Physiology and Reproduction

233  **Physiological mechanisms regulating metabolic efficiency in commercial broilers.** Laura Ellestad*, 1, Samuel Latzsch1, Michael Rothrock2, and Jean Guard2, 1University of Georgia, Athens, Georgia, United States, 2USDA-ARS, Athens, Georgia, United States.

Recent implementation of the Veterinary Feed Directive and increased demand from the public for food produced without antibiotics have created a need for development of strategies to maintain broiler production efficiency in the absence of antibiotic growth promoters. As feed accounts for almost 70% of total broiler production cost, understanding basic mechanisms regulating efficiency of feed nutrient use is crucial to developing these strategies and preserving production margins, particularly in antibiotic-free systems. In any given population of broilers raised under the same conditions, there is a natural variation in metabolic efficiency as measured by feed conversion ratio [FCR; g feed intake (FI)/g body weight gain (BWG)]. The objective of this study was to identify physiological parameters associated with hormonal regulation of nutrient uptake and utilization differing between high efficiency (low FCR) and low efficiency (high FCR) commercial broiler chickens. Male Ross 708 broilers (108 birds) were individually reared between post-hatch day (D) 7 and D35, and BWG, FI, and FCR were calculated on an individual bird basis from D7- D35. On D36, blood, liver, and muscle were collected from the 6 highest (HE) and lowest (LE) efficiency birds based on FCR for measurement of circulating hormones and expression of genes associated with hormonal action and nutrient partitioning (n = 6). Data were analyzed with a 2-tailed Student’s t-test. While there were no differences between HE and LE groups in terms of D35 final body weight, BWG, or FI, LE birds had a significantly higher FCR than HE birds (1.69 ± 0.03 vs 1.41 ± 0.007; P < 0.0001). Circulating levels of thyroxine (T4) were higher in the LE group (17.4 ± 0.42 vs 13.6 ± 0.62 ng/mL; P < 0.05), and levels of corticosterone (CORT; 558.8 ± 70.0 vs. 364.1 ± 72.8 pg/mL; P = 0.0787), growth hormone (GH; 1532 ± 251.9 v. 1043.9 ± 35.3 pg/mL; P = 0.1030), and insulin (INS; 7.0 ± 1.0 vs. 4.61 ± 0.4 µU/mL; P = 0.0788) tended to be higher in LE birds. Plasma levels of insulin-like growth factor 1 (IGF1) and triiodothyronine did not differ between groups. In muscle, mRNA levels of IGF1 receptor (2-fold), cationic amino acid transporter 1 (CAT1; 5-fold), and glucose transporter (GLUT) 5 (2-fold) were higher in HE birds (P < 0.05). Levels of mRNA for CAT1 (2-fold) and GLUT2 (1.2-fold) were also higher in the liver of HE birds (P < 0.05), while expression of sodium-glucose transporter 1 (1.8-fold) was higher in LE birds (P < 0.05). These data suggest that plasma T4, and to a lesser extent CORT, GH, and INS, may contribute to improved feed efficiency by altering expression levels of key amino acid and sugar transporters in metabolically important tissues such as liver and muscle.

**Key Words:** feed efficiency, microbiome, gastrointestinal tract, carbon metabolism

There is a natural variation in metabolic efficiency as measured by feed conversion ratio [FCR; g feed intake (FI)/g body weight gain (BWG)] in a given population of broilers. The objective of this study was to identify microbiological constituents associated with high efficiency (low FCR) and low efficiency (high FCR) commercial broiler chickens. Male Ross 708 broilers (108 birds) were individually reared between post-hatch day (D) 7 and D35, and on D36, jejunum, ileum, duodenum, and cecum were collected from the 6 highest (HE) and lowest (LE) efficiency birds based on FCR for microbiome analysis (n = 6). For phenotypic microbiome analysis, tissue samples were homogenized in tryptic soy broth (1:3 wt/vol dilution), filtered, normalized to a 1.0 OD600, and used to seed Biolog EcPlates to assess the microbial communities’ ability to utilize 31 different C-sources. For genotypic microbiome analysis, genomic DNA was extracted from homogenized tissue samples using the QiAamp DNA Stool kit, and 16S rDNA sequencing was performed using the Illumina MiSeq platform and processed using the QIIME2 pipeline. There were definite differences between gastrointestinal tract segments, regardless of feed efficiency, with the cecal microbial loads being more than 1 log higher than duodenal samples and more than 3 logs greater than ileal and jejunal segments. EcPlates indicated that microbial respiration was significantly higher, according to a 1-tailed, paired t-test, in LE birds (P < 0.0001) in cecal, duodenal, and jejunal segments, with metabolism of C-sources such as glycogen, a-D-lactose, glucose-1-phosphate, and g-hydroxyl-butyric acid differing between groups. Genotypic microbiome analysis showed that while gastrointestinal segment was the greatest driver of richness, diversity, and community structure within these samples, there were significant differences in taxa within each segment between HE and LE broilers, including those within the dominant Firmicutes and Bacteroidetes phyla. These data suggest that microbial signatures are associated with feed efficiency differences that can be linked on a genotypic and a phenotypic community-scale level. These microbial signatures need to be studied further, as they represent potential targets for future strategies to maintain or even improve current production efficiencies within the poultry industry.

**Key Words:** feed efficiency, microbiome, gastrointestinal tract, carbon metabolism

234  **Intestinal microbial ecology associated with metabolic efficiency in commercial broilers.** Michael Rothrock*, 1, Laura Ellestad2, and Jean Guard1, 1USDA-ARS, Athens, Georgia, United States, 2University of Georgia, Athens, Georgia, United States.

In the absence of antibiotic growth promoters, the broiler industry needs to develop new strategies to maintain broiler production efficiency. To better develop these strategies, a basic understanding of the microbiome effects on broiler feed efficiency is needed, with the hope that this knowledge can be leveraged to make production strategies more efficacious.

A 2X2 factorial designed experiment was completed to compare the effects of (a) 2 rearing growth curves/photostimulation ages (15 or 21 wks) with (b) 2 different feeding regimens, every-day spin feeding (EDS) vs skip-a-day (SAD), on broiler breeder production through 65 wks of age. For this experiment, 2,400 females and 360 male Cobb-500 broiler breeder chicks were randomly divided into one of 4 treatments, with 3 replicates per treatment. From 2 wks of age until photostimulation, birds were fed either on a SAD basis to reach target body weight (2.1 kg) for photostimulation at 15 wks (15-SAD) or 21 wks (21-SAD) with chain feeders; or EDS basis to reach target body weight at 15 wks (15-EDS) or 21 wks (21-EDS). Following obtainment of target body weight, at either 15- or 21-wks, the birds were transferred to breeding pens with 9 replicate pens (38 hens and 4 roosters each) per treatment, and photostimulated (14L:10D). The weight profile of the birds that were moved
236  Germ cell dynamics during nest breakdown and formation of primordial follicles in the domestic turkey (Meleagris gallopavo). George Hall*1, Julie Long2, Ben Wood1,3, and Gregory Bedecarrats1. 1University of Guelph, Guelph, Ontario, Canada, 2Beltsville Agricultural Research Centre USDA, Beltsville, Maryland, United States, 3Hybrid Turkeys, Kitchener, Ontario, Canada.

Within the avian ovary post-hatch, the breakdown of germ cell nests and the formation of primordial follicles marks a pivotal time in cellular depletion and reorganization. Since upon sexual maturation, birds lack a germinal bed with self-renewing oogonium, consequently, this cellular change heavily influences the finite number of ova available throughout their reproductive life span. This study aimed to historically assess the different subpopulations of germ cells within the cortex of the left ovary during germ cell nest breakdown in the domestic turkey. This was accomplished by measuring the size and density of pre-follicular germ cells and primordial follicles within ovaries collected from poults at 1, 3, 5, 7, 9, 11, 13, 15 and 21 d post-hatch. Whereas, the total germ cell count was determined from ovaries at 5, 9 and 15 d post-hatch. Four ovaries were analyzed per age group. Ovaries were fixed, sectioned (5 μm) and stained (H&E) for imaging. For size and density measurements, 6 to 16 sections per ovary were evaluated; for total germ cell counts, the entire ovary was assessed. Measurements were averaged for each ovary before analyses. Among age groups, a one-way ANOVA was used to test for significance (P ≤ 0.05); followed by a post-hoc test (Tukey) to determine which ages differed. Based on our results, germ cell nest breakdown was initiated 5 - 7 d post-hatch, as shown by the first decrease (P ≤ 0.001) in the pre-follicular germ cell density. This was accompanied by the appearance of primordial follicles on d 7. Initiation of nest breakdown is temporally followed on d 9 by the first increase (P ≤ 0.05) in size of both pre-follicular germ cells and primordial follicles. This growth ended by d 13, with no further change in size (P > 0.05). The majority (~90%) of nest breakdowns concluded by d 15, as evidenced by a lack of change in both pre-follicular germ cell and primordial follicle densities in older ovaries. Before the initiation of germ cell nest breakdown (d 5) and after the majority had broken down (d 15), the total germ cell count was 1,057,402 ± 194,627 and 169,578 ± 31,594 (mean ± SEM), respectively, which equates to an 84% decrease in the total germ cell population. The presence of pre-follicular germ cells at 21 d post-hatch suggests that a further decrease in the overall germ cell population may occur. It appears that, in the domestic turkey, the loss of germ cells during nest breakdown is comparable to the domestic chicken, but exceeds the average 2-thirds lost in mammalian species.

Key Words: germ cell, primordial follicle, nest breakdown, ovary, turkey

237  Biostrong® Libido improves semen quality of male breeders and increases female reproductive performance. Megan Koppen*1 and Jan Dirk van der Klis2. 1Delacon USA, Inc., Carlisle, Pennsylvania, United States, 2Delacon Biotechnik GmbH, Steyrregg, Austria.

Male fertility is critical in turkey breeding as it directly correlates to hatchability. Crucial for sperm quality and male fertility is an effective antioxidant system to counteract sperm cell membrane lipid peroxidation and reduction of free radical formation responsible for sperm DNA fragmentation. Phytogenics can increase resilience of spermatocytes by stimulating cellular antioxidant capacity thus upregulating cellular antioxidant response element (ARE), which results in increased production of antioxidant enzymes. On top, essential oils and saponins can support hormonal regulation of spermatogenesis and increase testosterone production. Biostrong® Libido (BSL) is a proprietary blend of essential oils, flavonoids, and quillaja saponins developed to improve male breeder fertility. A field trial was conducted to evaluate the efficacy of BSL on semen quality and quantity in turkey toms; and consequently, reproductive performance in female breeders. BSL was supplemented at 750 g/MT to 1,200 male turkey breeders fed corn-soy based diets starting at 22 weeks of age, immediately following selection (5 weeks before milking). Packed cell volume (PCV) and mLs per milked tom was evaluated over a period of 32 weeks. The weekly averages of the test barn were compared with a 9-cycle-average. Female reproductive performance was also evaluated. A house of female breeder turkeys (approximately 20,000 hens) was inseminated with semen from the BSL supplemented toms. Measurements gathered from these hens were from the first week of lay through end of production (over a period of 24 weeks). Reproductive performance was measured based on percentage of fertile eggs and were compared with 4 control barns. Due to trial design, statistics were unable to be performed. Percent differences among treatments were identified and used to demonstrate potential product efficacy in a commercial setting. BSL fed toms showed a 6.5% increase in PCV compared with the 9-cycle-average over the 32-week period. Additionally, mLs per milked tom increased by 5% compared with the control flocks. Turkey hen breeders inseminated with semen of toms fed BSL compared with the average of 4 control barns showed percentage of fertile eggs in the first half of production (1–13 weeks of lay) increased by 0.43% and by 0.19% during the second half of production (14–24 weeks). Female production for the entire 24-week period averaged 0.35% more fertile eggs in the BSL inseminated hens compared with the average of 4 control barns. Due to trial design, statistics were unable to be performed. Percent differences among treatments were identified and used to demonstrate potential product efficacy in a commercial setting. BSL fed toms showed a 6.5% increase in PCV compared with the 9-cycle-average over the 32-week period. Additionally, mLs per milked tom increased by 5% compared with the control flocks. Turkey hen breeders inseminated with semen of toms fed BSL compared with the average of 4 control barns showed percentage of fertile eggs in the first half of production (1–13 weeks of lay) increased by 0.43% and by 0.19% during the second half of production (14–24 weeks). Female production for the entire 24-week period averaged 0.35% more fertile eggs in the BSL inseminated hens. Based on the results of this field trial with turkey breeders, dietary supplementation with BSL may improve semen quantity and quality and exert positive effects on the reproductive performance of females.

Key Words: fertility, semen quality, turkey, phytogenics, antioxidant
Besides environmental, nutritional, and pathological conditions, oviductal functions also govern the egg production and quality. Recently, we identified several novel genes associated with albumen formation in the magnum, and eggshell biomineralization in the shell glands. The objectives of this study were to determine the enriched biological process and pathways, and solute carrier (SLC) genes that mediate the 1) albumen synthesis and secretion in the magnum, and 2) eggshell biomineralization in the shell glands. Segments of oviducts were collected from Hy-line laying hens at 3 h post-ovulation (p.o.) and 15–20 h.p.o., molter, and non-laying hens. Total RNAs isolated from the magnum of laying hens at 3 h.p.o. vs. non-laying hens (n = 3/group), from the shell gland of laying hens at 15–20 h.p.o. vs. non-laying hens (n = 3/group), respectively, were subjected to RNA-Seq. Biological pathways and SLC genes obtained from the RNA-Seq data were further validated in all the experimental groups (n = 6/group) using real-time PCR (qPCR). In the magnum, biological processes (L-serine biosynthetic process, regulation of immune system process, and proline transport) and metabolic pathways (superpathways of serine and glycine biosynthesis I, matrix metalloprotease inhibitors, asparagine biosynthesis I, asparagine degradation I and choline degradation I) were enriched. Using qPCR, we further confirmed that Interstitial Collagenase, Gelatinase B, Cingulin, and several family members of SLCs related to anion and cation exchanger, amino acid transporter were significantly higher in laying hens at 3 h p.o. Identified biological process and pathways may increase the bioavailability, transport of essential ions, amino acids and immune-related molecules in the lumen of magnum for albumen biosynthesis. In the shell gland, biological processes (serine biosynthetic, cellular sodium ion homeostasis) and pathways (calcium signaling, pantothenate, and CoA biosynthesis) were the most-enriched. Using qPCR, we further confirmed calcium signaling pathways (Otopetrin 2, Calcitomin, Stanniocalcin 2, and Plasma membrane Ca2+ transporting 2) and SLCs genes related to sodium/hydrogen exchanger, sodium bicarbonate cotransporter, zinc long-chain fatty acids transporter, and glucose-6-phosphate exchanger were significantly higher in shell gland at 15 h p.o. Identified biological process and pathways may increase the bioavailability, mineralization, and remodeling of calcium for the eggshell formation. In conclusion, this study identified the biological pathways involved in the albumen biosynthesis and eggshell formation, and can potentially be used to enhance the egg production traits in chickens.

Key Words: RNA sequencing, gene expression, biological pathways, magnum, shell gland/uterus

239 Organic matrix protein profile associated with changes in crystallographic texture and mechanical properties in guinea fowl eggshell. Nathalie Leroy1, Alejandro Rodriguez2, Lucie Combes-Soia3, Aurelien Brionne1, Valerie Labas2, Yves Nys*1, and Joel Gautron1, 1Boa, IN.R.A. Université de Tours, Nouzilly, France, 2UMR PRC IN.R.A85 CN.RS 7247, Université de Tours, Nouzilly, France, 3University of Granada, Departamento de Mineralogy y Petrología, Granada, Spain.

Guinea fowl eggshells have a particular ultrastructure and crystallographic arrangement that is different from other birds and is at the origin from its exceptional mechanical properties. It is a 2-layer structure with different microstructure: the inner layer (1/3) is constituted of columnar calcite crystal units which progressively develop preferred orientation as observed in hens but the outer layer is made of calcite microcrystals with varying crystallographic orientations and interlocking boundaries. The study of early shell mineralization in Guinea fowl revealed the formation of a transient amorphous calcium carbonate as recently observed in hens, then formation of larger crystals issued from space completion and elongation of the crystals due to inhibition by the organic matrix of faces parallel to the C axis. The shift from larger to smaller crystal is suspected to be controlled by the shell organic matrix proteins and proteoglycans. That was confirmed by a quantitative proteomic study (nanoLC-MS/MS) of the eggshell matrix of shell sampled before and after the changes in crystallography of guinea fowl shell (8 birds sampled/stage; The comparison of protein abundance carried out using weighted spectral count and the eXtracted Ion Chromatogram combined with Anova and Hierarchical clustering). The level of 149 proteins was quantified at 5 calcification stages. Neogasthæae (chicken, Guinea fowl, zebra finch…) have in common 54 proteins including 4 proteins related to the biomineralization process (Nucleobindin-2, extracellular serine/threonine protein kinase, Milk Fat Globule-EGF factor 8 protein and Calbindin D-28K). Among the 15 most abundant Guinea fowl proteins at the early stage of shell formation, 10 proteins were analog to the chicken (OVAL, OVM, OVOT, HPX, LYZ C, OC-17-like, HAPLN3 (Hyaluronan and proteoglycan link protein 3), CST3, HBAA and CLU). Nine proteins were unique to the Guinea fowl eggshell including 2 acidic proteins with putative calcium-binding domains LOC110408336 and CGRE1 and Dromaiocalcin-1-like, the homolog to Ovoceilen-17. 61 matrix proteins were present in the shift period. Among them are calcium binding proteins (NPNT-X1, CALBP1, Protein S100-A6, ANX1A2 and CDH2…), core proteins of proteoglycans (TSKU, GPC4…), and other proteins regulating the activity of proteins driving the mineralization (SSP1, OC-116GDF6…). The identification and quantification of the proteins of the eggshell matrix should allow a better understanding of the mechanisms of shell formation and the control of its mechanical properties. It might be used for determination of biological markers for the genomic selection of chicken layers with improved shell mechanical properties.

Key Words: guinea fowl, eggshell, crystallography, proteomic, shell matrix protein

240 The effect of cooling rate of broiler hatching eggs on embryonic development, hatchability and hatch time. Serdar Özlü* and Okan Elibol, Ankara University, Faculty of Agriculture, Ankara, Diskapi, 06110, Turkey.

The effect of the egg cooling profile of broiler hatching eggs after oviposition on embryonic development and hatchability of fertile eggs was studied. Hatching eggs were obtained from Ross 308 broiler breeders at 35 wk (prime) of age in Exp 1, and at 28 wk (young) and 64 wk (old) of age in Exp 2, respectively. A total of 120 eggs were collected and then randomly placed to cardboard egg trays in Exp 1. The eggshell and internal egg temperatures were measured intervals 40 min during egg cooling process. To measure the internal egg temperature, a 4-mm diameter hole was drilled into the top of the each egg after measured the eggshell temperature. In Exp 2, a total of 3,150 eggs that had been laid within a 15 min period were collected and then randomly assigned to 2 temperature controlled chambers with either control (360°- 480°) or rapid (45°-120°) cooling to 24 and 18°C EST, respectively. Eggs were remained in the chambers until the EST of both cooling groups were similar, then eggs were transported to hatchery and were stored for 6 d at 16°C and 75% RH. Each tray of 150 eggs was considered to be a replicate and there were 5 replicate trays per cooling profile treatment in each flock age. Some (25 embryos in each batch) of the eggs...
were opened before and after cooling profile treatment to determine the stage of the blastoderm. The eggs were randomly set in a single commercial incubator in Exp 2. The CORR procedure of SAS was utilized to determine correlation coefficient for eggshell temperature relative to internal temperature in Exp 1. Data from the completely randomized design were subjected to ANOVA using the GLM procedure of SAS in Exp 2. A significant ($P < 0.0001$) positive correlation ($r = 0.995$) existed between eggshell temperature and internal egg temperature. The stage of embryonic development was advanced by control cooling and by the older flock. In younger flock eggs, fertile hatchability was significantly decreased by rapid cooling due to higher early and late embryonic mortality ($P \leq 0.05$). However, early embryonic mortality and percentage of second grade chicks was reduced ($P \leq 0.05$) and fertile hatchability was numerically higher by rapid cooling compared with control in older flock eggs. In conclusion, the data from this study demonstrated that rapid cooling after lay retarded the stage of blastoderm development in eggs from both young and old broiler breeder flocks. This was apparently detrimental, as indicated by higher early and late embryonic mortality, in the case of the young flock but beneficial in the case of the old flock. The hatchability differences between young and old flock eggs by rapid cooling rate might depend on the differences of embryonic development at oviposition.

**Key Words:** hatching broiler egg, egg temperature, egg cooling rate, hatchability, hatch time

241 Development of a chicken intestinal organoid culture and its characterization. Mohan Acharya¹, Annie Donoghue¹, Joshua Lyte¹, and Narayan Rath*², ¹University of Arkansas, Fayetteville, Arkansas, United States, ²USDA/ARS, Fayetteville, Arkansas, United States.

Organoids are 3 dimensional constructs of tissues that can simulate tissue functions hence, have been studied for the purpose of regenerative medicine, analyzing physio-pathology of tissues and interaction with different agents including microbes. However, most advances in this area has been limited to mammalian species with few studies in avian models. Optimum maintenance of gut health and protection against pathogenic organisms is an impending issue in poultry production due to the restrictions in antibiotics growth promoters. To identify factors that may improve intestinal competency for growth and protection against pathogens, we sought to develop chicken enteric organoid culture for screening of selective agents and to examine interaction with pathogens. We isolated intestinal villi from day old chicks, purified, and cultured in DMEM-F12 media containing antibiotic/antimycotic, 10% fetal bovine serum, bovine pituitary extract, insulin, selenium, transferrin, and, polyamine supplements which favor the repair of the severed ends of villi to spheroids within 24 h of incubation. The structural changes in the organoids over 3 d were verified using scanning electron microscopy which showed these organoids budding and proliferating 3 dimensionally and contained vascular tissues. We examined the organoids by immunofluorescent staining with several different antibodies, lectins, and antigen ligands, which showed the presence of several epithelial antigens including cadherin, keratin, Na-K ATPase, mucin, and showed the cells with polarized localization of β-actin. The organoids could be infected with Salmonella, expressing green fluorescent protein (GFP). We tested the effect of selective agents that are known to affect intestine such as dextran sulfate (inducer of colitis), retinoic acid (vitamin A), 2,4 dinitrophenol (a weight reducing agent), deoxycholic acid a (bile acid), capsaicin and lipopolysaccharide, both, inflammatory agents, phorbol 12-myristate 13-acetate (a protein kinase C activator), β-glycophate (a pesticide), and indomethacin (a prostaglandin inhibitor), for their effect on organoids. We are currently evaluating the effects of enteric neurochemicals, such as serotonin, on organoid response to pathogens of relevance to the poultry industry. Our results show that these chemicals differentially affect the organoids some of which cause severe damage whereas others, showed little to no change. These results suggest that the intestinal organoids have potential to screen for chemicals and factors which can improve gut health, serve as antibiotic alternatives, provide a template to study nutrient absorption, host bacterial interactions, and other physiological mechanisms.

**Key Words:** chicken intestinal organoids, structure, immunohistochemistry, Salmonella