders that affect the quality, texture, and appearance of poultry meat; mainly the large breast muscle (*Pectoralis major*) and are mainly driven by intensive production. WS is characterized by white striations running parallel to the muscle fibers in the breast meat, caused by fat and connective tissue replacing necrotic muscle fibers. WB results in hardened breast muscles creating a rubbery texture, due to fibrosis and muscle degradation. A more recent myopathy is the Spaghetti Meat (SM) which is marked by soft, stringy muscle fibers that fall apart easily (linked to poor muscle structure and integrity). A similar occurrence in the 'tenderloins' (*Pectoralis minor*), called Feathering, has also been reported. Estimates of the prevalence of these myopathies in selected countries will be presented as well as trends seen by the industry (e.g., the recent reduction in WB). The underlying causes of these myopathies are not all understood but we know they are multifactorial and include factors such as rapid growth, high breast muscle yield, and metabolic imbalances that can put excessive stress on the growing bird. This can include reduced oxygen supply to muscle cells (hypoxia), oxidative stress, inflammation, and eventual muscle necrosis. Genetic, nutrition, environmental stress, and management practices (e.g., stocking density) can also play important roles. Meat affected by myopathies is often downgraded or rejected by processors, leading to significant economic losses. Also, concerns about production ethics are rising among consumers, highlighting the need for sustainable practices. To mitigate these issues, research is attempting to understand the exact causes and suggest solutions to

eliminate/minimize the problem. Long term solutions focus on genetic selection for slower growth, more resilient broilers, while short term solutions investigate improved feeding strategies, and better environmental conditions. Addressing these conditions requires a holistic approach involving the strategies mentioned above to promote muscle health and ensure high-quality poultry meat. Overall, today balancing growth efficiency with animal health is critical to ensure both productivity and sustainability in the expending poultry meat market.

Keywords: Myopathies, broiler, meat, genetics, Nutrition

Mycoplasma, antibiotics in lay and Anti-Microbial Resistance (AMR)

229S Shifting away from routine antibiotic use in breeders and layers is necessary and beneficial

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The routine administration of antibiotics in intensive poultry farming – primarily to prevent persistent mycoplasma infections such as Mycoplasma gallisepticum and M. synoviae – can conceal underlying challenges including suboptimal housing, inadequate hygiene, poor ventilation, insufficient biosecurity measures, and incomplete vaccination protocols. This dependence on antimicrobials promotes the development of antimicrobial resistance (AMR), constituting a notable One Health concern. The prevalence of AMR in avian mycoplasmas is rising[1], particularly in South-East Asia, necessitating increased dosages and revised treatment regimens (creative combinations; *pers obs.*). Resistance also disrupts the chicken microbiota and elevates the risk posed by zoonotic pathogens such as *Salmonella* and *Campylobacter*. Intensive poultry operations may use up to 3.65 tons of antibiotics annually per million layers under week-a-month practices[6], further exacerbating resistance and raising the potential transfer of resistant bacteria through contamination of meat and eggs and through manure used as fertilizer. Effective alternatives encompass the procurement of disease-free stock, utilization of live vaccines (e.g., against Coccidiosis, Salmonella, Mycoplasma), and enhanced biosecurity protocols. These strategies have demonstrated success in reducing antibiotic reliance and corresponding resistance rates, as evidenced by lowering erythromycin resistance within Australian poultry populations. Mordern production systems achieve robust performance and minimal disease incidence through comprehensive vaccination programs, all-in-all-out management, early chick care, supplementation with gut health promoters, probiotics, and diligent flock monitoring. The adoption of these sustainable approaches and accurate diagnosis[12] mitigates the risk of AMR while supporting animal health and productivity.

Keywords: Mycoplasma, Layers, Antimicrobial resistance, AMR, Breeders

230S In lay antibiotic use in broiler breeders in Southeast Asia. Results of second survey

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Assessing the use of antimicrobials in Asia reveals that veterinarians are eager to make progress in this area. While the results of this survey are encouraging, they also highlight the challenges of reducing and replacing antibiotics with alternative solutions. For the 2024 health survey, various solutions have been identified, along with some misconceptions about using ineffective antibiotics to address management issues such as ventilation, water quality and breeder over-phototimulation. Additionally, the survey brings attention to the concern surrounding the use of antibiotics in combination with live Mycoplasma vaccines for preventive measures. Overall, the findings suggest a reliance on antibiotics for preventing diseases.

Keywords: antibiotic use, broiler breeders

231S Understanding the critical components of effective antimicrobial stewardship in animal health

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Antimicrobial resistance is a clear and current global risk to human and domestic animal health and welfare. This risk is likely to increase unless measures are introduced to reduce our current level of use of antimicrobials. While imposition of restrictions or prohibitions on use of some antimicrobials is appropriate, this approach will not fully address the problem. Much more effective control is likely to be introduced by implementation of effective antimicrobial stewardship – a comprehensive suite of measures to ensure that the most appropriate antimicrobials are used and they are only used when they are the only possible solution to a bacterial disease problem. These measures include identification of the drivers of antimicrobial use and development of solutions to disease problems that do not rely on antimicrobial treatment. This will require more effective and accessible diagnosis of disease problems, and development introduction and promotion of the use of effective vaccines to control viral and bacterial diseases. When antimicrobial treatment is the only viable alternative, it is critical that animal health workers have access to appropriate decision support tools to assist them in making the most appropriate choice of drug, route of administration, dose rate and dose duration. In some situations, introduction of specific measures to encourage or “nudge” behaviours of animal health workers and farmers that reduce inappropriate antimicrobial use may have considerable benefit. The introduction of antimicrobial stewardship measures has been shown to achieve significant reductions in antimicrobial use in animal health without any compromise to the health and welfare of animals. Indeed, most of these measures are likely to enhance agricultural productivity and profitability.

Keywords: antimicrobial stewardship, vaccines, diagnosis, behaviour, disease control

232S MS control experience in yellow broiler breeders

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Background and efficacy of MS vaccination in yellow broiler breeders, relationship of field strain positive rate and antibody levels. Isolation, identification and MIC of MS, control measures, the role of biosecurity and farm management in MS control.

Keywords: Mycoplasma, MS vaccination, yellow broiler breeders, antibody levels, MIC of MS

233S AFCD's measures in addressing antimicrobial resistance in poultry in Hong Kong

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Antimicrobial resistance (AMR) represents a profound global health challenge, necessitating coordinated and multisectoral actions. In Hong Kong, the government set up a High Level Steering Committee on AMR to formulate strategies in collaboration with different sectors to tackle AMR threats. Adopting the principles of the World Health

Organization Global Action Plan and One Health Approach, the Hong Kong Strategy and Action Plan on Antimicrobial Resistance was first launched in July 2017. The Agriculture, Fisheries and Conservation Department (AFCD) leads efforts to combat AMR in the animal health sector guided by the One Health framework. Since 2019, the AFCD has established a comprehensive surveillance system monitoring antimicrobial usage (AMU) and AMR in local livestock farms, supported by voluntary farmer reporting, systematic audits, and rigorous laboratory protocols. From 2020 to 2024, poultry farms consistently reported low AMU, with no use of third- or fourth-generation cephalosporins in recent years. Surveillance of commensal *Escherichia coli* and *Enterococcus* spp. revealed relatively stable prevalence of extended-spectrum beta-lactamase-producing *E. coli* (ESBL-E) and carbapenem-resistant *E. coli* (CRE) over the years, with no vancomycin-resistant enterococci (VRE) detected. Detailed analysis of AMU and AMR data from poultry farms, including whole genome sequencing, demonstrated strong concordance between phenotypic and genotypic resistance, highlighting the predominance of plasmid-mediated resistance genes. Additional studies indicated that local poultry farm environments may serve as reservoirs for ESBL-E, prompting targeted interventions such as educational campaigns on feed handling, cleaning and disinfection, and the exploration of competitive exclusion via probiotics.

Keywords: AMR, AMU, Hong Kong

234S Antimicrobial susceptibility in mycoplasma gallisepticum and Mycoplasma synoviae in Asia

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Effective control measures on the short term include targeted antimicrobial treatment in order to reduce clinical signs and economical losses in case of mycoplasmosis. Antibiotic resistance in Mycoplasma gallisepticum and Mycoplasma synoviae, two major pathogens affecting poultry worldwide, has emerged as a significant challenge to animal health and food security, especially in the case of key antimicrobials in veterinary and human medicine, such as fluoroquinolones, macrolides, lincosamides and aminoglycosides. The development of resistance is often linked to ecological factors such as antibiotic misuse, lack of regulatory oversight, and environmental contamination. In countries with poorly enforced veterinary drug regulations, the overlap between human and animal antibiotic usage exacerbates the risk of cross-resistance. Moreover, migratory birds may facilitate the intercontinental spread of resistant strains, particularly in regions where avian migration routes intersect with major poultry-producing areas. The conventional agar or broth microdilution method for the determination of minimal inhibitory concentration (MIC) of antimicrobials against mycoplasmas is a time-consuming procedure requiring special expertise and the isolation of the mycoplasma strains, which limits its applicability for the support of rapid therapeutical decisions. Therefore, regular antimicrobial susceptibility testing of the local strains is recommended to follow changes in susceptibility profiles and promote targeted treatment. Techniques for the examination and trends in antibiotic susceptibility of M. gallisepticum and M. synoviae in Asia are discussed and compared based on previous experiences and on the study of recent strains (24 M. gallisepticum and 57 M. synoviae strains collected from China, India, Indonesia, Malaysia, Taiwan and Vietnam between 2022-2025). Addressing antibiotic resistance in M. gallisepticum and M. synoviae in Asia requires a multifaceted approach. Strengthening antibiotic stewardship, improving diagnostic capabilities, enhancing surveillance networks, and implementing sustainable farming practices are crucial. Regional cooperation is essential to monitor resistance trends, standardize treatment guidelines, and mitigate ecological drivers of resistance. Understanding the ecological dynamics that shape resistance emergence in M. gallisepticum and M. synoviae is critical to safeguarding poultry health and ensuring the long-term efficacy of available therapeutics in Asia and beyond.

Keywords: antimicrobial resistance, avian mycoplasma, MIC tests, minimal inhibitory concentration (MIC), regional trends

235S The development of antimicrobial resistance in *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

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Mycoplasma synoviae and *Mycoplasma gallisepticum* are major pathogens in poultry, responsible for respiratory, articular, and reproductive disorders that result in significant economic losses. While antimicrobials remain critical for managing clinical symptoms and reducing production losses, their effectiveness is increasingly compromised by rising antimicrobial resistance (AMR). The number of antimicrobial classes effective against mycoplasmas is limited due to their natural resistance to several drug types, including β-lactams and first-generation quinolones. Treatment typically relies on macrolides, tetracyclines, and fluoroquinolones, but growing AMR, as observed *in vitro*, raises increasing concern worldwide. Trends in antibiotic susceptibility of *M. synoviae* and *M. gallisepticum* in Europe and Asia, as well as temporal changes, are discussed. AMR in *M. synoviae* and *M. gallisepticum* is primarily associated

with chromosomal mutations affecting drug targets, although alternative mechanisms such as efflux pumps and biofilm formation also contribute. Phenotypic methods remain the most reliable approach for detecting AMR in *Mycoplasma* species; however, they are time-consuming, technically demanding, and results are often difficult to interpret. The absence of clinical breakpoints and limited epidemiological cut-off values (ECOFFs) further complicate AMR categorization. Advances in molecular techniques offer a promising alternative for faster AMR detection and prediction. The current knowledge of genetic mechanisms underlying AMR in clinically important *Mycoplasma* species affecting poultry is summarized, with emphasis on the role of molecular assays in identifying resistance-associated mutations. The potential of genome-wide association studies (GWAS) is explored as an emerging tool for linking genetic traits to phenotypic resistance patterns, offering new insights for enhancing resistance prediction in veterinary medicine.

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Keywords: antimicrobial resistance (AMR), avian mycoplasma, minimal inhibitory concentration (MIC), molecular detection, genome-wide association studies (GWAS)

236S Genetic studies of multi-resistance in MG and MS

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A selection of the recent multi-resistant *Mycoplasma gallisepticum* and *M. synoviae* strains made from commercial poultry in SE Asia was subjected to WGS, A lot of these strains were from local breeding programmes that have historically used antibiotics for mycoplasma control. Antimicrobial resistance was characterized by the microbroth dilution method to a range of antibiotics. In some resistant isolates SNPs associated with resistance in antibiotic target regions were absent. Additionally some genes were found associated with biofilm elaboration. Other interesting findings included characterization of resistant MS and MG isolates made from the same bird sample where shared sequences were demonstrated. In samples from countries where multiple antibiotics are used in a single generation mechanisms for resistance other than target modification appeared more common. When this pressure has been reduced these resistant strains could rapidly disappear (although the pressure could be maintained for all resistances by just the use of one antibiotic once established). Whether other bacterial resistance will respond in the same way with removal of antibiotic pressure is a different question.

Keywords: antibiotic resistance

237S Solution – Vaccination, freedom, biosecurity structural changes needed in the industry to control MG and MS

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Antimicrobial Resistance (AMR) is an emerging human health issue and has implications for the use of antibiotics in poultry and egg production and other avian species. Asia, inparticular has large problems with antibiotic usage that are currently being addrressed by veterinary authorites. These include control of the right to prescribe, elmination of antibiotics addition at feedmills, the use of off label antibiotics in food producing animals, and reactions to restrictions. The emergence of multi-reisistant *Mycoplasma gallsepticum* and *M. synoviae* strains are a special problem in the region. Antibiotics are not a vitamin-like additive for poultry and egg production but, in lay, a targetted attack to prevent the effects of mycoplasma. Mycoplasma free stock is now becoming easily obtainable in Asia and the prevention of mycoplasma contamination is best achieved by biosecurity and live mycoplasma vaccination.

Keywords: Avian mycoplasma control, Antibiotics in lay, AMR (human health), One health

Managing raw material quality, variability and digestibility for optimum broiler performance and gut health

238S Symposium Problem Introduction

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Abstract not required.

Keywords: broiler performance, gut health, raw material

239S Evaluation of raw material quality and Its impact on broiler performance

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Broiler performance is affected by several factors including genetic, disease control, nutrition, and management at farm levels. Feed plays an important role in providing sufficient nutrients to meet broiler requirements. It is, then, critical that data input, especially raw material compositions, are evaluated accurately. This evaluation should be conducted at the feed mill level to adjust the feed formula according to the ingredients used. Evaluation is commonly performed based on physical, chemical, and biological methods. Physical evaluation plays a significant role in quality control of raw materials, through evaluations of color, smell, appearance, etc. For example, quantification of damaged corn can be a useful method to adjust Metabolizable Energy value. A quick test on soybean meal color can also provide an indication of over or under-cooked of the product. Moreover, feed microscopy and other quick test methods are also practical in identifying adulteration of raw materials. Chemical methods have been used widely to estimate the quality and nutrient contents of raw materials. Proximate analysis is a common wet chemistry method to estimate fat, protein, ash, fiber, and moisture of raw materials. Depending on the capacity and capability of laboratories, a more sophisticated instrument, such as high-pressure liquid chromatography is available to quantify more specific nutrient contents, such as amino acids, vitamins, minerals, mycotoxins, and other anti-nutritive factors. With the advancement of technology, feed mills are relying on near infrared reflectance system as a more practical method of quantifying nutrient contents. However, it is critical to have an accurate calibration for a proper prediction of nutrient contents. Biological evaluation can be set up as *an in-vitro* or *in-vivo* system. *In-vitro* system may use an artificial model in the laboratories to mimic animal production environment, while *in-vivo* system is dedicated to feeding evaluation directly to broiler. Titration systems with different raw material inclusion levels have been common practices to evaluate raw materials *in-vivo*. Likewise, a digestibility trial using animal models can be performed to estimate nutrient digestibility of raw materials. Results from these chemical, physical, and biological evaluations can be used to develop a quality control system to not only adjust feed formulation but also accept or reject certain raw materials. These decisions ultimately allow companies to optimize animal performance and economic return.

Keywords: Physical, Chemical, Biological evaluation, Quality control, Feedmill

240S Optimizing feed quality and nutrient utilization: Enzyme solutions to synchronize digestive dynamics

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Balanced digestion of starch and protein is important for optimal broiler performance. However, starch is typically digested more rapidly and completely than protein. In the small intestine, starch shows higher digestibility at various points compared to protein, which tends to lag behind in both speed and extent of digestion. One reason for this discrepancy is the contribution of endogenous protein and amino acid flows, which reduce the apparent digestibility of protein. While the true digestibility of most proteins is high, a significant portion of the protein content in the small intestine originates from the animal itself, not the diet, making it appear less digestible. Another factor is the energy demand of the gastrointestinal tract. A substantial portion of dietary energy is used for digestion and absorption, and much of this energy comes from the breakdown of amino acids rather than glucose. In contrast, glucose is used less by the gut and more readily enters the bloodstream, leading to a rapid rise in blood glucose levels after feeding. This suggests that glucose is a more efficient energy source for intestinal cells than amino acids. Protein that is digested quickly may be less likely to be broken down within the gut lining, especially if it is absorbed early in the small intestine where glucose is still available as an energy source. This could help preserve amino acids for growth and other functions. Starch and protein digestion rates can be significantly influenced by a variety of factors, including the type of plant-based feed ingredients used, the harvest year and location, and the processing methods applied to by-product ingredients. These variables affect the nutritional quality and digestibility of feed, ultimately impacting broiler performance. This presentation explores how these factors shape the digestive dynamics of starch and protein and highlights the role of targeted feed enzyme strategies in balancing energy and amino acid utilisation.

Keywords: Broiler, Enzyme, Starch, Protein

241S Formulation techniques to maximize nutrient digestibility for broiler performance and gut health

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Feed optimization strategies in poultry have changed over time as influenced by the emerging animal nutrition concepts, which mainly focused on adaptation to genetic improvement, innovations in feed technologies and compliance to sustainability measures. Broiler diets must be then, dynamic, to align to the development of the species' nutrient requirements. The profitability of animal protein production greatly depends on the efficiency of production. In broiler production, efficiency entails a range of strategies to produce healthy chickens which will provide safe and sustainable meat at an optimum cost. And since feed contributes around 80% of total cost of production, the attention lies on efficient utilization of nutrients in raw materials to reduce feed cost and at the same time improve feed quality to promote gut health. The animal feed industry is constantly struggling to deal with these issues on raw materials: availability, variability and anti-nutritional factors.

These concerns in broiler feeds interplay with the prevailing animal health concerns nowadays, triggering further devastating consequences of feed and pathogens interaction. Dysbiosis in broilers has become an important global problem particularly with the fast-growing birds as a result of genetic selection. The presence of bacterial and viral challenges in global broiler production generates more pressure for the stakeholders to find ways to produce chickens in most efficient and profitable method. Amongst the many feed additive options available in the market, enzymes are so far, the most promising additive to improve nutrient utilization in feed materials. As it is one of the most scientifically investigated additives in the feed industry, enzymes have proved its worth in improving digestibility of raw materials and promote a healthy and resilient gut in broilers. The most expensive nutritional parameters in broiler diets are energy, amino acids and phosphorus. In economic terms, to maximize extraction of these nutrients from the feed is a clear indication of a feed cost reduction, while improving gut health and promoting sustainability of animal meat production. To fully utilize these expensive nutrients from the feeds, enzymes which are classified as carbohydrases, proteases and phytases are generally used in feed formulations. These catalysts mainly function to improve nutrient digestibility and reduce pathogenic microorganisms in the gut. With numerous available tools and technologies developed to benefit from enzymes, the feed formulators must be cautious in selecting the right feed enzymes to be used in broiler diets.

Keywords: broiler, formulations, gut health, digestibility, enzymes

242S Understanding ingredient evaluation and the use of alternative ingredients in poultry

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Ingredient evaluation remains a cornerstone of poultry nutrition, directly impacting productivity, health, and economic sustainability in broiler and laying hen production. Traditional feed formulation has relied on conventional ingredients such as corn and soybean meal; however, fluctuating costs, regional availability, and environmental pressures have driven interest in the use of alternative feedstuffs. Alternative ingredients show higher variability and require precision evaluation to replace traditional ingredients properly. This review examines current methods for assessing the nutritional values of feed ingredients, with a particular focus on carbohydrates, fiber fractions, fat, and protein (amino acids). The accurate characterization of starch and soluble and insoluble non-starch polysaccharides affects gut physiology, nutrient digestibility, the prediction of metabolizable energy (ME), and the proper exogenous enzyme application. Similarly, understanding the quality and digestibility of fat and protein feedstuffs allows the strategic application of fat digestion enhancers and proteases for meat and egg production. Fat quality and digestibility, influenced by fatty acid profile and degree of saturation, are pivotal for ME contribution. At the same time, anti-nutritional factor evaluation in protein ingredients allows adjustments in the formulation. Robust evaluation techniques, such as in vitro assays, near-infrared spectroscopy, and in vivo digestibility trials, are necessary to accurately estimate ME and standardized ileal amino acid digestibility in these ingredients. Additionally, this review contrasts key international feed evaluation systems and nutritional tables, highlighting their methodological differences in energy and amino acid estimation and the implications for ingredient selection and diet formulation. A deeper understanding of these evaluation systems, along with enzyme strategies to unlock the nutritional potential of alternative ingredients, improves the precision, cost-effectiveness, and sustainability of modern poultry production.

Keywords: Ingredient evaluation, fats, Carbohydrates, Enzymes, NSP

Steering nitrogen emission from poultry production through precision nutrition

243S Impact of nitrogen emission from poultry production on global warming and sustainability

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Excess dietary protein, an imbalance of amino acids in a feed formula, or use of proteins with low digestibility leads to nitrogen inefficiency. Poultry excrete nitrogen primarily as uric acid, which can be converted into ammonia (NH_3), nitrous oxide (N_2O), and nitrate (NO_3^-) contributing to environmental degradation. Inadequate manure handling and land application may cause nitrate leaching and gaseous emissions. The impact of nitrogen emissions spans three sustainability pillars: 1) Environmental: nitrate leaching can cause soil and water contamination, ammonia and odor emissions causing air quality degradation, and biodiversity loss due to eutrophication. 2) Economic: reduce nitrogen efficiency due to improper feed formulation, increasing cost of managing emissions and cost of meeting regulations, and risk of penalties due to tightening environmental policies (e.g. Kyoto agreement in 1992, Paris climate agreement in 2016, yearly climate change conferences (COPs; conference of parties), and European green deal). 3) Social: health impact on farmers, farm workers, and nearby communities as well as public pressure and consumer demand for more sustainable food. Contribution of feed to total global warming potential (GWP), acidification, and eutrophication of poultry production is reported to be 92, 76, and 95 percent. Thus, nutritional interventions among other tools can reduce the negative effects of nitrogen emissions. Amino acid balancing, low crude protein (CP) diets, use of local raw materials and raw materials from land use change free areas are efficient tools to reduce the eutrophication and acidification as two major parameters in life cycle assessment (LCA) besides the GWP. The GWP has been subject of studies which has been often descriptive and lacking replications. Nevertheless, recent data shows that low CP diets are possible to be applied to broiler diets with the potential to reduce feed GWP. Low and zero SBM diets reducing the GWP to half and consequently showing sustainability superiority compared to SBM based diets. Different recommendation systems and low CP could be utilized as tools to reduce the environmental impact of poultry meat production. Thus, nutritionists are advised to utilize SBM removal from feed and low CP strategies as efficient tools to improve the environmental, economic and social impact parameters of poultry industry while sustaining this industry in being able to feed the world with high quality proteins.

Keywords: nitrogen emissions, poultry production, sustainability, zero soybean meal, low crude protein

244S Impact of crude protein content in poultry diet on performance, metabolic efficiency, gut health and welfare

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Looking back at the history of advances in protein nutrition, academia and advanced industry leaders have evolved from using crude protein, total amino acids, and digestible amino acids to standardised ileal digestible amino acids, realising precision nitrogen nutrition since the 1960s. However, a significant proportion of customers in the region are still adopting crude protein and total amino acids for poultry feed production. Continuing research expands our understanding of nitrogen-precision nutrition, where the genetic potential for protein deposition is achieved through the supplementation of digestible amino acids, rather than through the supplementation of crude protein *per se* or excess amino acids by large volumes of protein meal inclusion. It is estimated that under current practice, more than 50% of nitrogen is excreted through uric acid and undigested forms, which contribute to carbon emission, soil acidification, and water eutrophication. Maintaining high protein levels in broiler diets has a twofold effect on broiler nutrition through (1) the increased levels of undigested protein that is supplying fermentable source of nitrogen to enteric pathogens, and (2) digested and absorbed but excess supplementation of amino acids mostly in form of non-essential amino acids that need to be metabolically processed and excreted in form of uric acid. Increasing levels of undigested protein are generally associated with the proliferation of enteric pathogens and encourage unfavourable microbial profiles. Moreover, the total amount of excess amino acids that are digested/absorbed but need to be excreted in the form of uric acid theoretically increases the maintenance energy requirement, which is an economically important aspect for efficient broiler production. Recent unpublished research demonstrated a 45 kcal AME increase per 1% CP reduction when the same amount of essential amino acids is maintained, suggesting that the metabolic energy requirement for 1% excess protein is economically significant. In addition, the formation of uric acid uses 2 molecules of water and hence increases litter moisture content, which is an important aspect for poultry health and welfare. This session will cover in detail the impact of undigested protein and excess amino acids supplemented through a high-protein diet on intestinal health, metabolic efficiency, and welfare using recent research data.

Keywords: crude protein, intestinal health, metabolic cost, welfare

245S Reducing nitrogen emissions through precision nitrogen nutrition: what we know so far

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Production of poultry-based food is associated with an increasing shortage of feed ingredients, competition between the use of crops as food or feed, and environmental impacts of nitrogen excreted by the animals and other environmentally relevant compounds. The nitrogen utilization efficiency (NUE), defined as nitrogen accretion per nitrogen intake, is a key figure to assess how precise nitrogen nutrition is. Higher NUE values indicate a more precise alignment of the for metabolic purposes available supply of nitrogenous nutrients and their requirements. Amino acids (AA) are the most relevant nitrogenous nutrients in this regard. Strategies to increase the NUE include to increase the gain:feed ratio, to consider more AA when diets are formulated as well as increasing predictability and the magnitude of AA digestibility. The potential to

increase the NUE by increasing AA digestibility currently is smaller than the potential to increase the NUE by increasing knowledge on AA requirements. In addition, prediction of AA digestibility still is imprecise. Possibilities to increase NUE by considering AA digestibility may gain relevance once predictability has improved. Formulating diets that meet the requirement of more AA than the current standard appears easiest to be put into practice in the near future. Meeting target concentrations of more AA in diets requires the use of a higher number and higher dietary inclusion of free AA. Downsides of that include the possibility of an increasing carbon footprint of diets because the carbon footprint of free AA is higher than that of most plant-based feed ingredients. In addition, free AA were often suggested not to be metabolically equivalent to peptide-bound AA because of a higher kinetic absorption rate, which may lead to increased AA oxidation. The NUE is a suitable key figure to evaluate such effects because NUE summarizes all processes in the metabolism, while performance traits, i.e., gain:feed ratio, can be affected by other influences than the nitrogen metabolism. Results of a recent project, where peptide-bound AA were substituted with free AA without affecting the digestible dietary concentration of all 20 proteinogenic AA, suggest that performance was impaired at 87 g free AA/kg. This must have been caused by other factors than an imbalanced availability of AA for nitrogen accretion because the NUE was not affected. Lower feed intake was identified as the most probable cause for impaired performance. However, performance and NUE was not affected up to 54 g of free AA/kg. This is a much higher inclusion of free AA than the standard in current practical feeds.

Keywords: amino acids, nitrogen utilization, requirements, digestibility, absorption

246S The role of reduced crude protein in AGP-free poultry diets towards enhanced sustainability

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The rising global demand for poultry requires sustainable production methods, particularly as the industry transitions towards antibiotic growth promoter (AGP)-free systems. This paper emphasizes how reducing dietary crude protein (RCP), carefully balanced with synthetic amino acids, serves as a versatile approach to enhance sustainability across environmental, economic, and animal welfare dimensions. Excess dietary protein results in increased nitrogen waste and ammonia emissions, which contribute to environmental pollution and adversely affect bird health. RCP diets assist in mitigating these emissions and reduce the overall greenhouse gases associated with feed ingredients such as soybean meal. Economically, RCP improves feed efficiency, thereby maintaining or enhancing performance metrics such as body weight gain and feed conversion ratio, ultimately leading to cost savings. From an animal welfare perspective, RCP encourages a healthier gut microbiome by decreasing undigested protein, which supports immune health and diminishes physiological stress, as evidenced by reduced serum corticosterone levels. Additionally, enhanced litter quality associated with RCP facilitates better footpad health. Successful implementation hinges on precise amino acid balancing and is substantiated by comprehensive commercial trials. In conclusion, RCP emerges as a scientifically validated, cost-effective, and indispensable tool for fostering a more sustainable, resilient, and profitable poultry industry devoid of antibiotics.

Keywords: amino acids, antibiotic-free, crude protein, sustainability, poultry

247S Advances in analytical technology to support precision nitrogen nutrition

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The excretion of excess nitrogen into the environment – alongside other nutrients such as phosphorus and carbon – is a global concern due to its role in eutrophication, which contributes to the degradation of aquatic ecosystems. Runoffs from agricultural land and livestock farming are some of the main sources and highlights the importance of managing nitrogen loading for a sustainable animal agriculture. Therefore, precision nutrition in animal feed formulation is a strategy that can be adopted to mitigate excess loading of nitrogen. Combined with an understanding of what constitutes an optimal diet for animals, analytical tools such as Near Infrared (NIR) spectroscopy can provide valuable insights on the feed ingredient nutrient profile, beyond just the proximate values. NIR spectroscopy is a cost- and time-efficient tool for real-time analysis of nutrients, including total and digestible amino acid content. In the context of precision nitrogen nutrition, NIR spectroscopy enables the optimisation of nutrient supply – particularly amino acids – in the feed formulation, to enhance animal health and productivity, whilst reducing the impact on the environment.

Keywords: NIR spectroscopy, Precision nutrition, Feed formulation, Nitrogen excretion/loading, Amino acids

Animal Welfare around the world – Different perspectives on a shared goal

248S Global perspective - The International Poultry Welfare Alliance

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The International Poultry Welfare Alliance (IPWA) was established to foster collaboration across academia, industry, and allied stakeholders in pursuit of science-based poultry welfare improvement that is relevant to commercial production systems worldwide. As a global, multi-stakeholder initiative, IPWA serves as a platform to connect diverse expertise and ensure that science informs welfare advancements while keeping them practically applicable across regions and production systems. Recent efforts highlight how IPWA bridges the gap between academic research and implementation at scale. The development of reference guides and training for key welfare indicators (KWIs) demonstrates the importance of creating outcome-based measures that are reputable, robust, and reliable, with the potential to be consistently applied at the commercial farm level and throughout global supply chains. These resources not only synthesize current scientific understanding but also provide practical tools to enable more consistent welfare assessment in commercial practice. Beyond technical resources, IPWA has prioritized global engagement through structured dialogues at major conferences highlighting North America, Europe, Asia, and Latin America. These activities have convened producers, researchers, veterinarians, allied industry, and welfare organizations to discuss the question of what poultry welfare will look like in 2050 and how to achieve it. The breadth of participation underscores the diversity of perspectives shaping poultry welfare and the necessity of a forum where shared values and regionally specific needs can coexist. For academics, IPWA offers both a resource and an opportunity. It provides pathways to ensure that research contributes directly to global practice while also guiding the next generation of welfare innovations. By serving as a conduit between scientific discovery and practical application, IPWA is an authority in shaping a shared global vision for poultry welfare that reflects both scientific innovation and the needs of modern poultry production systems.

Keywords: welfare, industry, global, outcome-based, key welfare indicator

249S Origin and content of the Scientific opinions of the European Food Safety Authority: welfare of laying hens and broiler chickens on farm

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In the context of the revision of EU regulation regarding animal welfare and the "End of cage age" citizen initiative in Europe, the European Commission has asked EFSA (European Food Safety Authority) to produce two scientific opinions. The two Scientific Opinions had to consider the welfare of domestic fowl (Gallus gallus) related to the production of meat (day-old chicks, broiler chickens and broiler breeders) and the production of eggs (pullets, laying hens and layer breeders). Request The objective was to identify the welfare consequences that broilers and layers might experience when reared/housed in the husbandry systems currently in use in the EU, and to identify the hazards responsible for these welfare consequences. The preventive measures that have to be proposed should avoid birds for experiencing any of the main welfare consequences identified. Animal Based Measurements (ABMs) to assess the welfare consequences should be proposed. Outcomes Husbandry systems currently used in the EU are described in the first part of the opinions. Following this, the opinions identified the highly relevant welfare consequences, e.g. restriction of movement, inability to perform comfort, foraging and exploratory behaviours, that can be seen as consequences of husbandry and management practices applied. Main hazards responsible of the different welfare consequences (e.g. too high stocking density, lack of enrichment) and main animal-based measures used (e.g. plumage damage, performance of comfort behaviours) are described. Recommendations are provided on quantitative or qualitative criteria to answer specific questions on the welfare of broilers and related to genetic selection, temperature, feed and water restriction, use of cages, light, air quality and mutilations in breeders. For laying hens, the welfare consequences of non-cage compared to cage systems are described and minimum enclosure characteristics are identified for laying hens, pullets and layer breeders. The adverse welfare consequences of beak trimming are described as well as the pros and cons associated with rearing of non-beak-trimmed layer flocks. Conclusions To improve the welfare of farmed broiler chickens and laying hens, EFSA recommended avoiding the use of cages, the practice of mutilation and feed restriction. The two scientific opinions include advice on stocking density of animals, enrichment, lighting, litter and structures such as covered veranda and elevated platforms.

Keywords: welfare, broiler chicken, laying hens, breeders, European Union

250S Australian perspective – Does free-range automatically ensure high animal welfare levels?

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Over the past 15 years, the consumer demand for free-range eggs in Australia has been on an upward trajectory with these eggs now making up the majority of retail sales. This is projected to increase as the 2022 ban on caged eggs slowly rolls out across the different states. Free-range systems are perceived by consumers to have higher hen welfare compared to caged or indoor barn systems as well as perceived to produce tastier and healthier eggs. But these systems have been a source of public contention due to misconceptions of the size of farms categorised as 'free-range'. In 2016 an egg labelling regulation was put in place to include both small and large-scale farms as 'free-range', as long as birds had 'meaningful and regular outdoor access' at a maximum outdoor stocking density of 10,000 hens per hectare. While consumer driven changes to supermarket labelling have resulted in greater transparency to the purchaser, does a 'free-range' laying hen system actually result in higher hen welfare? As with any housing system, there are inherent challenges in managing large numbers of birds and compromises to welfare must exist. Almost 90% of the 21-million-bird national flock of predominantly brown hens strains, resides in the states of New South Wales, Victoria, and Queensland. These states span a range of climatic zones including extreme cold and hot temperatures, but birds predominantly have continual outdoor access year-round. This behavioral freedom is one of the greatest attributes of the free-range housing system when considering contributions to animal welfare status and alignment with the Five Domains Model. We know from experimental and commercial tracking of individual hens, that extreme variation exists in how hens use the outdoor resource. Some hens choose to frequent daily, whereas others remain inside. There is also variation in the microenvironments on the range and areas that hens choose to occupy. The agency to the individual bird along with the access to different range areas that facilitate foraging and dust bathing strongly align with positive behavioural interactions with the environment. Furthermore, consumption of insects and forage on the range increases food variety creating positive nutritional opportunities. In contrast, having access to an outdoor area increases exposure to environmental extremes and reduces predictability in the daily routine when outdoor access has to be temporarily removed. This presentation will consider how free-range systems in Australia align with the Five Domains Model of animal welfare, the future of this type of housing system, and where research gaps in animal welfare currently lie.

Keywords: behavior, laying hen, Five Domains Model, agency, positive

251S Is functional structuring of the broiler barn increasing welfare?

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Conventional broiler chicken production typically involves housing large flocks in uniform, barren environments that provide only the basic resources: feed, water and bedding. However, the quality of the bedding often deteriorates rapidly as the birds grow, and the overall environment fails to support adequate expression of many species-specific behaviours such as foraging, dustbathing, exploration and perching. The result is unsatisfied behavioural motivation, contributing to prolonged periods of inactivity. Inactivity is particularly problematic in broiler chickens, as their rapid growth predisposes them to leg disorders and walking impairments. Encouraging movement through environmental stimulation can help mitigate these issues, promoting better physical health and welfare. Moreover, the uniformity of the environment with no dedicated areas for rest and activity, respectively, can increase bird-to-bird disturbances. This can further compromise animal welfare, especially in young birds, and reduce productivity due to an increased risk of carcass condemnation from scratches. A promising strategy to address these challenges is the creation of functional areas within broiler barns. Functional areas are defined as distinct zones within the housing environment of captive animals that are designed to meet different behavioural motivations. These areas can stimulate activity, reduce detrimental bird-to-bird disturbances, and allow birds to engage in a broader range of natural behaviours. Examples of functional areas include zones differing in light intensities, dark brooders for resting, hides for retreating, elevated platforms for perching, and substrates suitable for dustbathing and foraging. Access to covered verandas can also provide additional complexity to the environment. There is substantial evidence supporting the benefits of each of these individual measures used to create functional areas, e.g., from research in dark brooders. However, most of this research has been conducted in small-scale experimental settings. To effectively implement these findings in commercial production, large-scale studies are needed to determine the optimal design, distribution and space allocation of functional areas. This includes understanding the space requirements per bird to ensure equitable access to the resources and maximize the benefits of environmental complexity. Incorporating functional areas into broiler housing holds the potential of being a significant step toward more welfare-friendly and sustainable broiler production.

Keywords: Activity, Broiler chicken, Functional area, Species-specific behaviour, Welfare

252S Technological Perspective – How can PLF support poultry welfare?

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Animal welfare has become a growing concern in the poultry industry. To ensure and enhance poultry welfare, stakeholders across the industry, research institutions, and animal welfare organizations have been working diligently toward shared goals. Since true animal welfare must be maintained continuously and corrected promptly when compromised, one of the major challenges is the lack of real-time, continuous assessment systems on poultry welfare that can notify producers in a timely manner and offer actionable recommendations. Although protocols for welfare evaluation exist, inconsistencies in their experience and interpretation by different assessors often result in data variability, making it difficult to compare results within and across studies. These challenges underscore the need for continuous, objective, and automated technologies that can assess poultry welfare reliably and provide immediate feedback to producers. This presentation at “Animal Welfare Around the World – Different Perspectives on a Shared Goal” aimed to identify the key challenges in assessing and improving poultry welfare and to explain the role of Precision Livestock Farming (PLF) technologies (including sensing systems, artificial intelligence, and machine learning) in addressing these issues. Our previous research and applications of PLF in poultry welfare will be presented as illustrative examples. For example, we conducted a survey to understand the perspectives of both industry producers and academic researchers on PLF and poultry welfare, and found that producers often define welfare in terms of meeting basic needs like feed and water, while researchers emphasize behavioral and psychological well-being. We have also used objective technologies to assess welfare, integrated multiple sensors to monitor environmental conditions, and employed cameras and wearable devices to continuously track poultry behavior as an indicator of welfare status. While these technologies show promise, few have reached the level of commercialization, largely due to challenges related to cost and user-friendliness. In conclusion, PLF represents a promising solution for enhancing poultry welfare through real-time, objective monitoring, but further development and practical implementation are needed to ensure these tools can be effectively adopted on commercial farms, where the majority of birds are raised.

Keywords: Precision Livestock Farming, Poultry, Welfare, Production, Behavior

253S Individual Perspective – Flock vs. individual welfare on farm and in research

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Animal welfare is a condition whose promotion in each individual animal is the benchmark. A scientific approach to welfare places the individual animal in the focus of assessment. While this is more feasible in mammals—due to smaller group sizes, visible facial expressions, and the ability to carry heavier sensors—technological advances are now making individual welfare monitoring in poultry increasingly viable. Tools such as RFID and computer vision enable welfare assessments across the chicken's lifetime, depite retrospectively at slaughter. Continuous, real-time welfare data are essential for timely intervention, adaptation of environmental conditions, and ultimately for integrating welfare indicators into breeding decisions alongside performance traits. Technologies for individual monitoring in poultry are advancing rapidly and becoming lighter. RFID tags, now embedded in leg or wing bands, are suitable for adult birds and are moving toward early-life application. Tracking individuals over their lifetime supports a welfare-oriented life cycle assessment, capturing the development of welfare indicators over time and across generations. In breeding, continuous data on weight, growth, and egg-laying performance are important traits. The addition of welfare indicators—such as foot pad and keel bone health, plumage condition, and behavioral traits—offers new dimensions for selection and management by revealing meaningful correlations between welfare and productivity. Welfare-performance profiles can be generated, for example, via electronic nests, which automatically record individual weights, nest visit durations, and laying events. This enables egg traceability, chick identification, and the longitudinal tracking of entire hen lineages with integrated welfare and performance data. RFID also allows tracking of individual movement, such as use of outdoor runs or spatial preferences within housing systems. Despite these developments in research, the on-farm use of RFID remains largely restricted to sentinel animals. For flock-level assessment under commercial conditions, computer vision remains the most applicable technology. Although current systems do not yet allow individual tracking or direct linking of performances or welfare traits to specific birds, AI-supported video monitoring delivers substantial benefits: continuous observation, early warning, and decision support for timely interventions. In future, AI integration may enable predictive analytics that enhance both animal welfare and production efficiency in poultry systems.

Keywords: Precision Livestock Farming, Animal Welfare, Tracking, RFID, Individual performance

The current and future perspective of ChickenGTEx Project and its applications in precision breeding

254S The genetic regulation of multi-tissue transcriptome in chickens

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Chicken is a valuable model for understanding fundamental biology and vertebrate evolution, as well as a major source of nutrient-dense and lean-protein-enriched food globally. Although it is the first non-mammalian amniote genome to be sequenced, the chicken genome still lacks a systematic characterization of functional variation. Here, through integrating bulk RNA-Seq of 7,015 samples, single-cell RNA-Seq of 127,598 cells and 2,869 whole-genome sequences, we presented a pilot atlas of regulatory variants across 28 chicken tissues, including millions of regulatory effects on primary expression (protein-coding genes, lncRNA and exon) and post-transcriptional modifications (alternative splicing and 3' untranslated region alternative polyadenylation). We highlighted distinct molecular mechanisms underlying these regulatory variants and their context dependence and utility in interpreting genome-wide associations of 39 chicken complex traits. Finally, we compared gene expression and regulation between chickens and mammals and demonstrated how this resource can assist with cross-species gene mapping of complex traits.

Keywords: ChickenGTEx, complex trait, molecular QTL

255S Sex-biased gene regulation across 30 chicken tissues

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The domestic chicken (Gallus gallus) is a key model for understanding sexual dimorphism, vertebrate evolution, and agricultural genetics. However, a systematic exploration of its sex-biased regulatory landscape remains incomplete. To address this, we integrated 7,997 bulk RNA-seq samples, 280 whole genomes, and 93 single-cell/nucleus RNA-seq datasets from a sex-balanced, uniformly reared chicken population (32 tissues), constructing a multi-tissue atlas of regulatory variants. We identified widespread tissue-sharing of non-primary eQTLs (promoter-enriched), indicative of conserved transcriptional control across tissues. Sex-biased gene (co)expression and cell-type proportions revealed pervasive Z-linked dosage imbalance and cell-autonomous regulation, driving dimorphism in bone development, immunity, and neural networks. Notably, endocrine tissues (e.g., fat) exhibited pronounced sex-biased genetic regulation, mediated by polarized co-expression networks, Z-linked transcription factor imbalances, and sex-divergent cellular composition. Beyond SNPs, structural variants (SVs) disproportionately contributed to tissue-specific regulation, with 112 sex-biased SV-eQTLs identified. Finally, we linked these regulatory variants to 865 serum lipid traits and 26 complex agricultural traits, uncovering sex-biased genetic mechanisms underlying metabolism and economically important phenotypes. Our work provides a comprehensive resource for decoding sex-dimorphic regulation in vertebrates and agricultural genomics.

Keywords: gene regulation

256S Bridging the omics knowledge gap: Integrative metrics for host-microbe interaction networks driving phenotypes

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Rapid advances in sequencing technologies have led to an explosion of data, yet analytical capabilities and depth of interpretation have not kept pace, resulting in an "affluent data but impoverished knowledge" paradox. To address these challenges, our research team has been conducting extensive exploration and efforts in the deep application of AI, improvements in data sharing and multi-omics integration, iterative wet-dry experimental cycles, and the cultivation of interdisciplinary talent. Traditional analyses often focus on single omics layers. We proposed that fecal microbiota inadequately represent the state of microbes across intestinal segments, distinguished individual enterotypes and identified key microbes influencing growth, energy metabolism, and disease. Integrating genome and microbiome, we performed microbial heritability estimation and GWAS, revealing the Christensenellaceae family exhibits high cross-species heritability. We pioneered the concept of microbiability in chickens to quantify microbial contributions to phenotypic variance. Duodenal microbial communities explained 24% of abdominal fat weight (AFW) variance in broilers and 26% of hepatic steatosis variance in laying hens. For genomic-transcriptomic integration, molQTL mapping is the current mainstream approach, widely adopted by major projects like HumanGTEx and FarmGTEx, and now extended to sc-ranscriptomics. We innovatively proposed "regulatability" (rb2) to partition the contribution of expression profiles to phenotypic variation and identified key regulatory tissues. Significant regulatory effects of the pituitary and liver on yolk weight were identified. Applying rb2 to microbes revealed prominent effects in Bifidobacteriaceae, *Bifidobacterium*, and *Lactobacillus vaginalis*. By integrating these approaches, we identified pleiotropic genes regulating hen phenotypes and microbes, constructing a multi-dimensional interaction network. Our discovery elucidated the complete pathway whereby CHST14 influences *L. salivarius* abundance and activity, regulates BSH production and downstream bile acid metabolism, and ultimately drives abdominal fat deposition. Our research, through the integration of multi-omics data and innovative analytical methods, provides in-depth insights into the complex interaction networks and regulatory mechanisms underlying host gene-gut microbiota-metabolic phenotype relationships. Deepening such integrated research will precisely reveal the principles of biological regulation, pioneering novel avenues for intervening in crucial economic traits and human metabolic diseases.

Keywords: multi-omics integrative analysis, microbiability, regulatability, host-microbe interaction, abdominal fat weight

257S Mapping the regulatory genetic landscape of complex traits using advanced intercross line

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Complex traits exhibit a highly polygenic architecture that complicates gene mapping and molecular characterization. As a model organism for birds, chickens possess high-quality reference panels, functional annotations, and molecular quantitative trait locus maps. However, the genetic mechanisms underlying growth traits have not been systematically analyzed. Here, we develop a 16-generation advanced intercross line of chickens to enhance informative recombination and identify 154 single-gene quantitative trait loci. We use multiple co-localization methods to establish a network landscape of tissue-specific regulatory mutations and functional gene relationships. We leverage gene-clustering and restoration quantitative trait loci within the omnigenic model framework to elucidate the genetic regulation system of growth traits. Cross-species comparisons show the conserved functions of growth-related genes and divergent features of regulatory mechanisms in mammals and birds.

Keywords: complex traits, co-localization, advanced intercross line, omnigenic model

258S Winding back the clock – The genome edited re-wilded chicken and how to identify functional polymorphisms relating to domestication

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The rise of conservation genetics offers the possibility of the ‘de-extinction’ of species, as well as the potential for transgenic insertion of genetic diversity in critically endangered species. To establish a precedence for this, we are attempting to transgenically alter a domestic chicken to resemble it’s wild/ feral counterpart. This will be performed by editing up to 20 300kb genomic edits. This presentation will address how these regions are being selected. The selection of these regions to edit will be based on two approaches – a single cell approach using a laboratory advanced intercross between wild and domestic lines, and samples from two free-living feral populations from Hawaii and Bermuda. In the case of the laboratory intercross, 45 samples were each sampled using 10X Multiome chips (1 sample/ chip), with 200k cells generating 200k transcriptomes and 200k ATAC-seq genomes. These were then used in Genome Wide Association Study (GWAS) to identify cis and trans regulation of gene expression and open chromatin. For the feral populations 465 individuals were whole genome sequenced and phenotyped for a variety of morphological (bone allocation, behaviour, bodyweight, amongst others) and gene expression traits (400 hypothalmic transcriptomes). Selective sweeps were mapped in the feral populations, as well as GWAS studies performed. For the single cell analysis, over 40 clusters (putatively cell types) were identified when clustered based on ATAC-seq. Around 300 cis eQTL were identified per cluster, with several thousand trans eQTL identified per cluster. In particular, we focus on genes that have been identified as previously affecting anxiety-related behaviour in this intercross, and the overlap between ATAC and expression QTL for these gene candidates. For the feral system, selective sweep analysis identified 1181 genes present in sweep regions for the Hawaiian population, and 1190 genes present in the Bermudian population, with 261 genes shared between these two populations. Considering just the hypothalamic eQTL, 86 cis eQTL and 37 trans eQTL were found for the genes in the sweep regions. GO analysis of these sweep regions found a significant enrichment for neuronal and behaviour-related processes. The results demonstrate how we will proceed with the selection of specific 300kb regions with which to create the re-wilded chicken line, as well as how both laboratory and natural populations can be used to in conjunction with single cell and other sequencing technologies to try and identify functional elements for gene regulation.

Keywords: single cell, Gene regulation, ATAC, feralisation, genome editing

Hormonal regulation of growth and body composition in poultry

259S Mechanisms regulating induction of pituitary growth hormone production during chicken embryonic development

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One of the hormones regulating growth in chickens is growth hormone (GH), as evidenced by multiple reports that natural mutations in the GH receptor result in dwarf chickens. Pituitary GH cell differentiation occurs around embryonic day (e) 14 in the chicken. GH cell differentiation can be induced *in vitro* by treatment with e14 serum, and the hormone responsible for this effect is the adrenal glucocorticoid corticosterone (CORT). Plasma CORT levels naturally increase between e12 and e14. CORT can prematurely induce GH gene expression *in vitro* and *in vivo*. Induction of GH in embryonic pituitary cells occurs through a transcriptional mechanism. CORT induction of the GH promoter is mediated by a GC-responsive region (GCRR) located between -1045 and -890 of the GH gene. The GCRR contains an Ets-1 site, an Ets-like protein 1 (Elk1) site, and two non-canonical, degenerate glucocorticoid response elements (dGRE). Mutation of the Ets-1, Elk1, and distal dGRE sites, but not the proximal dGRE site in the GCRR abolished CORT responsiveness. CORT regulates binding of Ets-1 and glucocorticoid receptor (GR) to the GCRR and binding of GR and Pit-1 to the Pit-1 sites of the endogenous GH gene. Pharmacological evidence indicates that CORT induction of GH requires activation of the cell signaling kinase ERK1/2, and CORT increases ERK1/2 activity in embryonic pituitary cells. Ets-1 is a direct target of ERK1/2 in embryonic pituitary cells, and CORT and ERK1/2 activation phosphorylate Ets-1. Using single-cell RNA sequencing technology, a population of embryonic pituitary cells co-expressing GH and POMC (cortico-somatotrophs) was identified, and CORT increased the abundance of these cortico-somatotrophs. Taken together, our findings indicate that CORT induction of GH in chicken embryonic pituitary cells involves both canonical genomic mechanisms and non-canonical activation of cell signaling cascades, and that somatotrophs might differentiate directly from corticotrophs during chicken embryonic development.

Keywords: growth hormone, corticosterone, embryonic development

260S Studies on the mechanism of growth axis genes affecting the balance of adipogenic, myogenic, and osteoblastic differentiation in chickens

Hongmei LI*[1] [1]*South China Agricultural University, Department of Animal Genetics and Breeding, Guangzhou, Guangdong, China*

Mesenchymal stem cells (MSCs) belong to pluripotent cells and play an important role in specific states during the process of individual development and maturation and in the process of tissue repair.We obtained MSCs from the tibia of 7-day-old Mahuang chickens and cultured them to the third generation for in vitro chemical induction. (1) Through identification by oil red O staining, immunofluorescence, alizarin red staining, the results indicated that the induction was successful and chicken BMSCs had multi-directional differentiation potential; (2) Through qRT-PCR determination of key osteogenic genes such as Osterix and BMP2, the results indicated that the key genes were significantly expressed at each stage ($P < 0.05$); (3) During the process of inducing osteogenic differentiation, IGF-1 and IGF-2 genes were over-expressed or interfered with respectively, and the expression levels of osteogenic differentiation related genes of BMSCs were detected by qRT-PCR. The results showed that under the condition of over-expression of IGF-1 and IGF-2, the expression levels of Osterix, BMP2, and RUNX2 all increased. Under the conditions of interfering with IGF-1 and IGF-2, the expression levels of Osterix, BMP2 and RUNX2 all decreased. This indicates that over-expression of IGF-1 and IGF-2 can promote the osteogenic differentiation of BMSCs, and interference with IGF-1 and IGF-2 can inhibit the osteogenic differentiation of BMSCs. The research also found that the upstream regulatory gene GHR of IGF1 can inhibit the adipogenic and myogenic differentiation of chicken BMSCs by suppressing mitochondrial biogenesis and mitochondrial function. Interference with the expression of GHR in DF-1 cells, mitochondrial biogenesis and the expression of key regulatory factors encoded by mitochondrial DNA (mtDNA), down-regulation of oxidative phosphorylation (OXPHOS) gene expression, accompanied by a decrease in the expression of enzymatic active proteins of phosphorus oxide complexes in SLD chicken skeletal muscle and GHR knockout cells. We found that the down-regulation of GHR could reduce the number of mitochondria and change their structure, thereby affecting the development of bones and muscles in chickens. Moreover, GHR affects mitochondrial function and lipid metabolism in chickens by regulating the AMPK-PGC1α-PPAR signaling pathway. In conclusion, a series of studies we have conducted have shown that the GH-GHR-IGF1 growth axis gene regulates the growth and development of chickens by influencing mitochondrial function and regulating the adipogenic, osteogenic and myogenic differentiation balance of mesenchymal stem cells.

Keywords: GH-GHR-IGF1, mitochondrial biogenesis, mitophagy, bone marrow mesenchymal stem cells

261S Signaling and epigenetic networks of growth axis genes in chicken myogenesis and muscle development

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Objectives: This study aimed to investigate the signaling pathways and epigenetic regulatory networks underlying growth axis genes in chicken embryonic myogenesis, focusing on the mechanisms of myoblast proliferation, differentiation, and muscle fiber formation, with implications for improving poultry muscle production. Materials and methods: In vivo experiments, the effect of injection of IGF-1 and testosterone (T) on chicken embryonic muscle development was measured. The relative regulated mechanism was studied by using in vitro culture model of chicken embryo myoblasts. Tet2 was knocked down via siRNA to assess epigenetic regulation. And the effect of siTet2 on 5-azacytidine (5-AZA), or vitamin C (VC)-induced muscle differentiation was studied by qRT-PCR, Western blot, immunofluorescence, and flow cytometry. Inhibitors LY294002 (PI3K) and KP372-1 (Akt) were used to validate signaling pathways. Results: Firstly, IGF-1's role in chicken embryonic myogenesis was investigated. Results showed IGF-1 dose-dependently promoted myoblast proliferation, but the pro-proliferative effect was abolished by PI3K/Akt inhibitors, confirming reliance on the PI3K/Akt pathway. In vivo, exogenous IGF-1 stimulated myoblast proliferation and skeletal muscle growth, with upregulated myogenic regulatory factors (MRFs) indicating involvement in early embryonic muscle development. In addition, exogenous T promoted embryonic muscle growth by upregulating androgen receptor (AR), PAX7, and p-Akt expression. T-enhanced myoblast proliferation, cell cycle progression, and PAX7-positive cell ratio were blocked by PI3K/Akt inhibitors, indicating dependence on the AR-mediated PI3K/Akt pathway. Concurrently, Tet2 expression increased during myoblast differentiation,company with elevated DNA demethylation (5hmC) and reduced histone methylation (H3K9me2/H3K27me3). Tet2 knockdown decreased genomic 5hmC, upregulated H3K9me2/H3K27me3, and inhibited MRFs and myotube formation, highlighting Tet2-mediated active demethylation in activating myogenic genes. Furthermore, 5-azacytidine (5-AZA) and vitamin C (VC) upregulated TET2 and MyHC, promoting DNA/histone demethylation. However, Tet2 knockdown attenuated these effects, suggesting 5-AZA/VC act through Tet2 to facilitate differentiation. Conclusion: IGF1 and T drives myoblast proliferation via PI3K/Akt signaling, while Tet2 modulates epigenetic landscapes to promote differentiation. These insights reveal coordinated roles of hormonal signaling and epigenetic regulation in chicken embryonic muscle development, offering targets for optimizing poultry muscle production.

Keywords: chicken, myogenesis, differentiation, TET2, DNA demethylation

262S IGF2 promotes the differentiation of chicken embryonic myoblast by regulating mitochondrial remodeling

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Skeletal muscle is crucial for animal movement and posture maintenance, and it serves as a significant source of meat in the poultry industry. The number of muscle fibers differentiated from myoblast in the embryonic stage is one of the factors determining the content of skeletal muscle. Insulin-like growth factor 2 (IGF2), a well-known growth-promoting hormone, is crucial for embryonic and skeletal muscle growth and development. However, the specific molecular mechanism underlying its impact on chicken embryonic myoblast differentiation remains unclear. To elucidate the molecular mechanism by which IGF2 regulates chicken myoblast differentiation, we manipulated IGF2 expression in chicken embryonic myoblast. The results demonstrated that IGF2 was upregulated during chicken skeletal muscle development and myoblast differentiation. On the one hand, we found that IGF2 promotes mitochondrial biogenesis through the PGC1/NRF1/TFAM pathway, thereby enhancing mitochondrial membrane potential, oxidative phosphorylation, and ATP synthesis during myoblast differentiation. This process is mediated by the PI3K/AKT pathway. On the other hand, IGF2 regulates BNIP3-mediated mitophagy, clearing dysfunctional mitochondria. Collectively, our findings confirmed that IGF2 cooperatively regulates mitochondrial biogenesis and mitophagy to remodel the mitochondrial network and enhance mitochondrial function, ultimately promoting myoblast differentiation.

Keywords: Insulin-like growth factor 2, mitochondrial biogenesis, mitochondrial function, mitophagy, myoblast differentiation

263S Thyroid hormone regulation of the insulin-like growth factor system in avian skeletal muscle

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Thyroid hormones are essential for optimal growth and body composition in poultry, partially through well-established crosstalk with the somatotropic axis at both central and peripheral levels. Recent work conducted with broiler chickens has demonstrated that consistent correlations exist between circulating thyroid hormones and expression levels of several components of the insulin-like growth factor (IGF) system in breast muscle. This relationship has been demonstrated by our research group using multiple *in vivo* and *in vitro* experimental models, providing evidence that local interactions between the thyrotropic and somatotropic axes are important for muscle growth and development. Broilers genetically selected for high growth potential and muscle accretion were shown to have decreased levels of circulating triiodothyronine (T_3), elevated expression of insulin-like growth factor binding protein (IGFBP) 4, *IGFBP5*, and *IGFBP7*, and reduced expression of *IGFBP3* when compared to those that have low growth and muscle accretion potential. Further, plasma T_3 and thyroxine (T_4) were lower in commercial broilers exhibiting a high feed efficiency phenotype, and this was accompanied by higher mRNA levels of *IGF1*, *IGF2*, and the IGF1 receptor (*IGF1R*) in breast muscle, alongside elevated expression of *IGFBP2*, *IGFBP4*, and *IGFBP5*. In broilers exposed to elevated ambient temperature, plasma T_3 was reduced as were breast muscle levels of mRNA for *IGF-1, IGF2, IGFBP2, IGFBP4, and IGFBP5*. Bioinformatics analysis indicates that there are several putative thyroid hormone response elements (TREs) in the 5'-promoter region of multiple IGFBPs, suggesting that thyroid hormones could directly regulate their expression and activity within breast muscle. Treatment of undifferentiated avian myoblasts with T_3 or T_4 induced expression of *IGFBP3*, *IGFBP5*, and *IGF2* after both short- and long-term timeframes, providing evidence that at least some of the putative TREs are functional. Fewer effects were observed when cells were induced to differentiate into multinuclear myotubes, likely due to the observation that levels of deiodinase 2 necessary for conversion of T_4 to more bioactive T_3 and thyroid hormone receptor alpha were higher in proliferating myoblasts cultured in an undifferentiated state. Higher levels of *IGF2*, *IGF1R*, *IGFBP3*, and *IGFBP5* were also observed in undifferentiated cells. Together, these findings demonstrate that thyroid hormones can affect local expression of IGFBPs in avian muscle tissue, which in turn may regulate paracrine IGF signaling and, ultimately, influence growth and development of avian skeletal muscle.

Keywords: broiler, breast muscle, insulin-like growth factor binding proteins, triiodothyronine, thyroxin

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