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POSTER PRESENTATIONS

*AuthorPresenting Paper

T1 In ovo feeding of glutamine to chicks. A. A. Pedroso*, V. T. Barbosa, I. B. Maciel, C. E. Barbosa, and K. L. Martins, *University of Goias, Goiania, Goias, Brazil.*

An experiment was carried out using 650 fertilized eggs. The eggs were identified, weighed, and allocated into five incubators regulated at 65% RH and 37.5°C. At the 16th incubation day, eggshells were drilled at the air chamber region and test solution was injected manually with a sterile syringe. The experimental treatments were: 0, 10, 20 and 30 mg of glutamine and a control group represented by intact eggs. The glutamine was dissolved in 200 ml of saline solution (0.9%) and deposited into amniotic liquid. The experiment consisted of five treatments with 130 eggs used in each treatment. Each egg represented an experimental unit. After inoculation, the eggs were packed individually in tulle and relocated into the incubators until hatch. Glutamine levels did not improve hatchability, chick:egg, and chick weight. Glutamine levels did not affect embryonic mortality, but embryos supplemented with glutamine hatched faster.

Key Words: embryos, chicken, amino acid

T2 The effect of Glutamine or Glutamic acid supplementation in combination with antibiotics on the growth performance of broiler chickens. K. Nakagawa*, I. Shinzato, and H. Sato, *Ajinomoto Co., Inc., Tokyo, Japan.*

Two broiler trials were conducted to evaluate the effect of dietary Glutamine or Glutamic acid on the growth performance with or without antibiotics. In the initial trial (EXP 1), 900 broiler chicks (Arbor Acres) at 0 d were allocated to one of six treatments with six replicates (25 birds per pen). The experimental diets consisted of either 1.0% Glutamine, 1.0% Glutamic acid or 1.0% L-Ala (nitrogen source) supplementation with or without antibiotics (CP 22.5%). Chicks were fed each experimental diet from 0 to 21 days of age, with body weight and feed intake per pen measured at 7, 14 and 21 days of age. Without antibiotics, body weight and feed conversion ratio were significantly improved by supplementation of 1.0% Glutamine and 1.0% Glutamic acid ($P < 0.05$). When feeding antibiotics, there was no additional benefit of supplementing Glutamine or Glutamic acid in regards to body weight and feed conversion ratio. In the subsequent trial, 480 broiler chicks (Arbor Acres) at 0 d were allocated to one of five treatments with six replicates (16 birds per pen). Similar diets as in EXP 1 were used with 1.0% Glutamine, 1.0% Glutamic acid or 1.0% L-Ala supplementation without antibiotics, while antibiotic fed groups were only supplemented with 1.0% Glutamine or 1.0% L-Ala. Phases of the trial were as follows: CP 22.5% starter: d0-d21; CP 20.0% grower: d21-d42; CP 18.9 finisher: d42-d56. Chickens were fed experimental diets from 0 to 56 days of age, and measuring body weight and feed

intake per pen weekly. Glutamine and Glutamic acid could not show any improvement in starter and finisher, but in grower, Glutamine supplementation could show the significant improvement ($P < 0.05$) and Glutamic acid supplementation could show the tendency of improvement ($P < 0.20$) under the condition without antibiotics. Therefore, these results suggest that Gln and Glutamic acid supplementation have possibility to improve growth performances as replacement of antibiotics in broiler chickens.

Key Words: Glutamine, Glutamic acid, antibiotics

T3 Dried porcine solubles (DPS) and coccidiosis vaccines on morphometric parameters in lower intestine. P. H. D. Tomasi and J. L. Andriquetto*, *Universidade Federal do Paraná, Curitiba, Paraná, Brazil.*

The objectives were to compare the effectiveness of two different coccidiosis vaccines and their effect on the intestinal morphometry of broilers, and to evaluate the interaction of dried porcine solubles, a source of peptides, on the intestinal parameters of the vaccinated broilers. Vaccines against coccidiosis cause a reaction on the mucosa of the small intestine decreasing performance. A vaccine made of attenuated oocysts (Paracox® - Schering Plough) was given to 1-day old chicks (active immunity), and a vaccine based on antigens isolated from gametocytes of *Eimeria* (Coxabic® - Abic) was given to the breeders, and the chicks were used in the trials (passive immunity). DPS was supplied, for the first 21 days, in an attempt to reduce the deleterious effects of the vaccines, as a source of peptides rich in glutamine (6%), in a 2 x 2 factorial design: T1(Paracox/DPS; n=420), T2 (Paracox only; n=420), T3 (Coxabic/DPS; n=420), T4 (Coxabic only; n=420). Animals were fed equal levels of energy, protein, and amino acids. On day 7, there was an interaction between type of vaccine and DPS in the vilus height (VH) and in the relation between vilus height and crypt depth (V:C) in duodenum and V:C in ileum. In these interactions, the use of Paracox resulted in a lesser VH and V:C, if compared against Coxabic, but DPS improved these parameters ($P < 0.05$). DPS, on day 7, increased VH in ileum ($P < 0.05$). On day 14, Coxabic produced better parameters in VH and V:C of duodenum, VH, crypt depth (CD) and V:C of jejunum and CD and V:C of ileum ($P < 0.05$). The use of DPS produced a bigger V:C in duodenum and jejunum ($P < 0.05$). At 21 days of age, the analysis of interactions showed that the use of Paracox resulted in a worse CD and V:C in jejunum and a worse VH in ileum and, with DPS these parameters were improved to the level of Coxabic ($P < 0.05$). DPS improved VH and V:C of duodenum, VH of jejunum and V:C of ileum ($P < 0.05$). To conclude, Paracox produced bigger intestinal lesions than Coxabic, but the use of DPS significantly decreased such lesions.

Key Words: DPS, intestinal morphometry, coccidiosis vaccine

T4 Efficacy of probiotic supplementation in broiler diets.

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Two experiments were conducted to evaluate the efficacy of a bacillary probiotic feed additive (Ecobiol®) containing 1×10^9 CFU/g of *Bacillus coagulans* CECT 5940 on performance of broiler chickens. A randomized complete block design was applied in each experiment. The experimental design was applied to 308 Ross male broilers in both the starter (0-21d) and the grower (22-42d) phases, from day-old to slaughter at 42 days of age. In experiment I, there were four treatments, a basal diet (control), and three levels of inclusion of the bacillary probiotic: 10, 100, and 1,000 mg/kg, applied to 12 cage-pens of 12 broilers each. Mortality was considered normal (mean 4.3%) and was not affected by treatment. During the grower phase, probiotic supplementation improved feed to gain ratio (1.99 vs 1.97, 1.96, and 1.96 g gain/g feed, for control vs 10, 100 and 1,000 mg/kg, respectively; $P \leq 0.05$). Improvements were also observed over the global period, and broilers supplemented with the probiotic converted better than control birds (1.80, 1.79, 1.78, and 1.78 g feed/g gain; for control, 10, 100, and 1,000 mg/kg, respectively; $P \leq 0.05$). No differences were observed among the different doses. In experiment II, two experimental treatments with 12 floor-pens of 60 broilers each were used: 1) basal diet (control), and 2) basal diet with 1,000 mg/kg of probiotic, the recommended commercial dose. Mortality was considered normal (mean 4.5%) and was not affected by treatment. During the grower phase, probiotic supplementation improved feed to gain ratio (1.91 vs 1.88 g gain/g feed; $P \leq 0.05$). For the global period, broilers supplemented with the probiotic converted better (1.83 vs 1.80 g feed/g gain; $P \leq 0.01$) and tended to eat less feed (102.7 vs 100.2 g/d; $P < 0.10$) than control birds. In conclusion, the data from these studies provide evidence that bacillary probiotic supplementation improves performance in broilers.

Key Words: *bacillus coagulans*, probiotic, broilers

T5 Evaluation of a mineral chelate for late-cycle laying hens.

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To determine the effect of a commercial chelated mineral product (Bioplex Poultry®) on the egg quality of late-cycle laying hens, 360 55 week-old Babcock White Leghorns were fed either a control diet (C) or a supplemented diet containing Bioplex Poultry® (BP). The BP was added sequentially at 4 week intervals in a completely randomized design with dietary treatment (C, BP 55 weeks (BP55), BP 59 weeks (BP59), BP 63 weeks (BP63) and BP 67 weeks (BP67)) as the main factor. Feed consumption, body weights and hen-day production were measured monthly and egg quality data was measured bi-weekly. BP had no effect ($P > 0.05$) on feed consumption, body weights or hen-day production. Hen-day egg production was 82.5%, 82.2%, 84.4%, 84.9% and 79.1% for C, BP55, BP59, BP63 and BP67, respectively. BP had no effect ($P > 0.05$) on egg specific gravity, egg weight, albumin height or percent egg shell. Egg specific gravity was 1.080, 1.081, 1.081, 1.082 and 1.081, while percent egg shell was 8.8%, 8.7%, 8.3%, 8.7% and 8.7% for C, BP55, BP59, BP63 and BP67 respectively. For the late-cycle laying hens in this trial BP did not improve the eggshell quality.

Key Words: mineral chelator, laying hen, egg quality

T6 Effect of oligofructose and inulin on calcium and phosphorus content in tibia bone of growing broiler chickens.

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Numerous investigations performed in animal models have shown repeatedly that prebiotics, such as inulin, oligofructose, stimulate mineral absorption, mainly calcium. The objective of this study was to determine the effect of oligofructose and inulin intake on calcium and phosphorus content in tibia bone of broiler chickens in growing in a commercial farm. A total of 30,000 1-day old male Ross broiler chicks were randomly allocated into two groups. The birds were fed a control diet or a control diet supplemented with oligofructose 2% (Raftifeed® OPS) from the chicken reception to 7-days-old chicks and inulin (Raftifeed® IPS) 0.1% from 8-days-old chicks to 42-days-old chicken. A random sample (10 birds per treatment) was taken and culled by cervical dislocation at 1, 7, 14, 21, 28, 35 and 42 days of age to evaluate calcium and phosphorus content in bone. The inclusion of oligofructose and inulin improved calcium content in tibia bone by 18% on day 14, 7.8% on day 21 and 42 and in 5.7% on day 28 ($P < 0.05$). No significant differences were observed in phosphorus content. However inulin and oligofructose inclusion improved the molar Ca:P ratio of bone on day 7, 14, 21, 28 and 42 ($P < 0.05$). These results indicate the inclusion of oligofructose and inulin improve calcium concentration in bone from growing broiler chickens.

Key Words: inulin, bone, calcium

T7 Effect of mannan oligosaccharides and enzymes on antibody titers against Gumboro.

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This study evaluated the effect of including mannan oligosaccharides (MOS) and/or enzymes in broiler diets on antibody titers against Gumboro disease. A total of 750 one-day-old Cobb male chicks with initial weight 41.51 ± 0.59 g were used in the trial. The birds were distributed into a completely randomized experimental design in a $2 \times 2 + 1$ factorial scheme with two levels of MOS (0 and 0.1% until 21 days and 0.05% from 22 to 42 days of age), two levels of enzymes (0 and 0.05%) and a positive control diet with 125 ppm of colistin sulfate and 10 ppm of virginamycin as growth promoters and 51 ppm of salinomycin as anticoccidial, totaling five treatments and five reps. Enzyme contained cellulase, protease and amylase. Birds were obtained from flocks vaccinated against Gumboro at 14, 25 and 36 days and 10, 15 and 22 weeks of age. All vaccines contained live virus. The birds were vaccinated against Gumboro at 7 (attenuated live virus, Lukert sample) and 21 days of age (live virus, strong sample, strain V877), through drinking water. For serum analyses, blood samples were collected weekly through jugular vein puncture in two birds per replicate. The first and last collections were done at 7 and 42 days of age, respectively. Blood samples were centrifuged and serum was analyzed for antibody titers. There was no significant interaction ($P > 0.05$) between antibiotic and factorial (MOS and/or enzymes) or MOS and enzymes on antibody titers. The inclusion of MOS resulted in increased antibody titers against Gumboro in the 4th ($P < 0.03$) and 5th ($P < 0.02$) weeks. The titers in broilers fed MOS were 23.71 and

8.76% higher, respectively during the 4th and 5th weeks, compared with birds of the other treatments. These results demonstrate the positive influence of MOS on the immune system of birds. It can be concluded that MOS was effective in stimulating the immune response against Gumboro vaccine virus.

Key Words: additives, immune modulation, prebiotic

T8 Availa[®]Zn and Availa[®]Mn improve performance and intestinal strength of broilers fed plant-based diets. S. Davis¹, T. Cheng^{*2}, and T. Ward², ¹Colorado Quality Research, Wellington, Colorado, ²Zinpro Corporation, Eden Prairie, Minnesota.

A total of 882 Ross x Cobb 500 chicks were randomly placed into 49 pens and fed only plant-based diets. The treatments consisted of (1) Sulfates [100 ppm Zn from ZnSO₄ and 110 ppm Mn from MnSO₄]; (2) ISO 40 Availa-Zn [Availa-Zn zinc amino acid complex replaced 40 ppm Zn from ZnSO₄]; (3) ISO 80 Availa-Zn [Availa-Zn replaced 40 ppm Zn from ZnSO₄]; (4) + 40 Availa-Zn [Availa-Zn provided 40 ppm Zn on top of ZnSO₄]; (5) ISO 40 Availa-Mn [Availa-Mn manganese amino acid complex replaced 40 ppm Mn from MnSO₄]; (6) ISO 80 Availa-Mn [Availa-Mn replaced 80 ppm Mn from MnSO₄], and (7) + 40 Availa-Mn [Availa-Mn provided 40 ppm Mn on top of MnSO₄]. Broilers from the ISO 40 Availa-Zn treatment had numerically heavier 56-day body (3.84 vs. 3.74 kg) and breast meat (0.69 vs. 0.68 kg) weights while those from the + 40 Availa-Zn treatment showed significantly ($P < 0.05$) heavier 56-day body (3.94 vs. 3.74 kg) and breast meat (0.72 vs. 0.68 kg) weights when compared to the Sulfate control. All manganese treatments showed numerically heavier 56-day body (3.79, 3.75, 3.83 vs. 3.74 kg) and breast meat (0.69, 0.69, 0.69 vs. 0.67 kg) weights when compared to the Sulfate control. Intestinal strength was numerically higher for birds from the + 40 Availa-Zn treatment when compared to the Sulfate control (0.046 vs. 0.036 kg). Broilers fed plant-based diets can benefit from Availa-Zn and/or Availa-Mn supplementation.

Key Words: zinc, manganese, broilers

T9 Effect of sodium chloride (NaCl) in drinking water on performance and eggshell quality in Hy-Line laying hens. H. Collazos*, L. Barrantes, and J. Barbudo, *Universidad Nacional Abierta y a Distancia-UNAD, Bogota, Cundinamarca, Colombia.*

An 8 week trial was designed to determine the influence of sodium chloride drinking water supplementation on Hy-Line laying hens performance, egg yields and eggshell quality. Two hundred 40 week-old laying hens were allotted one of four treatments (controls, 0.4, 0.8 and 1.2 g of NaCl/L in drinking water) in a completely randomized design with five replicates and 10 birds per replicate. Birds were reared in bird cages. Diets were formulated to meet or exceed NRC recommendations. Water and feed were provided for ad libitum. Feed consumption was measured weekly while feed efficiency was calculated. Egg production, egg weight, and egg mass were recorded daily from 40 to 48 weeks of age. Random samples of 8 eggs from each treatment were collected weekly to measure egg yields, haugh units and eggshell quality such as egg specific gravity, eggshell thickness, eggshell breaking strength. Production parameters such as feed intake, egg production, egg weight, feed efficiency, body weight variation, and egg mass were not affected ($P > 0.05$). eggshell parameters: Eggshell breaking strength, shell weight, eggshell thickness, and yolk percentage

were not affected ($P > 0.05$). Haugh units and specific gravity were affected by treatments ($P < 0.01$). The results indicated that the Hy-Line hens were relatively insensitive to intakes of NaCl from drinking water at concentrations of 1.2 g/L.

Key Words: drinking water, eggshell quality, sodium chloride

T10 Influence of source and particle size of fibrous ingredients on performance of broilers. E. Jiménez-Moreno, J. M. González-Alvarado, D. González-Sánchez, R. Lázaro, and G. G. Mateos*, *Universidad Politécnica de Madrid, Spain.*

We evaluated the inclusion of fiber in the diet on productive performance of Cobb 500 broilers fed low-fiber diets from 1 to 21 d of age. There were six treatments and five replicates (a cage with 14 chicks) per treatment. The control diet was based on rice, fish meal, soy protein concentrate, yellow grease, and 3% of sepiolite and contained 3,095 kcal ME/kg, 1.31% total lysine, and 1.53% crude fiber. Three additional diets were formulated to be similar to the control diet but the sepiolite was substituted (wt/wt) by either oat hulls (OH), sugar beet pulp (SBP), or microcrystalline cellulose (CEL). There were two extra diets that were identical to the OH- or SBP- containing diets but in which the fiber source was ground to pass through a 0.5-mm screen instead of a 2.0-mm screen. Particle size of the fiber source did not affect any of the productive traits studied and no interactions of fiber source × particle size were observed. From 1 to 21 d of age, the inclusion of additional fiber improved average daily gain (ADG) (31.4 vs. 29.3 g; $P < 0.05$) and feed to gain ratio (F:G) (1.299 vs. 1.362 g/g; $P < 0.01$). Broilers fed OH had better ADG than broilers fed SBP with broilers fed CEL showing an intermediate growth rate ($P < 0.01$). The inclusion of OH or CEL in the diet increased feed intake with respect to the inclusion of SBP ($P < 0.05$). Also, broilers fed OH had better F:G than broilers fed CEL with broilers fed SBP showing an intermediate value ($P < 0.01$). From 15 to 21 d of age, the inclusion of SBP in the diet tended to impair ADG ($P = 0.06$) and F:G ($P = 0.06$) with respect to the inclusion of OH. We hypothesize that fermentable fiber sources such as SBP might reduce feed intake and impair broiler performance late in the productive cycle but that insoluble fiber sources such as OH might be beneficial, especially during the first stages of life. Therefore, young broilers might have a requirement for a minimal amount of insoluble fiber in the diet.

Key Words: inclusion of fiber, particle size, broiler

T11 Influence of β-mannanases on the metabolizable energy and performance of broilers fed nutritionally marginal corn-soy diets. H. Schulze*¹, V. Ravindran², and P. J. Moughan³, ¹Danisco Animal Nutrition, Leiden, The Netherlands, ²Institute of Food, Nutrition and Human Health, Palmerston North, New Zealand, ³Riddet Centre, Massey University, Palmerston North, New Zealand.

β-mannan is a non-starch polysaccharide found in soybean meal and may lower nutrient utilization in corn-soy diets fed to broiler chickens. The present study was conducted to evaluate the effects of 3 mannanase (MAN) products on the performance, N-corrected apparent metabolizable energy (AME_n) and excreta scores of broilers fed corn-soy diets that were marginal in metabolizable energy and amino acids. Each of the 3 MAN products was tested at 2 inclusion levels (MAN1, 100 and 1000 mg/kg; MAN2, 100 and 1000 mg/kg; MAN3, 20 and 1000 mg/kg) and compared with the unsupplemented

diet. Each of the 7 dietary treatments was randomly assigned to 12 individually-caged birds and fed from d 7 to 28. Total excreta collection was carried out from d 24 to 27 for the determination of AME_n, and excreta were scored daily during these 4 days for stickiness and wateriness on a scale of 1 to 5. MAN products had no effects ($P > 0.05$) on weight gain and feed intake, but significantly ($P < 0.05$) influenced feed/gain of broilers. Both inclusion levels of MAN1 and MAN2 caused improvements in feed/gain over the unsupplemented control, but the differences were significant ($P < 0.05$) only with MAN1 at 1000 mg/kg and MAN2 at 100 mg/kg. All 3 MAN products, irrespective of the inclusion level, increased ($P < 0.05$) the AME_n values. Excreta scores of broilers tended ($P < 0.10$) to be lowered by supplemental mannanases. The present results suggest that β -mannanase supplementation may improve performance, energy utilization and manure quality of broilers fed corn-soy diets.

Key Words: β -mannanase, metabolizable energy, broilers

T12 The effect of vitamins supplementation on broiler breeder performance, hatchability, and methionine metabolism in 18 day-old chick embryos. J. W. Lu* and C. N. Coon, *University of Arkansas, Fayetteville.*

Thirty four week-old Cobb 500 females were fed 8 different diets for a 12 week period: basal (positive control), basal - B₆, basal - riboflavin, basal - B₁₂, basal - folic acid, basal - choline, basal - vitamin A; and basal without each of the test vitamins. The hatching eggs were collected after weekly AI with 50 million sperms. The isotopic tracers, [2,3,3-²H₃]-L-serine, [²H₁₁]-L-betaine and [1-¹³C]-L-methionine were administered as a single bolus dose by amnion routine injection into 18 day-old chick embryos from breeders after 12 weeks feeding with positive control, missing folic acid or B₆-missing diets. Six chick embryos were killed at time 0, 30, 60, 120 min after injection, respectively. Hepatic amino acids concentrations and enrichments were investigated by using HPLC and HPLC/MS methods, respectively. The lack of B₆, folic acid, B₁₂, or riboflavin supplementation showed a significantly negative affect on hatchability (about 10% lower) within 1-4 weeks feeding prior to decreasing egg production about 10% in 5-8 weeks. Each of the test vitamins significantly increased egg weight with 1.5-2.0 gram, which was consistent with the previous observation that all nutrients tightly involved in methionine metabolism play a critical role in egg weight. About two fold elevation of hepatic homocysteine in chick embryos from breeders fed diets without added folic acid or B₆ indicated an impaired methionine metabolism. The peak area of M+0, M+1 and M+2 forms of free methionine significantly increased with time after isotopes were injected suggesting the chick embryo can absorb and utilize methionine provided by amnion routine injection. [²H₃]-methionine was not detectable suggesting remethylation via MHMT is the primary pathway of the two remethylation reactions. The findings in the present study may indicate that methionine metabolism has a critical role in breeder performance and thereafter chick embryonic development. Supplying enough vitamins in broiler breeder diets is necessary for optimal breeder performance, hatchability, and chick quality.

Key Words: vitamins, broiler breeder, isotopes, methionine metabolism

T13 Effects of method of whole wheat feeding on the performance and gizzard development of broiler chickens. A. Amerah, V. Ravindran*, R. G. Lentle, and D. G. Thomas, *Massey University, Palmerston North, New Zealand.*

Whole grains can be offered to poultry in either a mix feeding (MF) system where the whole grain is added substituting part of the ground grain in a complete, pelleted diet or a free choice feeding (FCF) system where the birds have the choice to select the whole grain or protein concentrate offered in separate feeders. The present study was conducted to investigate the effects of feeding whole-wheat through MF or FCF on the performance and gizzard development in broilers. The following 3 treatments were employed: GW, ground wheat diet with 60-69% wheat; MF, GW diet with 49-50% wheat and 10-20% whole wheat; and FCF, whole wheat and protein concentrate offered in separate feeders. The GW diets and the protein concentrate were offered as pellets. Each diet was fed to six pens of 36 birds each from day 7 to 35. On d 35, 24 birds from each treatment were killed to obtain gizzard weights. No differences ($P > 0.05$) were observed in the weight gain, feed intake and feed/gain of broilers on the GW and MF treatments. Birds on the FCF treatment had the lowest ($P < 0.05$) weight gain and feed intake, and the highest ($P < 0.05$) feed/gain. During week 1, the protein concentrate was consumed more than the whole-wheat (69 vs. 31%), which led to the amount of concentrate offered being restricted during subsequent weeks. Over the trial period, the average consumption of protein concentrate remained high (56% of the total intake). Both whole wheat treatments increased ($P < 0.05$) the relative gizzard weights. Factors that affect diet selection such as learning and previous experience, visual differences between the foods, texture and, flavor of the food and palatability may explain the lower whole-wheat intake in the FCF treatment. In this study, whole wheat was introduced only on d 7 and it is possible that the birds may have to be trained to experience choice feeding from the first week of life. However, the present results appear to suggest that FCF may not be an appropriate feeding system for fast-growing modern broilers.

Key Words: whole wheat, free choice feeding, broilers

T14 Effect of an evolved thermo-tolerant phytase on performance and bone ash in broiler chickens. R. Angel*¹ and C. Wyatt², ¹*University of Maryland, College Park,* ²*Syngenta Animal Nutrition, Research Triangle Park, North Carolina.*

Two trials were conducted to determine the effect of different levels of an evolved thermo-tolerant phytase (Q, Quantum Phytase) on performance, nutrient retention and bone mineralization, using male Ross 308 broiler chickens. Trial 1 consisted of a 10-d battery pen trial (1 to 10 d of age) using four pens/treatment (8 b/pen) to determine the impact of adding Q on tibia mineralization, Ca and P absorption and retention from diets formulated to be at NRC (1994) non-phytin P (nPP) recommendations or below. Tibia ash wt (dry-defatted basis) in broilers fed the control (C) diet (0.45%) was similar ($P > 0.05$) to that of broilers fed diets containing 250 and 2500 U Q/kg (was 1.59, 1.80 and 1.84 g respectively). Broilers fed the diet containing 2500 U Q/kg had higher ($P < 0.05$) ash weight than those fed the diet containing the same level of nPP (0.45%) but no added phytase. Tibia ash wt increased from 1.34 to 1.66g when birds fed a low nPP diet (0.33%) were offered Q at 250 U/kg ($P < 0.05$), with this enzyme treatment returning tibia ash wt to that of the C diet (0.45% nPP). The equivalency as calculated against P from monocalcium phosphate, of 250 U Q/kg

was 0.127 % nPP. Trial 2 (H to 45 d) was done to determine pelleting survivability, performance and bone ash, of two phytases [Q and R (Ronozyme (P) CT®)] in a floor pen trial (8 pens/Trt, 59 b/pen). An industry level of nPP was used in the control diets (C). This level was reduced by 0.12% nPP with no added phytase (NC) or with 250, 500, 1000 and 2500U Q/kg or 250 and 500 U R/kg in pelleted (80 to 85C) diets. Over the 45 d of the trial, broilers fed the Q1000 diets had similar gain as those fed the C diets (2.89 and 2.88 kg) and had the same feed conversion (1.75). Broilers fed the R500 diets performed similarly to those fed the Q250 diets but not as well ($P < 0.05$) as those fed the Q500 diets. Femur ash wt was similar in birds fed the Q250 and R500 diets and higher ($P < 0.01$) for those fed the Q500, Q1000, and C diets. Average pelleting survivability was 62.5 and 24.9% for Q and R, respectively. This study demonstrates there are differences in phytase sources and response in the bird.

Key Words: phytase, pelleting, broiler chickens

T15 Long term feeding of conjugated linoleic acid and fish oil to laying hens: Effects on egg quality, production performance, tissue fatty acids and hen liver pathology. D. Gonzalez*, K. S. Ryu, M. P. Goeger, and G. Cherian, *Oregon State University, Corvallis.*

The long term feeding of conjugated linoleic acid (CLA) and fish oil on egg quality characteristics, production performance, liver pathology, plasma prostaglandins, tissue and egg fatty acid content of laying hens is investigated. Single comb white leghorn hens ($n=112$; 22 weeks old) were kept in cages and randomly assigned to four diets (28 hens/diet, 4 replicates of 7 hens) containing 3.0% yellow grease (control), 2.75% yellow grease + 0.25% CLA (CLA-YG), 2.5% yellow grease + 0.25% CLA + 0.25% fish oil (CLA-FO), and 2.75% yellow grease + 0.25% fish oil (FO-YG). The CLA preparation consisted of cis-9, trans-11 and trans-10, cis-12 fatty acid isomers as free fatty acids in a ratio of 1:1. The diets were fed for eleven months. Eggs were collected every 28 days for 11 months. Feed consumption, hen day egg production and feed efficiency was monitored. At the end of the trial, blood, cardiac, hepatic and adipose tissues were collected. No effect of diet was found on feed consumption, hen day egg production, feed efficiency, egg weight, yolk weight, shell weight or haugh unit. The CLA-YG and CLA-FO diets produced an increase in CLA and saturated fatty acids in the adipose, heart and liver tissues and egg yolk with a concomitant reduction in monounsaturated fatty acids ($P < 0.05$). Feeding CLA-FO and FO-YG increased the omega-3 fatty acids in egg yolk, liver and heart of hens. Incorporation of CLA was highest in CLA-FO eggs (4.2 mg per g fat or 21 mg per egg) ($P < 0.05$), which is higher than that in ruminant-derived foods (3 mg CLA per g fat). No difference was observed in the number of fat vacuoles or total fat content of hepatic tissue in hens. Plasma prostaglandin E2 was lowest in FO-YG-fed hens ($P < 0.05$). As the hens aged, egg weight and yolk weight increased significantly in all the diets ($P < 0.05$). No difference was noticed in shell weight due to age. The current study demonstrates that "healthy" eggs with increased omega-3 fatty acids and CLA can be produced by minor diet modifications without affecting the production performance or health of birds.

Key Words: egg, conjugated linoleic acid, Omega-3 fatty acid

T16 Effect of different levels of pigment and sources of fat with or without enzyme on egg yolk color and performance of laying hens. F. Zaefarian*, M. Shivazad, and M. Abdollahi, *Tehran University, Karaj, Iran.*

An experiment was conducted to evaluate the effects of different levels of pigment in diets which contained three sources of fat, with or without enzyme on egg yolk color and laying hens performance. All of the diets were isocaloric and isonitrogenous. The experiment was arranged in a completely randomized design (CRD) with a $3 \times 2 \times 3$ factorial trt arrangement. The experiment consisted of 18 treatments with three replicates containing 6 Hy-line W36 laying hens from 23 to 31 weeks of age. The treatments consisted of three basic diets contain sunflower oil, tallow or fatty acid with or without enzyme and three levels of pigments (0, 1100 ppm red pigment with 125 ppm yellow pigment, 1500 ppm red pigment with 225 ppm yellow pigment). The evaluation of different traits were measured daily or periodically. The main effect of pigment levels, enzyme and sources of fat and also their interaction on egg production, egg weight and feed conversion were not significant. Main effect of pigment, fat sources and enzyme for these traits were also not significant ($P > 0.05$). The main effect of pigment level, enzyme and fat sources and also their interaction on blood cholesterol were not significant ($P > 0.05$), but the differences between averages of the three fat sources was significant ($P < 0.05$). Tallow with the average of 188.78 mg/dl had the highest and fatty acid with the average of 146.46 mg/dl had the lowest effect on blood cholesterol. The main effect of pigment and enzyme and also their interaction on egg yolk cholesterol was not significant ($P > 0.05$), but main effect of fat on yolk cholesterol was significant ($P < 0.05$). The effect of sunflower oil on yolk cholesterol was the highest and tallow was the lowest. Analysis of variance for main effect of pigment showed significant effect on yolk color in the first week ($P < 0.05$). The significant effect of pigment continue until the end of experimental period. Main effect of enzyme on yolk colour was not significant ($P > 0.05$), but the main effect of fat on yolk colour was significant ($P < 0.05$).

Key Words: laying hen, pigment, yolk colour

T17 Effect of xylanase or xylanase, amylase and protease in combination with phytase on the nutritional value of a corn/soy-based diet for growing broiler chickens. A. J. Cowieson*¹, N. K. Sakomura², N. A. A. Barbosa², and M. Hruby¹, ¹*Danisco Animal Nutrition, Marlborough, Wiltshire, United Kingdom*, ²*Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, São Paulo, Brazil.*

The aim of this work was to study the effect of a single xylanase or a combination of amylase, protease and xylanase with phytase in corn-soybean diets formulated with or without nutrient reduction on the nutrient ileal digestibility of broiler chicks. A total of 2400 day-old male broiler chicks were allocated to 6 treatments with 10 replicates of 40 chicks each. The experimental design was completely randomized arranged in a 2×3 factorial. The six diets consisted of two controls (Positive (PC)- without nutrient reductions and Negative (NC)- with nutrient reductions), each fed with or without one of two supplemental enzyme treatments. The combination of enzymes (Danisco Animal Nutrition) evaluated were: Phyzyme XP (phytase; Phy) with Avizyme 1505® (xylanase, amylase, protease; XAP) and Phyzyme XP with Avizyme 1310® (xylanase; X). Ileal digesta were collected at 42 days of age to determine digestible energy (ADE) and digestibility

coefficients of protein (CPD), dry matter (DMD), calcium (CaD) and phosphorus (PD). There was no effect ($P > 0.05$) of enzymes or diet (PC vs. NC) for DMD. The XAP+Phy improved CPD (78.18%) compared to controls (76.03%) but the X+Phy did not elicit a response (77.06%). As expected, the ADE of the PC was higher ($P < 0.01$) than that of the NC. However, XAP+Phy or X+Phy did not improve ($P > 0.05$) the ADE of the NC but improved ($P < 0.05$) the ADE of the PC by 3.5% and 2% respectively resulting in a significant diet*enzyme interaction. There was no difference between PC and NC for Ca retention but a lower ($P < 0.05$) P retention in the NC compared with the PC. The X+Phy improved ($P < 0.05$) Ca and P retention in the NC but had no effect in the PC. The XAP+Phy improved ($P < 0.05$) P retention in the PC and NC but had no effect on Ca retention in either control diet. It can be concluded that there are differences in bioefficacy between X and XAP in corn/soy-based diets and the way that the two enzyme systems interact with phytase.

Key Words: enzymes, combination, digestibility

T18 Effect of xylanase or xylanase, amylase and protease in combination with phytase on corn/soy-based diet for growing broiler chickens. N. K. Sakomura*¹, N. A. A. Barbosa¹, A. J. Cowieson², and M. Hruby², ¹Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, São Paulo, Brazil, ²Danisco Animal Nutrition, Marlborough, Wiltshire, United Kingdom.

The aim of this study was to establish the effect of a single xylanase or a combination of amylase, protease and xylanase with phytase in corn-soybean diets formulated with or without reduction in energy, Ca and P concentration on the performance of broiler chicks. A total of 2400 day-old male broiler chicks (Cobb) were allocated to 6 treatments with 10 replicates of 40 chicks each. The experiment was conducted as a completely randomized design arranged in a 2x3 factorial. The six experimental diets consisted of two controls (Positive (PC)- without nutrient reductions and Negative (NC)- with nutrient reductions), each fed with or without one of two supplemental enzyme treatments. The combination of enzymes (Danisco Animal Nutrition) evaluated were: Phyzyme XP (phytase; Phy) with Avizyme 1505® (xylanase, amylase, protease; XAP) and Phyzyme XP with Avizyme 1310® (xylanase; X). The chicks fed NC showed lower ($P < 0.01$) feed intake, body weight and higher feed conversion than the PC. The addition of either of the two combinations of enzymes to the NC improved ($P < 0.01$) feed intake, body weight and feed conversion such that the birds fed on the NC with supplemental enzymes had a performance that was not statistically different from those fed on the PC. Supplementation of the PC with either enzyme did not elicit a significant response in terms of performance. It can be concluded that there are differences in bioefficacy between X and XAP in corn/soy-based diets and the way that the two enzyme systems interact with phytase. These results are likely to also depend on corn quality and it may be that, for some corns, X+Phy is sufficient to improve performance whereas for other corns XAP+Phy would yield a more consistent and larger effect. This may be related to rate and degree of starch degradation and protein quality of both corn and soybean meal.

Key Words: enzymes, combination, performance

T19 Effect of fructooligosaccharides and inulin on intestinal bacteria and pathogens in cecal contents from broiler chickens.

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The colon of humans and animals harbors a complex microbial ecosystem that is essential for the well being of its host. Prebiotics, such as the blend of fructooligosaccharides plus inulin (FOS-I), are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species in the colon that can improve the hosts health. The aim of this work was to evaluate the effects of dietary administration of FOS-I on cecal microbial populations in broiler chickens. Broiler chickens were kept under standard commercial conditions in a poultry farm in Veracruz, Mexico. Flocks (15,000 birds) were randomly assigned to experimental (conventional diet supplemented with FOS-I) and control (without FOS-I) groups. After 6 wks, 10 birds in each group were sacrificed, cecal contents were collected and cultured, and selected microbial populations were enumerated by standard microbiological methods: *Lactobacillus* spp, *Bifidobacterium* spp, *Escherichia coli* and *Campylobacter* spp.

Cecal bifidobacteria concentrations increased ($p=0.007$) in FOS-I fed birds compared with the control group. The experimental diet had no effect on concentrations of *E. coli* and *Lactobacillus* spp. Although not statistically significant, cecal *Campylobacter* populations (4.64 vs 3.19 log₁₀ ufc/g, $p=0.08$) decreased 1.5-fold in prebiotics-treated birds. Addition of FOS-I to a commercial diet increased the density of bifidobacterial populations and may have the potential to improve the health and performance of broiler chickens under commercial conditions. Our data provide further evidence that FOS-I can stimulate the increase in bifidobacterial populations in birds grown under the stress of commercial conditions. Additionally, results suggest that FOS-I may be contributing to reduce shedding of *Campylobacter* spp, which is important to prevent transmission to humans and thus, decrease the risks to public health.

Key Words: bifidobacteria, campylobacter, prebiotics

T20 Effects of lipopolysaccharide dosage and injection frequency on broiler performance and organ weights. P. Sirimongkolkasem* and K. C. Klasing, University of California, Davis.

Two experiments were conducted to investigate the effect of *Salmonella typhimurium* lipopolysaccharide (LPS) dosage and injection frequency on male broiler performance and organ weights. In both experiments birds were fed diets formulated to contain 120% of NRC-recommended nutrient levels and were injected with LPS s.q. at 8 d of age (d0 of expt). Experiment 1 had a 3x2 factorial design with the main effects being LPS dose (1, 4 and 7 mg LPS/kg BW) and number of LPS injections (once on d0 or twice on d0 and d1). In experiment 2, a single LPS injection with 3 doses (0.1, 0.5 and 4 mg LPS/kg BW) was used to determine the effect of lower doses. Uninjected birds served as a negative control in both experiments. There was a significant decrease in average daily gain, feed intake and feed efficiency 24hrs after the first LPS injection in both experiments. The decreased performance was dose dependent, with the highest dose giving the largest depression. In experiment 1, a significant decrease in average daily gain and feed intake due to LPS persisted until d5, however, the dose dependent effect of LPS was not significant after d1. In experiment 2, the decrease

in gain and feed intake due to LPS injection was only observable for the first 24hrs after injection. In both experiments, there was no difference in feed efficiency between LPS and control groups after d1. The second LPS injection did not increase the impact of the first injection on any parameters measured. 24hrs after the first LPS injection an increase in the relative liver weight was evident in all treatment groups, whereas a decrease in the relative semitendinosus weight occurred only in those injected with 4 mg LPS. Neither the LPS dose nor the number of injections significantly affected relative organ weights. These results indicate that increasing LPS injection frequency does not prolong or increase its impact on growth performance while a high single dose (greater than 4 mg/kg BW) can prolong the depression in feed intake and average daily gain. Additionally, the dose dependent effect of LPS can only be observed for 24 hrs.

Key Words: LPS, broiler, dose

T21 Comparative thermostability of phytase products in pelleted feeds. N. E. Ward*¹, D. Campbell¹, and A. Korsbak², ¹DSM Nutritional Products Inc., Parsippany, New Jersey, ²DSM Nutritional Products Inc., Oerbaek, Denmark.

The stability of commercial phytase products is important to manufacturers of pelleted feeds. Different techniques are used to stabilize phytase products against the heat and moisture of feed milling, and not all are equally effective. Thus, two experiments at different locations determined the stability of phytase products when conditioned and pelleted in commercial type feeds. In Experiment 1 (Danish Tech. Inst., Kolding Denmark), four phytase products (Ronozyme P (CT), Phytase N, Phytase P, and Phytase Q) were pelleted in replicates at 75, 85, 90 and 95°C in wheat-based diets. Non-supplemented mash and pellets at each temperature were taken to adjust for wheat phytase. In Experiment 2 (Kansas State Univ., Manhattan KS), these phytase products were pelleted at 70, 80 and 90°C in corn/soybean meal diets. Conditioning was for 30 seconds in both experiments. The results for Experiment 1 are noted below. At the highest temperature (95°C), the 89% retention of Ronozyme P (CT) was superior ($P < 0.05$) to all other phytases, followed by 62% for Phytase N, 5% for Phytase P and 9% for Phytase Q. The superior survivability ($P < 0.05$) of Ronozyme P (CT) was consistent across all temperatures. Phytase N retention was greater ($P < 0.05$) than for Phytase P and Phytase Q, while the latter two did not differ ($P > 0.05$). Experiment 2 mirrored the results of Experiment 1, but with lower retentions across all phytase products. At 90°C, the retention for Ronozyme P (CT) was highest (75%), followed by Phytase N (34%), Phytase Q (8%) and Phytase P (5%). As pelleting temperature increased, the decrease in phytase survivability was precipitous for Phytases N, P, and Q, as opposed to Ronozyme P (CT). The thermo-stability of phytase products differed substantially. The decline in stability with increased pelleting temperatures was gradual for Ronozyme P, and hence, is less likely to be affected as pelleting temperatures fluctuate throughout the day or across seasons in commercial feed mills. Further, these data confirm previous work that the stability of phytase products may not be the same across different pelleting systems due to inherent characteristics of these systems.

Table 1. Pelleting Retention (%) of Phytase Products

Phytase Product	75°C	85°C	90°C	95°C
Ronozyme P (CT)	96	90	85	89
Phytase N	82	70	65	62
Phytase P	82	42	15	5
Phytase Q	89	54	27	9

Retention determined by comparing phytase level at each temperature with level in mash feed. 12 samples analyzed per temperature-phytase. $P < 0.05$

Key Words: phytase, pelleting, stability

T22 Production performance of Pearl Grey guinea fowl pullets fed diets with varying concentrations of dietary metabolizable energy and crude protein. S. N. Nahashon*, N. Adefope, A. Amenyenu, and D. Wright, *Institute of Agricultural and Environmental Research, Tennessee State University, Nashville.*

This study was undertaken to assess dietary metabolizable energy (ME) and crude protein (CP) concentrations for optimum production performance of Pearl Gray guinea fowl replacement pullets. In a 3 x 3 factorial arrangement, 540 1-day old Pearl Gray guinea keets were randomly assigned to experimental diets with 2,900, 3,000 and 3,100 kcal of ME/kg of diet; each contained 20, 22 and 24% CP, respectively, from 0-8 wk of age (WOA). From 9-16 WOA, experimental diets had 3,000, 3,100 and 3,200 kcal of ME/kg of diet, and each contained 17, 19 and 21% CP, respectively. At 17-22, 23-27 and 28-56 WOA all experimental birds were fed the same diet at each age period and the diets contained 3,000, 2,900 and 2,800 kcal of ME/kg of diet, respectively. These diets had 18, 17 and 16% CP, respectively. Each dietary treatment was replicated 4 times, and feed and water were provided ad libitum. Body weights were measured weekly from hatch to 22 WOA and at 28-56 WOA the experimental birds were observed for feed consumption (FC), hen-day egg production (HDEP), egg weight (EW), egg mass (EM), feed conversion ratio (FCR), internal egg quality (IEQ), shell thickness (ST) and body weight (BW) at the end of each 28-day lay period for 7 consecutive periods. Mortality was recorded as it occurred. Overall, BW gains were significantly higher ($P < 0.05$) in birds fed 3,000 and 3,100 Kcal of ME/kg of diet and 24% CP from 0-8 WOA than other dietary treatments. Percent HDEP, EM and IEQ were higher ($P < 0.05$) and FCR was lower ($P < 0.05$) in pullets fed diets containing 3,000 and 3,100 kcal of ME/kg of diet than those on 2,900 kcal of ME/kg of diet at 0-8 WOA. Birds on 22 and 24% CP diets also exhibited higher HDEP, EM and lower FCR than those on 20% CP diets. However, differences in mean EW, BW and ST were not significant ($P > 0.05$) among dietary ME and CP concentrations. Thus, feeding 3,000-3,100 Kcal of ME/kg of diet and 22-24% CP at 0-8 WOA; and 3,100-3,200 Kcal of ME/kg of diet and 19-21% CP at 9-16 WOA improved HDEP, EM, IEQ and FCR of Pearl Grey guinea fowl laying pullets at 28-56 WOA.

Key Words: pearl gray guinea fowl pullets, metabolizable energy, crude protein

T23 Keratinase supplementation in the soybean and cottonseed meal containing diet improves growth performance and nutrient digestibility of broiler chickens. H. Y. Wang¹, Y. M. Guo^{*1}, and J. C. H. Shih², ¹China Agricultural University, Beijing, China, ²North Carolina State University, Raleigh.

Effects of bacterial keratinase (KE) on growth performance and nutrient digestibility were studied in broiler chicks fed diets with soybean meal (SBM) and cottonseed meal (CSM) as the main protein sources in a 2×2 factorially arranged experiment. Four different diets were formulated: soybean meal diet (S), S plus KE (SK), cottonseed-soybean meal diet (C) in which 50% of SBM was replaced by CSM, and C plus KE (CK). The crude preparation of KE, or VersazymeTM, was added at 400,000 EU or 1 g/kg feed. The supplementation of KE birds significantly improved body weight (BW) at age 21 d by 8% (S vs. SK) and 10% (C vs. CK) ($P < 0.05$). During the whole growing period, the presence of KE always significantly reduced feed conversion rates (FCR) ($P < 0.01$). KE also helped reduced mortalities of the birds: 4.79% (S) vs. 4.11% (SK) and 5.29% (C) vs. 0.98% (CK). As expected, protein digestibility was significantly improved by KE in diets ($P < 0.01$). They were 58% (S) vs. 69% (SK) and 54% (C) vs. 64% (CK). To explore the possible mechanism of the beneficial effect of KE, the digestive enzymes and intestinal morphology were studied. The activities of amylase and trypsin in broilers fed SBM or CSM diet tend to be improved by KE in different growth periods. The 2X increase of chymotrypsin activities by KE supplement in both kinds of diets and both ages is interesting. Microscopic examinations of the small intestine including the sections of duodenum, jejunum and ileum were conducted. It is of great interest that KE supplementation appeared to stimulate the growth of villi in the lumen at age 21 d, but not in older birds at 42 d. In conclusion, dietary supplementation of KE has beneficial effects on growth performance and nutrient digestibility in broilers. The positive effects on intestinal digestive enzymes and intestinal development warrant further studies. In the presence of KE, the replacement of 50% SBM with CSM caused no negative effect on broilers. It is possible that KE can elevate the nutritional value of some less digestible protein source, in this case, CSM.

Key Words: keratinase, cottonseed meal, broiler chicks

T24 Developmental gene expression of nutrient transporters in the small intestine of chickens divergently selected for high or low juvenile body weight. C. R. Miller^{*}, P. B. Siegel, K. E. Webb, Jr., and E. A. Wong, *Virginia Tech, Blacksburg.*

The objective of this study was to evaluate the developmental gene expression of nutrient transporters in the small intestine of chicken lines that had undergone long term selection for high (HH) or low (LL) 8-week body weight and the reciprocal crosses (HL and LH). Nutrient transporters investigated were the peptide transporter PepT1, amino acid transporter EAAT3, and monosaccharide transporters GLUT5 and SGLT1. Chicks were reared in batteries with ad libitum access to feed and water. Chicks were weighed and killed on embryonic day 20 (e20), day of hatch (DOH with no access to feed), and days 3, 7, and 14 post hatch. Duodenum, jejunum, ileum and liver were collected. DNA extracted from liver was used to sex birds by PCR. RNA was extracted from the intestinal segments of four males from each line and time point except e20 HL (n=3). Expression of nutrient transporters was assayed by real time PCR using the relative quantification method. All genes were induced on DOH relative to e20 in all lines. A line difference in PepT1 was seen with populations with L sires. Lines

with L sires (LL and LH) had greater PepT1 RNA expression than lines with H sires (HH and HL) ($P < 0.01$). Expression of PepT1 RNA ($P < 0.01$) and EAAT3 RNA ($P < 0.01$) were greatest in the ileum. Expression of SGLT1 RNA ($P < 0.01$) and GLUT5 RNA ($P < 0.01$) were greatest in the distal intestine. These results show that long term selection for high or low body weight in chickens has had little impact on developmental gene expression of EAAT3, GLUT5, and SGLT1 between the selected lines and the reciprocal crosses. In contrast, developmental gene expression of PepT1 was impacted.

Key Words: chicken, intestine, nutrient transporters

T25 The effects of dietary copper source and concentration on chick growth, tissue copper concentrations, mucosal copper and iron mineral transporters and copper excretion. J. H. Skaggs^{*1}, M. E. Persia¹, B. D. Humphrey², and W. W. Saylor¹, ¹University of Delaware, Newark, ²University of Maryland, College Park.

An experiment was conducted to determine the effects of Cu source and concentration in corn-soybean meal diets fed to male broiler chicks from 7-21d. The seven dietary treatments included: basal diet (no added Cu, B); B + 25 mg/kg inorganic (IN) Cu from CuSO₄•5H₂O (25IN); B + 25 mg/kg organic (OR) Cu from BioplexTM Cu 10% (25OR); B + 100 mg/kg IN Cu (100IN); B + 100 mg/kg OR Cu (100OR); B + 250 mg/kg IN Cu (250IN); and B + 250 mg/kg OR Cu (250OR). Male Ross 708 chicks were weighed, wing banded and assigned to one of the seven diets. Chicks were housed 4 birds per pen with 8 replicate pens for the B, 25IN and 25OR diets and 6 replicate pens for remaining diets resulting in 192 total birds in 48 pens. Performance data were collected over the 7 to 21 d period. Excreta were collected from 15 to 21 d. On d 21, chicks were bled via heart puncture before being euthanized for collection of liver and duodenal mucosa. Cu concentration was determined in plasma, liver, mucosa and excreta samples. Mineral transporter mRNA abundance was quantified in mucosal samples. Body weight gain and feed intake of birds fed the 250OR diet were 14% lower ($P < .0001$) than that of birds fed the basal diet. Birds fed 250IN and 250OR diets had 61% and 92% higher ($P < .0001$) liver Cu concentrations, respectively, than those fed the basal diet. Total excreta Cu increased ($P < .0001$) with increasing dietary Cu. The OR diets resulted in a 21, 37, and 28% increase in Cu excretion for dietary inclusions of 25, 100, and 250 mg Cu/kg, respectively. Dietary Cu treatment had no significant effect on mRNA expression of mucosal transporters CTR-1, and DMT-1, and ATP7A. In this study, feeding 250 mg Cu/kg in the OR form resulted in growth depression that was not observed when the IN form was fed, and that could not be fully accounted for by reduced feed intake.

Key Words: broiler, copper, organic

T26 The effect of feeding quinoa on the Omega-3 PUFA contents of chicken eggs. N. P. Johnston^{*1}, G. Aduviri², A. Parker¹, R. Hall¹, B. T. Slauch³, and R. T. Davidson¹, ¹Brigham Young University, Provo, Utah, ²University of San Andres, La Paz, Bolivia, ³Eggland's Best, King of Prussia, Pennsylvania.

In recent years many findings have been published denoting positive health benefits to humans from the greater inclusion of omega-3 PUFAs in the diet. Since quinoa is a pseudo grain whose lipid fraction is reasonably high in alpha linolenic acid (ALA) (8.3%), an omega-3 PUFA (18:3 n-3), it was hypothesized that its inclusion in the diet of

laying chickens would alter the omega-3 fatty acids levels of table eggs. To test the hypothesis fifteen Single Comb White Leghorn laying chickens were equally divided into three treatments according to the level of quinoa in the grain fraction of the diet (0%, 50%, 100%). Feeding commenced at 20 wks of age and continued for 16 weeks. At periodic intervals nine eggs were collected from each treatment and divided into three pooled replicate groups of three eggs each and analyzed for their omega-3 contents by gas chromatography. Replacing 50% of the corn with quinoa resulted in a significant ($P < 0.05$) increase in the omega-3 content of eggs from 47.9 to 82.1 mg/egg. Replacing all of the corn with quinoa resulted in an additional increase ($P < 0.05$) to 95.9 mg/egg. The respective levels of individual omega-3 PUFAs (ALA 18:3 n-3, EPA 22:5 n-3, DHA 22:6 n-3) were 0% quinoa - 12, 6, 26 mg/egg, 50% quinoa - 28, 6, 44 mg/egg and 100% quinoa - 38, 7, 47 mg/egg. Adding quinoa at both the 50% and 100% level resulted in progressive increase ($P < 0.05$) in 18:3 ALA (12, 28, 38 mg), and an increase ($P < 0.05$) in DHA 22:6 (26, 44, 47 mg) at 50% quinoa but no additional increase at 100%. Adding quinoa at either level did not alter EPA 22:5 (6, 6, 7 mg) concentrations of the egg over the corn-based control. As hypothesized, a valued characteristic of quinoa feeding in lieu of corn is the substantial increase in omega-3 PUFAs egg content.

Key Words: quinoa, Omega-3, PUFA

T27 Bleed-out and mechanical carcass washing impact on chiller water color, pH, chlorine level and carcass bacteria. D. L. Brinson^{1,2}, R. J. Buhr^{*1}, and J. K. Northcutt¹, ¹USDA-ARS, Russell Research Center, Athens, Georgia, ²The University of Georgia, Athens.

Commercial broilers were processed to compare the impact of slaughter and carcass washing treatments on chiller water characteristics including residual color ($L^* a^* b^*$), pH, total chlorine, turbidity, and carcass bacteria populations. On each of two processing days, eight broilers were electrocuted and not bled and eight broilers were stunned-and-bled conventionally. All carcasses were triple-tank scalded, mechanically defeathered and eviscerated. Four of the electrocuted carcasses and four stunned-and-bled carcasses were washed in an inside-outside carcass washer. The remaining four from either treatment were not washed. All carcasses were chilled for two–20 min periods in ice (2 L) and water (2 L of 20 ppm total chlorine) using an individual bag chilling method. Postchill carcass rinsates were collected and evaluated for *E. coli*, coliforms, *Enterobacteriaceae*, and total aerobes. Colorimetric and spectrophotometric readings were used to assess residual chiller water color and turbidity. In addition, final pH and total chlorine were measured postchill. Rinses of carcasses slaughtered by electrocution or stunned-and-bled did not differ significantly in post-chill bacterial populations. However, the levels of *E. coli* and *Enterobacteriaceae* recovered in carcass rinses were significantly lower from washed carcasses compared to those that were not washed, while numbers of coliforms and total aerobes were reduced by carcass washing. Chill water color and turbidity readings could not differentiate between carcass slaughter or washing treatments indicating similar residual water soluble proteins and suspended solids in the chiller water. For the first chill period, residual chlorine levels were lower for wash carcasses, but all other chill water characteristics were not significantly different between the slaughter or washing treatments. This study indicates that carcass bleed-out during slaughter had no affect on these chill water characteristics evaluated.

Key Words: bleed-out, carcass wash, chiller water color

T28 Nutritional composition of grilled and raw enhanced or non-enhanced chicken breast fillets. J. Kiker^{*1}, J. Howe², J. Holden², C. Alverado¹, J. Boyce¹, A. Luna¹, and L. Thompson¹, ¹Texas Tech University, Lubbock, ²Beltville Human Nutrition Research Center, Beltville, Maryland.

USDA Nutrient Database for Standard Reference for poultry products was last updated in 1979. Research was conducted to determine the nutritional value of enhanced (E) and non-enhanced (NE), raw or grilled breast fillets and to update the database. Two trials were conducted, forty fillets per trial were E by injection with a 0.6% NaCl and 0.4% sodium triphosphate solution and forty fillets were not E. Cooking losses in the E-grilled fillets (36.6%) were lower than in the NE-fillets (41.2%, $P < 0.05$). Percentage moisture was significantly higher in raw fillets (75.3%) than cooked fillets (E-grilled 67.7%; NE-grilled 65.8%, $P < 0.05$). LSMeans for percentage protein were greater in grilled fillets (NE-fillets, 31.8%; E-fillet, 28.1%) than raw fillets (NE-fillets, 22.2%; E-fillets, 19.9%). E-fillets had a higher ash content (cooked, 2.37 mg/100 g; raw, 1.8 mg/100 g) than NE-fillets (cooked 1.4 mg/100 g; raw, 1.1 mg/100 g). Fat content was lowest in E-grilled fillets (2.17%) compared to the other three treatments (three-mean average = 2.62%, $P < 0.05$). Sodium and phosphorus contents were significantly higher in the E-fillets (Na, 410.2 mg/100 g; P, 332.2 mg/100 g) than NE (Na, 157.5 mg/100 g; P, 263.01 mg/100 g). NE-grilled fillets had the highest K content (365.5 mg/100 g), followed by E-grilled (335.3 mg/100g). Enhancement (7.26%) of fillets reduced cooking losses, increased moisture, sodium (166.2%) and phosphorus (29.9%) in the cooked product. Referring to this study, a 100-g serving of E-grilled breast fillet would provide 56.7% of the daily reference value (DRV) for protein, 3.33% (DRV) for fat, 18.63% (DRV) for sodium, and 37.4% of the reference daily intake (RDI) for P. In contrast, a 100-g serving of non-enhanced grilled fillet would provide 63.6%, 4.15% and 7.79% of the RDV for protein, fat and sodium, and 29.4% of the RDI for P.

T29 Nutritional composition of two flavors of rotisserie chicken obtained from four regions of the United States. S. M. Mueller^{*1}, J. C. Howe², J. M. Holden², C. Z. Alvarado¹, L. M. Boylan¹, A. M. Luna¹, D. B. Wester¹, and L. D. Thompson¹, ¹Texas Tech University, Lubbock, ²Beltville Human Nutrition Research Center, Beltville, Maryland.

Nutrient data reported in the USDA National Nutrient Database for Standard Reference (SR) was last updated in 1979 and does not contain values for whole, ready-to-eat rotisserie chicken. The objectives of this study were to update this database, compare current (2004) rotisserie chicken composition results to those given for roasted chicken in the SR, and to compare the composition of rotisserie chickens from four regions of the United States. Retail locations in 12 states, three in each of four regions, were chosen for original flavor rotisserie chicken procurement. Chickens were dissected into breast, thigh, drum, wing, back, and skin, combined into location, regional, and national composites for each part, and analyzed for nutrient content. Total fat content was 3.5% in breast meat and 37.1% in skin, and did not significantly differ from the total fat content of roasted chicken reported in 1979 ($P \geq 0.05$). Saturated fat content decreased in breast meat and skin from 1.01% and 11.42% to 0.84% and 9.99%, respectively. Polyunsaturated fat content was significantly higher in all parts than values reported in the SR. Cholesterol values were 88.9, 130.1, 140.6, and 159.9 mg/100 g for breast meat, thigh meat, skin, and drum meat, respectively, and were all significantly higher than values reported in

the SR. Sodium and phosphorus contents of all parts were significantly higher than those reported in the SR. Sodium content ranged from 336.7 mg/100 g in thigh meat to 417.0 mg/100 g in drum meat, and phosphorus content ranged from 216.5 mg/100 g in thigh meat to 256.9 mg/100 g in drum meat. This research indicates current rotisserie chicken is very different from roasted chicken in 1979.

T30 Comparative anti-bacterial activity of egg white protein extracts from domestic chicken, turkey, duck and goose. O. W. Labadie*¹, J. Picman¹, and M.T. Hincke², ¹University of Ottawa, Ottawa, ON, Canada, ²Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada.

Egg white protein from the eggs of domestic chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), duck (*Anas platyrhynchos*) and goose (*Anser anser*) was evaluated in order to compare their food safety. A dozen fresh eggs from each species were washed with deionized water, cracked open and egg whites sampled. Samples were dialysed and lyophilized. Protein samples were analysed by SDS-PAGE, western blotting and antimicrobial assays. Lysozyme content was estimated from enzymatic activity measured by turbidity. SDS-PAGE revealed the presence of a 78kDa and 45kDa band, identified as ovotransferrin and ovalbumin by western blotting, in all species. C-type lysozyme (14 kDa) was present in chicken, turkey and duck egg white samples. The growth of *B.subtilis* was completely inhibited by all egg white samples (10mg/ml) regardless of the presence of 10mM sodium citrate/50mM sodium bicarbonate. Turkey egg white sample inhibited the growth of *S. aureus* and *Paeruginosa* in the absence of salts. In the presence of 10mM sodium citrate/50mM sodium bicarbonate, chicken and turkey egg white samples inhibited the growth of *S.aureus*. In the presence of 50mM sodium citrate, all egg white samples inhibited the growth of *P.aeruginosa* while chicken, turkey and duck samples inhibited the growth of *E.coli*D31. At lower concentrations (300ug/ml), all egg white samples significantly reduced populations of *B.subtilis*. Bactericidal activity was found to correlate with estimated lysozyme content, with chicken egg white showing the greatest bacterial reduction, as well as the highest lysozyme content, followed by turkey, duck and goose egg white. The egg white of the Galliformes (chicken and turkey) was found to possess greater antimicrobial activity than that of the Anseriformes. Interestingly, turkey egg white possessed the greatest bacteriostatic activity while that of chicken showed the greatest bactericidal activity. This study suggests that the Galliform egg is a safer product than the Anseriform egg due to more active ovotransferrin and higher levels of the broad specificity c-type lysozyme in the egg white. Supported by NSERC and PIC.

Key Words: albumen, antimicrobial, bacteriostatic

T31 Development of a direct fed microbial to control pathogens associated with turkey poult production. S. Gebert*, C. Kromm, and T. Rehberger, Agtech Products, Inc., Waukesha, Wisconsin.

Avian Pathogenic *Escherichia coli* (APEC) encompass a division of pathogenic *E. coli* that cause colibacillosis in young turkey poults. *Clostridium perfringens* is an enteric bacterial pathogen and is the major contributing factor associated with necrotic enteritis in poultry. These two pathogen groups were studied and targeted for the development of a *Bacillus*-based direct fed microbial (DFM) product for turkey poults at a commercial turkey company in Iowa. One hundred and five turkey poults at various ages were collected during three samplings over a four month period. Gastrointestinal samples were plated on

CHROMagar and Perfringens Agar Base for the enumeration of *E. coli* and *Clostridium*, respectively. Up to five colonies of each pathogen per bird were collected for further analysis. Multiplex PCR utilizing primers for the *iss*, *iucC*, *tsh*, and *cvaC* genes were used to identify APEC from the *E. coli* isolates. *Clostridium perfringens* was identified using a multiplex PCR reaction containing the four major toxin genes α , β , ϵ and *iota*. A total of 510 *E. coli* and 92 *Clostridium* were collected. Multiplex PCR identified 194 APEC isolates and 23 *C. perfringens* type A isolates. RAPD PCR analysis indicated that at a similarity coefficient of 80%, the 194 APEC isolates belonged to 34 clusters and the 23 *C. perfringens* type A isolates belonged to six clusters. Representative isolates from each cluster were used in a bacteriocin assay to select candidate *Bacillus* organisms for a DFM product. Six *Bacillus subtilis* strains were tested for their antimicrobial activity against 52 APEC and seven *Clostridium perfringens* type A isolates. Three of the six *Bacillus* strains showed the highest level of activity and the broadest spectrum by inhibiting 46 of the APEC isolates and four *Clostridium perfringens* isolates at greater than 50%. The 46 APEC isolates and the four *Clostridium* isolates represent 91.2% and 82.6% of their respective dendrograms. Future testing will incorporate these three *Bacillus subtilis* strains into a commercial product to determine its capability to control APEC and *Clostridium* within turkey poult production.

Key Words: *E. coli*, *Clostridium*, direct fed microbial

T32 Where molecular pathogenesis and NMR spectroscopy collide: An integrated approach towards the development of a CPS-based therapy for *Campylobacter jejuni*. D. J. McNally¹, H. C. Jarrell¹, M. Lamoureux¹, R. A. Coleman*¹, A. V. Karlyshev², B. W. Wren², J-R. Brisson¹, and C. M. Szymanski¹, ¹NRC Institute for Biological Sciences, Ottawa, ON, Canada, ²The London School of Hygiene and Tropical Medicine, London, United Kingdom.

The Gram-negative, spiral-shaped bacterium *Campylobacter jejuni* is one of the leading causes of bacterial gastroenteritis worldwide. As a result, there is intense effort to elucidate the virulence factors associated with this mucosal pathogen. Capsular polysaccharides (CPSs) are the outermost structure on the bacterial cell and therefore play key roles in the interaction between the pathogen and host as well as its environment. While the *Neisseria meningitidis* vaccine that is currently in use targets the conserved CPS structure of Group C organisms, for *C. jejuni* where at least 60 different CPS structures exist, there is no dominant structure associated with pathogenic strains. However, using high resolution magic angle spinning (HR-MAS) NMR, we identified a novel phosphoramidate (CH₃OP(O)(NH₂)(OR) (MeOPN) modification decorating *C. jejuni* CPS structures and demonstrated that 70% of isolates recovered from a variety of clinical presentations, animal sources and geographical locations express this rare modification. Furthermore, HR-MAS NMR analysis of cecal contents from *C. jejuni* infected chickens showed that the MeOPN can be readily detected, without purification or subculturing, from the natural bacterial host. Through HR-MAS NMR screening of the *C. jejuni* mass mutagenesis library, a gene cluster responsible for MeOPN biosynthesis has been identified and includes *cj1421c* and *cj1422c*, the putative transferases that add MeOPN to CPS. By examining the genetics and corresponding structure of the MeOPN modification, we have shown that MeOPN is a specific CPS marker for *C. jejuni* and therefore, a potential therapeutic and diagnostic target for this bacterium.

Key Words: system biology, therapeutic, campylobacter

T33 Novel method for rapid construction of *Campylobacter jejuni* deletion mutants. C. Hansen* and Y. M. Kwon, *University of Arkansas, Fayetteville.*

Campylobacter jejuni is the leading bacterial agent of human foodborne gastroenteritis throughout the developed countries and poultry is the most common source of human infection by this pathogen. Recently various genetic tools have been developed and utilized to identify *C. jejuni* genetic factors required for colonization and virulence. One of the techniques essential to assess gene function is to construct a deletion mutant. Current methods depend on the construction of a suicide vector harboring a deleted target sequence and its use in subsequent transformation, which requires multiple steps of PCR and cloning. In an effort to simplify deletion mutant construction in *C. jejuni*, we used the extension-overlapping PCR protocol to amplify target gene sequence in which an internal fragment was replaced by an antibiotic cassette (chloramphenicol resistance gene). The resulting PCR product can then be introduced into electrocompetent *C. jejuni* to select for chloramphenicol-resistant mutants in which the wild type allele has been replaced by the PCR product. We used this novel approach to successfully construct *C. jejuni* 81-176 mutant with deletion in the Cj0618 gene. Since a transposon insertion in Cj0618 has previously shown to reduce cecal colonization in chicks, we will test the cecal colonization of the deletion mutant. This novel method should simplify the construction of *C. jejuni* deletion mutants and thus facilitate functional characterization of many *C. jejuni* genes.

Key Words: *Campylobacter jejuni*, deletion mutant, PCR

T34 A survey on the hatchability of broiler and turkey eggs in the United States from 1985 through 2005. T. Schaal* and G. Cherian, *Oregon State University, Corvallis.*

A survey was conducted to investigate hatchability of broiler and turkey eggs set in the US hatcheries from 1985 through 2005. A total of 11 billion broiler eggs and 343 million turkey eggs were set in 2005. This represents a 50% and 25% increase in the total respective number of broiler and turkey eggs set since 1985. However, hatchability during this period remained at 79 to 82% for broiler eggs and 76 to 80% for turkey eggs. The advancements in nutrition, genetic selection and management of broiler and turkey flocks that occurred during this period did not result in any increase in hatchability. Considering the selling price to farmers of day old broiler chicks at 0.21 dollars and day old turkey poults at 1.15 dollars, the losses associated with hatchability cost the US poultry industry over 500 million dollars in the year 2005.

Key Words: broiler chicks, turkey poults, hatchability

T35 The efficacy of nipple drinkers and a direct-fed microbial on large white commercial turkey performance. S. M. Russell* and J. L. Grimes, *North Carolina State University, Raleigh.*

Concern over the use of antibiotics has led the poultry industry to find alternatives. PrimaLac® (Star Labs, Clarksdale, MO) is a direct-fed microbial (DFM) that contains viable *Lactobacillus/Streptococcus* sp. In the turkey industry, the use of nipple drinkers is being examined to enhance the growing environment. The objective of this study was to test the efficacy of nipple drinkers and DFM on turkey performance. A 2 by 6 factorial design was used (6 drinker types & 2 feed treatments):

1) control Plasson Minibell (T₁), 2) Plasson Easy Start (T₂), 3) Lubing Traditional (T₃), 4) Lubing Easy Line (T₄), 5) ValCo Turkey Drinker (T₅), and 6) Ziggity, Big-Z Activator (T₆). All drinkers remained in use through 18 wk except T₃, changed to the T₁ at 6 wk, and T₆, changed to T₁ at 14 wk. Typical turkey diets were formulated with and without PrimaLac®. Large White Hybrid Converter® (Kitchener ON, Canada) hens (30/pen) were reared to 18 wk. Feed consumption (by pen) and BW were determined at 1, 3, 5, 6, 8, 10, 12, 14, 16, & 18 wk. Feed conversion (FC) was calculated. Data were analyzed using the GLM procedure of SAS, Inc, and LS Means procedure was used to separate treatment means ($P \leq 0.05$). There were no feed by drinker type interactions. Six wk BW of birds reared on T₁ and T₅ were higher than birds on T₂ and T₆ with T₄ being intermediate. T₃ yielded lower 6 wk BW compared to all other drinkers. There were no longer any differences in BW due to drinker type by 16 wk. BW was higher and FC was improved for birds fed DFM through 8 wk. The T₂ had higher percent litter moisture beneath the drinker compared to all other drinkers with T₄ being intermediate at 6 wk. We conclude that some nipple drinker systems are effective through the brooding period for turkeys with some systems being capable of carrying birds through to market age. We conclude that the direct-fed microbial used herein is a viable alternative candidate to dietary antibiotics for rearing turkeys.

Key Words: nipple drinker, turkey, direct-fed microbial

T36 The influence of probiotics combined with mushroom extract on broiler chickens performance. W. Willis*, O. Iskhumen, Z. Liu, and M. Johnson, *North Carolina A&T State University, Greensboro.*

A study was conducted to evaluate the effect of combined Shitake mushroom (*Lentinus edeods*) extract with probiotics (Primalac®) on the performance of broiler chickens. In trial 1, 540 day of hatch chicks were randomly assigned to six treatment groups, and replicated three times with 15 males and 15 females per pen for 3 wks. Dietary probiotics and mushroom treatments were as follows: 1) Control feed + *ad libitum* tap water; 2) Control feed + skip-a-day mushroom water; 3) Control feed + *ad libitum* mushroom water; 4) Probiotic feed + *ad libitum* tap water; 5) Probiotic feed + skip-a-day mushroom water. 6) Probiotic feed + *ad libitum* mushroom water. Body weight gain, feed consumption and efficiency, mortality, bursa, liver, and spleen relative weights of chicks were taken. In trial 2, the performance of broilers from 3 to 7 wks of age withdrawn from the mushroom extract was evaluated with ten males and ten females per pen from trial 1. Mortality, weight gain, feed consumption and efficiency, carcass yield, fat pads and bursa weights were assessed. In trial 1, significant $P < 0.05$ differences in female weight gain (trt. 4- 0.62 vs trt. 1- 0.54 kg) and male spleen weights were observed. In trial 2, significant differences were observed in male weight gain (trt. 2- 2.40 vs trt. 4- 1.12 kg), male and female fat pads, male bursa wts (trt. 3- 0.15 vs trt. 6- 0.39), female carcass yield percentage (trt. 1- 77.8 vs trt. 4- 66.4), and feed consumption and efficiency. Body weights were severely depressed in the male broilers receiving the probiotics feed in treatments 4, 5, and 6 but not in the females. These results indicate that differences in gender occur with additives during different grow-out periods in broiler production. It appears that probiotics and mushroom extract offered no combination potential because weight gain was compromised in this study.

Key Words: probiotics, broiler chickens, mushroom extract

T37 Evaluation of drinking water or post-pelleting feed application of a *Lactobacillus*-based probiotic on broiler performance. N. Eckert*, D. Hyatt, J. Lee, S. Stevens, P. Anderson, S. Anderson, and D. Caldwell, *Texas A&M University, College Station.*

The present investigation was conducted to evaluate the effects of either drinking water or post-pelleting feed application of a probiotic on broiler performance during a 48 day grow-out. The trial consisted of 1,000 straight run broilers placed in 40 pens. Broilers were placed on litter consisting of half used litter from a commercial broiler house and half fresh pine shavings. Feed and water were provided to all broilers ad libitum. Four treatments were used in this trial, including a non-treated control, continuous probiotic water application, intermittent probiotic water application, and post-crumble or pelleting feed application. Each experimental group had 10 replicate pens. All broilers were fed a standard corn-soy ration containing Coban-60® (90 grams per ton) in a four diet feeding program (starter, grower, finisher, and withdrawal). Drinking water application of probiotic was by a medicator system in place within the grow-out barn. An increase in body weight was not seen in association with any of the probiotic experimental groups as broilers in the probiotic groups had final weights similar to the broilers in the control group. Broilers in the feed applied and continuous water applied probiotic groups showed a trend of reduced feed conversion between day 19 and 48 of grow-out but these values did not prove to be statistically different following analysis. The trends observed in this trial suggest that this *Lactobacillus*-based probiotic applied to the feed following crumpling / pelleting or continuously in the drinking water, may be conducive to reduced feed conversion and potentially heavier body weights in commercially-reared broilers, but further investigation is required. This trial is currently being replicated on completely non-medicated feed to investigate potential protection by this probiotic against microbial challenge from the rearing litter.

Key Words: probiotic, drinking water application, feed application

T38 In house composting and its effect on foodborne pathogens. K. S. Macklin*, O. A. Oyarzabal, R. A. Norton, J. B. Hess, and S. F. Bilgili, *Auburn University, Auburn, Alabama.*

In house composting of litter is one possible method to reduce the number of foodborne pathogens that reside within a poultry house. Reducing these numbers should reduce the amount that enters the processing plant and eventually onto the final processed product. In this experiment used litter was obtained and then formed into three compost piles. Temperature probes were used to measure the internal and external temperatures with the data being recorded hourly. Initial bacterial counts to determine the amount of *Salmonella*, *Campylobacter* and *Cl. perfringens* that was present in the litter was performed. These initial results show that there was no *Salmonella* or *Campylobacter*, even after enrichment; on average there was determined to be $2.6 \log_{10}$ cfu/g of *Cl. perfringens*. From each compost pile two 50g samples were removed and inoculated with 10^8 cfu of the following: (2) *S. enteritis*, *S. typhimurium*, *S. kentucky*, *S. heidelberg*, (3) *Cl. perfringens*, *Ca. coli* and (2) *Ca. jejuni*. The number in parenthesis denotes how many different strains for each species was used. These six 50g inoculated samples were then wrapped in cheesecloth and one was placed in the interior and exterior of each compost pile. After seven days the inoculated samples were collected and tested to determine the number of inoculated bacteria that survived. Additionally each sample was enriched to verify that all of the inoculated organisms were destroyed.

No detectable *Salmonella* or *Campylobacter* were detected in the inoculated samples that were placed inside the compost pile. Additionally no *Campylobacter* was recovered from the samples that were on the exterior of the compost pile. *Salmonella* in the exterior samples survived; however they were only detected after enrichment. Biochemical testing of this enriched sample indicated that *S. kentucky* was the sole *Salmonella* that survived. *Cl. perfringens* was recovered from the interior and exterior samples; however the amount recovered was less than $1 \log_{10}$ cfu/g. The results show that in house composting of litter is an effective way in either eliminating or reducing foodborne pathogens from a poultry house.

Key Words: foodborne pathogens, compost

T39 The effect of quicklime (CaO) on litter condition and broiler productivity. V. Ruiz¹, D. Ruiz¹, A. G. Gernat*¹, J. L. Grimes², J. G. Murillo¹, M. J. Wineland², K. E. Anderson², and R. Maguire³, ¹*Escuela Agrícola Panamericana, Zamorano, Tegucigalpa, Honduras,* ²*North Carolina State University, Raleigh,* ³*North Carolina State University, Raleigh.*

Waste management and disposal is among the most critical issues confronting confined animal feeding operations. There is an urgent need for innovative methods of collecting, processing, and disposing of manure, mortalities and by-products such that the environmental impact is minimized. The objective of this study was to develop a process using quicklime (CaO) that will result in a pathogen reduced manure that can re-used as a treated bedding material which is economically important in modern broiler production and have physical characteristics acceptable for land application. A common source of used broiler litter was treated with CaO at different percentages. The used treated and untreated litters were stockpiled separately for 10 days before allocation to assigned pens. The four litter treatments were T1) fresh wood shavings; T2) used broiler litter; T3) used litter with 10 % CaO, calculated on weight per volume basis; and T4) used litter with 15 % CaO calculated on weight per volume basis. The four litter treatments were allocated in a randomized complete block design to the 16 experimental pens providing four replicates per treatment. Body weight (BW), cumulative feed consumption, and feed conversion (feed:BW) were determined on a weekly basis through 42 days of age. Bird foot pads and breasts were observed for potential burns and blemishes on a weekly basis through 42 d of age. A second trial was conducted using the same procedures. Mortality was recorded daily. Carcass weights and percentages of carcass yield without giblets were determined prechill. Litter was sampled for pH, total phosphorus (P), nitrogen (N), soluble P, percent moisture and total plate counts (TPC) from the different litter treatments days 1 and 10 prior to bird placement and days 7 and 42 after bird placement. No significant differences were found for body weight, feed consumption, feed conversion, mortality, carcass weight or yield nor were blisters observed on the foot pads or breasts. The addition of CaO to used broiler litter before the bird placement increased pH and reduced soluble P and TPC. T4 had significantly ($P < .001$) higher pH on day 7 (11.1) and day 42 (8.7) when compared to T1 (day 7 = 5.6, day 42 = 7.6). Soluble P (ppm) was significantly ($P < .001$) lower for T4 (2.7) when compared to T1 (42.2), T2 (439.2), and T3 (35.0). Though not significant, T4 had lower TPC cfu/g in comparison to the other treatments on day 7 and 42. The authors conclude that the use of CaO as a treatment for broiler litter will initially reduce bacteria load in the litter and reduce soluble P without negatively affecting bird productivity per the conditions of this study.

Key Words: broiler, litter, lime

T40 Effect of drinking water coliform on broiler performance. P. Sedlacek*, A. B. Batal, B. D. Fairchild, and C. W. Ritz, *University of Georgia, Athens.*

Standards for poultry drinking water state that there should be fewer than 100 CFU/ml total bacteria and fewer than 50 CFU/ml coliform bacteria. Two experiments were conducted to evaluate the effects of coliform presence in broiler drinking water on performance. Cobb 500 by-product male broiler chicks were housed in Petersime batteries equipped with a nipple water system. Chicks were fed a standard corn-soybean meal diet and allowed ad libitum access to feed and water. Chick weight, feed intake, and water consumption were recorded weekly throughout the 35 and 21 day Experiments. In both Experiments, five replicate pens of ten birds were allotted to the water treatments which consisted of 50 and 500 CFU/ml of coliforms, and 4 replicate pens of ten birds were allotted to the water treatments consisting of 0 and 5000 CFU/ml of coliform contaminated water. Water treatments were mixed on a weekly basis. Water analysis for total coliforms and total plate counts were conducted weekly and the samples were taken directly from the water nipples. The average of the coliform counts for the 0, 50, 500, and 5000 CFU/ml treatments were 7.4, 111.6, 704.4, and 1924, respectively, for Experiment 1 and 6.1, 125.3, 530.3 and 5360 respectively, for Experiment 2.

Addition of 5000 CFU/ml of coliforms in the drinking water of broiler chickens significantly reduced weight gain during the first week in Experiment 1. However, overall coliform addition in the drinking water up to 5000 CFU/ml of coliforms had no effect on weight gain, feed intake, feed efficiency (gain:feed) or water intake in both experiments. Based on these results, up to 5000 CFU/ml did not impact broiler performance however, levels of 5000 CFU/ml or greater may reduce broiler performance during the first week posthatching. Based on previous research the negative effects of coliform bacteria were observed only in combination with other water contaminants or environmental stresses which confirms the findings herein and suggest that bacteria contamination alone may not be the reason for poor broiler performance but it is likely due to a combination of water contaminants and environmental stresses.

Key Words: broilers, water, coliforms

T41 Association of IL-10 gene clusters and Salmonella response in chicken. S. B. Ghebremicael^{1,2}, J. R. Hasenstein¹, and S. J. Lamont*¹, ¹Iowa State University, Ames, ²Wageningen University, Wageningen, The Netherlands.

Salmonella enteritidis (SE) lipopolysaccharide stimulates IL-10 gene expression in chickens. We investigated associations of single nucleotide polymorphisms (SNP) of IL-10 and linked genes with response to Salmonella. Genes in the IL-10 cluster (PIGR, IL-10, MAPKAP2, and LGTN) and DYRK1A were investigated using the F8 generation of two related advanced intercross lines (AIL) of the Iowa salmonella response resource population (ISRRP). The ISRRP was generated by crossing outbred broilers with dams of two highly inbred lines (Leghorn and Fayoumi). Intercrossing continued within the two dam lines. The F8 chicks (n=132) were intraesophageally inoculated at one d with SE. At d 7 or 8, both spleen tissue and cecal contents were cultured to quantify SE load. First, about 800 – 1000bp of each gene were sequenced from 3 birds per line. The SNP numbers in the sequenced region of PIGR, IL-10, MAPKAP2, LGTN and DYRK1A were 8, 7, 4, 7 and 16, respectively. The F8 population was genotyped for one SNP per gene by using Multiplexed SNaPshot

assay. Association of genes with SE bacterial burden was analyzed by general linear model. The pIGR, IL-10 and LGTN SNP had no significant associations. MAPKAP2 was highly significantly associated with SE burden in spleen tissue ($P < 0.0001$) and SE burden in cecal luminal contents ($P < 0.01$) in the broiler X Fayoumi AIL, but not the broiler X leghorn AIL. DYRK1A and SE count in spleen tissue and cecal content were significantly associated ($P < 0.05$) in the broiler X Fayoumi AIL. Genetic differences between the AIL imply that sufficient genetic variation exists to select for reduction of SE burden in poultry. The two significant genes are reported to play roles in gene expression regulation and cell proliferation, including stress and inflammatory responses. The results suggest that SNP in both genes are associated with Salmonella burden, and may be valuable in generating resistant birds.

Key Words: lipopolysaccharide, IL10 gene cluster, salmonella

T42 A database for chicken full-length cDNAs. Y Wang, Z. G. Wang, and F.C. Leung*, *Department of Zoology, The University of Hong Kong, Hong Kong.*

Collection of full-length cDNAs is essential to functional genomics studies. Projects for constructing the human and mouse full-length cDNAs database have generated a large amount of data and such databases are important research resources and tools for the scientific community. With the completion of the chicken genome sequencing, the same project in chickens can be initiated and we introduce a database that may facilitate future research work in chicken system biology. In the database, chicken ESTs were aligned over human and mouse full-length cDNAs or ORFs on the basis of high similarity in BLAST alignment. The alignment data are helpful to experimental researchers to confirm the corresponding full-length cDNAs in chicken. A total of over 540,000 chicken ESTs were used to find alignment positions on about 160,000 full-length templates from the NEDO, RIKEN and MGC databases. A part of the templates have known biological functions, and their homologous chicken genes in the EMBL database are given in the database. We also collect known chicken full-length cDNAs and continue to update the database for public use.

Availability: <http://bioinfo.hku.hk/chicken/>

Key Words: chicken, cDNA, database

T43 Characterization of Duck Pit-1 cDNA and genomic DNA. N. Kansaku*¹, T. Ohkubo², D. Guemene³, U. Kuhnlein⁴, and D. Zadworny⁴, ¹Azabu University, Sagamihara, Kanagawa, Japan, ²Kagawa University, Miki-Cho, Kagawa, Japan, ³INRA-SRA, Nouzilly, France, ⁴McGill University, Ste. Anne de Bellevue, PQ, Canada.

Pituitary transcription factor (Pit-1) involves the expression of growth hormone and prolactin. Our previous study demonstrates the association between genetic variation of chicken Pit-1 intron1 and egg production traits. Since intron1 contains the alternative transcription starting site, expression of Pit-1 isoform (gamma) may be related to variation and traits. In birds, only chicken and turkey pit-1 was cloned and sequenced. It is thus necessary to obtain information of Pit-1 sequence in the other birds to investigate the mechanism of Pit-1 gamma expression and association between genetic variation and egg production traits. Accordingly, this study aimed to clone and characterized the duck Pit-1 cDNA and genomic DNA.

Based on the chicken and turkey Pit-1 cDNA sequence, primers were designed. Reverse-transcribed product was subjected to PCR amplification. After sequencing of product, 5' and 3' regions were cloned by RACE. Genomic structure of duck Pit-1 gene was characterized by using newly designed primers. Based on the sequence of intron1, Pit-1 gamma specific primer was designed and used to examine the isoform expression in the anterior pituitary gland.

Of the 2575 nucleotides which were sequenced, 71, 1499, and 1005 bases represented 5', 3', and open reading frame which encoded a peptide of 335 amino acids. Approximately 12.6 kb of duck Pit-1 gene was sequenced and exon-intron boundary was characterized. Duck Pit-1 gene consists of 7 exons and 6 introns as in turkey. Intron1 of duck Pit-1 consist of 3Kb and shows 0.7kb longer than these of chicken and turkey. Both Pit-1alpha and gamma was detected by RT-PCR in the duck anterior pituitary gland. These result may indicating that inserted 0.7 kb sequence in the duck Pit-1 intron1 does not involve the expression of Pit-1 gamma. In conclusion, sequence information of duck Pit-1 cDNA and genomic DNA allows future determination of the genetic variation. This may be important since variation of chicken Pit-1 intron1 have been associated with egg production trait. Genetic variation of duck Pit-1 may explain or associate the different egg production trait in the various duck strains and breeds.

Key Words: duck, Pit-1, isoform

T44 Cloning and expression of chicken AMP-activated protein kinase subunit genes. M. Proszkowiec-Weglarczyk, M. P. Richards*, and S. M. Poch, *USDA, ARS, ANRI, Growth Biology Laboratory, Beltsville, Maryland.*

AMP-activated protein kinase (AMPK) is involved in maintaining cellular energy homeostasis and, on the whole animal level, in the regulation of energy balance and food intake. The AMPK enzyme complex consists of one catalytic (alpha) and two regulatory (beta and gamma) subunits. There are two known isoforms for the alpha and the beta subunits and three for the gamma subunit, each of which is encoded by a separate gene. Because so little is known about chicken AMPK subunit genes, the present study was designed to clone, sequence and characterize the seven gene homologues. A molecular cloning strategy involving RT-PCR and 5'-RACE was developed to sequence cDNAs corresponding to complete coding regions and portions of the 5'- and 3'-untranslated regions of chicken AMPK subunit mRNA transcripts. AMPK alpha-1, alpha-2, beta-1 and beta-2 genes code for predicted proteins of 560, 552, 273 and 272 amino acids, respectively. The beta-2 gene produces multiple transcript variants that differ at the 5'-end; however, all code for the same predicted amino acid sequence. The presence or absence of one coding exon in AMPK gamma-1 gene transcripts results in two variants that code for predicted proteins of 298 or 276 amino acids. Use of an alternate promoter and transcription initiation site and/or alternative splicing of gamma-2 gene transcripts results in four different variants that code for predicted proteins of 567, 452, 328 and 158 amino acids. Alternative splicing of exon 3 in the gamma-3 gene results in a shift of the open reading frame and the production of two transcript variants that code for predicted proteins of 382 or 378 amino acids. Each of the AMPK subunit genes exhibited a unique tissue-specific expression pattern. In general, chicken AMPK subunit genes displayed similar structures and high sequence homology as compared to corresponding mammalian genes. Understanding chicken AMPK subunit gene structure and expression patterns provides new insight into the role of the AMPK pathway in metabolic regulation in poultry.

Key Words: AMP-activated protein kinase, gene structure, chicken

T45 Gastrointestinal maturation is accelerated in turkey poult supplemented with a mannan-oligosaccharide yeast extract (Alphamune™). F. Solis de los Santos*¹, M. B. Farnell², A. M. Donoghue³, G. R. Huff³, W. E. Huff³, N. C. Rath³, and D. J. Donoghue¹, ¹University of Arkansas, Fayetteville, ²Texas A & M University, College Station, ³Poultry Produ Agricultural Research Service, USDA, Fayetteville, Arkansas.

Alphamune™ is a yeast extract antibiotic alternative that has been shown to stimulate the immune system and increase BW in pigs. The influence of Alphamune™ on gastrointestinal tract (GIT) development of turkey poults has not been reported. Two trials were conducted to evaluate the effects of Alphamune™ on gut maturation of 7 and 21 d old turkey poults. Poults were fed a standard control unmedicated turkey starter diet or the same diet supplemented with either 1 lb/ton or 2 lb/ton Alphamune™ (n = 18 poults/group). On d 7 and 21, a 2-cm section was collected from the mid-point of the duodenum, jejunum and ileum of each bird (9 poults/day/treatment) and fixed in a 10% formalin solution for 72 h and then stained. Twenty measurements of villus height, villus surface area, lamina propria thickness and crypt depth were taken per section per poult. On d 7, villus height and surface area of the duodenum and ileum were consistently greater for the 2 lb/ton Alphamune™ group. The crypt depths were deeper and the number of neutral goblet cells higher for all areas of the GIT evaluated in the Alphamune™ supplemented birds compared to controls on d 7. The intestinal morphology of the duodenum was not different between the controls and treated birds on d 21. However, in the jejunum villus height, villus surface area, lamina propria thickness and crypt depth were increased for both doses of Alphamune™ supplementation compared to controls in both trials. The ileal villus height was higher in the 2 lb/ton Alphamune™ birds and ileal surface area was increased for both doses of Alphamune™ compared to controls in both trials. The ileal crypts were deeper for all treatments compared to controls. These results suggest that feed supplemented with Alphamune™, a mannan-oligosaccharide, may accelerate gastrointestinal maturation in turkey poults.

Key Words: Alphamune™, gastrointestinal tract, yeast extract

T46 Adrenal hormones play important roles to tolerate acute heat stress in broilers. M. Kikusato*, K. Kato, A. Ohtsuka, and K. Hayashi, *Kagoshima University, Kagoshima, Japan.*

When broilers are exposed to a high temperature (38°), most of them die within 3 hours. However, some of them can tolerate the high temperature, indicating that an adaptation mechanism exists. We hypothesized that adrenal activation and glucose supply to the heart play key roles in the adaptation due to the following information. Heat dissipation by panting is supported by increased cardiac rate and blood flow. Epinephrine (EP) increases plasma glucose concentration. Norepinephrine (NE) increases cardiac glucose uptake and corticosterone (CTC) enhances lipolysis and glyconeogenesis. Therefore, we observed the effects of the hormones on adaptation to high temperature in broilers. Twenty-eight or twenty nine days old broiler chickens were divided into control (Cont) and EP groups with 6 replicates. They were fed basal diet (CP30%, ME3030 kcal / kg) made mainly from maize and soy protein isolate for 4 days. EP (0.1mg / kg BW) was injected subcutaneously, followed by exposure to heat (38°) for 6 hours. Water was offered ad libitum during the heat exposure. Birds were decapitated and blood was collected when body temperature exceeded 46° to measure hepatic and cardiac glycogen contents and

plasma glucose concentration (Exp. 1). Exp. 2 and 3 were conducted similarly as Exp. 1 except for those described below. CTC (50mg / kg BW) was injected 4 hours before the heat exposure in Exp. 2, and NE (1mg / kg BW) was injected 1 hour before the heat exposure in Exp. 3. The birds were tolerant when EP was injected. Hepatic glycogen was significantly increased by EP while cardiac glycogen was decreased significantly. Plasma glucose tended to be increased. The birds were also tolerant when CTC was injected. Hepatic and cardiac glycogen contents were increased significantly by CTC while plasma glucose concentration was not changed. The birds seemed to be more tolerant when NE was injected (mortality: 1/6). Hepatic glycogen and plasma glucose were not changed while cardiac glycogen tended to be increased by NE. In conclusion, adrenal hormones, especially NE, play important roles in tolerance of acute heat stress in broiler.

Key Words: heat stress, adrenal hormones, broiler

T47 Study of the effects of dietary lutein on semen parameters in roosters. H. Pizzey* and G. Y. Bedecarrats, *University of Guelph, Guelph, ON, Canada.*

Antioxidants such as vitamin E have been shown to improve sperm quality and fertilizing ability by protecting sperm plasma membranes against lipid peroxidative damages. A preliminary study conducted in our laboratory indicated that supplementing the diet of broiler breeders with lutein, a xanthophyll carotenoid with anti-oxidative properties, improved hatchability. However, whether this effect was due to increased fertility was not determined. Thus, the objective of this study was to determine if dietary lutein can improve sperm quality in mature roosters. Fifteen Barred Rock roosters housed in individual battery cages were stimulated with a 14h photoperiod at 18 weeks of age. At 40 weeks of age, birds were randomly assigned to 3 experimental groups (n=5 per treatment) and fed ad libitum a standard diet supplemented with 0, 30, or 120 ppm lutein. Semen was collected from each rooster by abdominal massage twice a week starting two weeks before the beginning of the experiment, and sperm analyses were performed on individual semen samples collected the day before (d0) and 19 (d19) and 29 (d29) days after introduction to diets. Sperm concentration was determined by spectrophotometer and sperm motility assessed by CASA (computer-assisted sperm analyser). Sperm viability was determined using SYBR-14 and PI fluorescent staining, with a manual count of viable and non-viable sperm under a fluorescent microscope. Data were analyzed using repeated measures ANOVA. Although no significant difference in sperm concentration, motility, and viability was observed between treatments, roosters fed the 120 ppm lutein diet tended to display a higher sperm motility and viability at d19 and d29. In conclusion, unlike vitamin E, dietary lutein supplementation does not significantly improve sperm quality in mature roosters.

Key Words: lutein, rooster, sperm

T48 3 β -HSD and cAMP in GC of hens subjected to heat stress. H Taira* and M. M. Beck, *University of Nebraska, Lincoln.*

Heat stress (HS) interferes with reproduction in laying hens, at least in part through reductions in LH, P₄ and E₂. In earlier studies, granulosa cells (GC) from hens subjected to acute and chronic HS produce less P₄, even when stimulated by LH, and that 3 β -HSD activity is correspondingly reduced; stimulation with LH and, to a greater extent,

with LH+FSH, increases the activity. In addition, strain differences in response to the hormone treatments have been shown. In order to further investigate mechanism(s) by which HS affects steroidogenesis, this study was conducted in two parts: 1) effect of HS on gene expression of GC 3 β -HSD; and 2) effect of HS on GC cAMP, as the second messenger operational in the steroidogenic pathway. Three strains of Hy-Line[®] laying hens were used in the first study (W36, W98 and Browns); subjected to 22C, 35C (24h, acute HS) or 35C (2wk, chronic HS); and GC from the largest 3 follicles were collected. RNA was extracted and reverse transcribed using oligo dt primers and superscript III reverse transcriptase. Samples were subjected to RT using primers for 3 β -HSD and GAPDH and JumpStart Ready Mix Taqman polymerase. Log transformed data were normalized using GAPDH. Expression of 3 β -HSD RNA was suppressed by acute (P=0.0077) and chronic (P=0.028), but there were no strain differences. In the 2nd study, Hy-Line[®] W98, W36 and CV20 hens were subjected to 22C or chronic (2wk) HS (35C). GC from the largest follicles were isolated and enzymatically dispersed. Aliquots of 100,000 viable cells were incubated with or without LH+FSH and cAMP was measured. HS decreased cAMP compared to TN by ~34% (P<0.0001). No strain differences were observed in response to 22C (W36 vs W98; P=0.0945, W36 vs CV20; P=0.5267, and W98 vs CV20; P=0.2869). However, under HS, cAMP levels of CV20 were lower than either W36 (P=0.0020) or W98 (P=0.0112). LH+FSH did not improve cAMP levels (P=0.4528). In previous studies, HS reduced 3 β -HSD activity and LH+FSH restored it differentially by strain and LH has been shown to increase cAMP in hen thecal cells. The lack of response to LH+FSH in this study suggests that the relationship between cAMP and 3 β -HSD in HS GC is not direct.

Key Words: 3 β -HSD, cAMP, heat stress

T49 Effect of blindness on reproductive performance in a White Leghorn chicken line. P. M. Kirby* and G. Y. Bedecarrats, *University of Guelph, Guelph, ON, Canada.*

The objective of this study was to evaluate the reproductive performance in a chicken line displaying naturally occurring blindness. Smoky Joes are genetically modified pigmented White Leghorn chickens which harbor a sex-influenced autosomal recessive mutation resulting in retinal degeneration. In this experiment, 2 successive generations were used. Chickens were raised in brooding battery cages under an 8h photoperiod until 17 weeks of age, and then hens were transferred to individual battery cages and stimulated with a 14h photoperiod. Egg production for the 1st and 2nd generations was recorded daily until the 42nd and 7th week post-photostimulation, respectively. In addition, egg weight was also recorded for at least 7 weeks. Sexual maturation was estimated by determining the age at first egg, and overall egg production was calculated as hen-egg-day. In both generations, blind hens reached sexual maturity earlier than sighted birds, with over 93% of blind and 76% of sighted hens laying their first egg before 22 weeks of age. Similarly, blind hens reached peak production on average 1 week earlier than sighted hens, though no difference in peak production was observed. However, blind hens also declined from peak of lay earlier than sighted birds, and after a 42 week production period, blind hens' laying rate was 12% compared to 21% for sighted hens. Overall, total egg production during the 42 weeks period was similar between blind (131 \pm 51) and sighted (145 \pm 50) hens. For both generations, no significant differences in weight were observed between eggs laid by blind and sighted hens. In conclusion, it appears that the lack of retinal stimulation in blind Smoky Joe hens results in a

shift in egg production with an advanced sexual maturation as well as an advanced drop in production.

Key Words: chicken, blindness, reproduction

T50 Effects of thermal stress during incubation on some embryo physiological parameters. R. D. Malheiros*¹, V. M. B. Moraes², M. Macari², J. Buyse³, and E. Decuyper³, ¹Universidade Estadual Paulista-UNESP, Dracena, SP-Brazil, ²Universidade Estadual Paulista-UNESP, Jaboticabal, SP-Brazil, ³Lab for Livestock Physiology and Immunology-KUL, Leuven, Belgium.

The aim of the present study was to verify the effects on embryo metabolism when subjected to 4 hours of heat stress (39.0°C). By that way 400 eggs were set to incubation (200 eggs each group), one group was designated as control (CONTROL) and second was designated as heat stress (HEAT). On the 13th day of incubation, the HEAT group of eggs was transferred to another incubator where they were submitted to a heat challenge of 39.0°C for 4 h. At the end of heat treatment on Day 13, 10 eggs were withdrawn from each group, and a blood sample was obtained for measurement of plasma glucose, triglyceride, uric acid and corticosterone levels and creatine kinase (CK) activity. A similar procedure was repeated on embryonic Days 14, 15, 16 and 17. In essence, the duration of heat treatment of embryos ranged between 4 h on Day 13 to 20 h for eggs remaining to the end of the 17th Day. In CONTROL embryos, plasma glucose started to increase significantly from Day 16 onwards. In HEAT embryos, this increase in plasma glucose occurred already on Day 15 and was of a larger magnitude, resulting in significantly higher plasma glucose in HEAT embryos from Day 16 onwards (heat treatment by age interaction; P<0.05). There were no significant overall differences between heat treatment neither in plasma corticosterone and uric acid levels nor in plasma CK activities. For all these plasma indices, a significant increase with embryonic age was observed from Day 15 or 16 onwards, irrespective of temperature. In contrast, HEAT embryos were readily characterized by significantly lower plasma triglyceride and this did not fluctuate significantly with embryonic age in either temperature treatment. Based on these first observations, it can be inferred that the age-related increase in circulating corticosterone is a causative factor for the age-related hyperglycemia, hyperuricacidemia and deteriorating muscle membrane integrity (increased CK activity) as already demonstrated in postnatal broilers. In conclusion, profound changes do occur in intermediary metabolism (hormonal-driven) in late-stage embryos and these ontogenic changes are subject to environmental temperature.

Key Words: incubation, embryo stress, embryo metabolism

T51 Characterization of *Clostridium* from broiler farms experiencing recurrent outbreaks of gangrenous dermatitis. T. Neumann*¹, D. Ritter², S. Dunham¹, J. Skalecki¹, and T. Rehberger¹, ¹Agtech Products, Inc., Waukesha, Wisconsin, ²Mountaire Farms of Delaware, Inc., Millsboro, Delaware.

Gangrenous dermatitis (GD) is a complex disease characterized by necrosis of the skin, subcutaneous tissue, and underlying musculature. Although a variety of microorganisms have been isolated from clinical specimens, *Clostridium* has been the bacterium most often associated with the incidence of GD. The purpose of this study was to better understand the etiology of this disease by comparing clostridial isolates from the environment of diseased farms as well as from organs and

tissues of affected birds. A total of 76 samples were collected over a two month period from broiler farms in Delaware that were currently experiencing outbreaks of GD. Samples were from a variety of different sources including wet and dry litter, gastrointestinal tracts, blood, livers, joints, and skin lesions. Most organs and tissues were harvested from recently deceased birds although a few sample sets were collected from clinically infected live birds. The samples were plated on Perfringens agar and isolated *Clostridium* colonies were picked. Multiplex PCR, targeting both α -toxin genes of *C. perfringens* and *C. septicum*, was used to verify species identification. Both *C. perfringens* and *C. septicum* were observed over the course of the trial. RAPD PCR was performed and used to construct dendrograms to determine the genetic diversity. The dendrograms, containing 115 isolates of *C. perfringens* and 84 isolates of *C. septicum*, indicated an index of diversity equal to 2.03 and 1.53 respectively. Although both species were similarly prevalent, *C. perfringens* was more likely to be isolated from litter samples. Litter isolates appear to be distinct from the majority of isolates that were isolated from the birds. Both *C. perfringens* and *C. septicum* were isolated from skin lesions, GI tracts, and joint samples however only *C. septicum* was isolated from liver samples. The results of this study seem to suggest a selective proliferation of environmental isolates through the gastrointestinal tract leading to a bacteremic state prior to the bird's death.

Key Words: gangrenous dermatitis, *Clostridium*

T52 Natural presence of *Campylobacter* and *Salmonella* in the spleen, liver/gallbladder and reproductive tract of commercial Leghorn laying hens. N. A. Cox¹, R. J. Buhr*¹, M. T. Musgrove², L. J. Richardson¹, and P. J. Fedorka-Cray³, ¹USDA-ARS-PMSRU-Russell Research Center, Athens, Georgia, ²USDA-ARS-ESQRU-Russell Research Center, Athens, Georgia, ³USDA-ARS-BEAR-Russell Research Center, Athens, Georgia.

Campylobacter and *Salmonella* are known to cause acute bacterial gastroenteritis in humans and poultry products have been implicated as a significant source of these infections. The objective of this study was to determine whether *Campylobacter* and *Salmonella* could be isolated from the reproductive tissues, lymphoid organs, liver/gallbladder and ceca of commercial leghorn laying hens. Two flocks were sampled. Hens (n=15) were obtained at 58 and 104 wk of age from separate facilities. Hens were euthanized, de-feathered and necropsy aseptically performed. To minimize the possibility of cross-contamination between samples, the ovarian follicles, spleen, liver/gallbladder, upper (infundibulum, magnum, and isthmus) and lower (shell gland and vagina) reproductive tracts were aseptically removed prior to the ceca. Individual samples were placed in sterile bags, packed on ice and transported to the laboratory for evaluation. In this study naturally colonized *Campylobacter* spp. were found in 3/10 ovarian follicles, 1/15 spleens, 4/15 liver/gallbladders, 7/10 upper reproductive tracts, 7/10 lower reproductive tracts, and 12/15 ceca. Naturally colonized *Salmonella* were found in 1/10 ovarian follicles, 1/15 liver/gallbladders, and 1/15 ceca but was not recovered from the spleen, upper or lower reproductive tract. *Campylobacter* were present in 93% of the hens sampled and *Salmonella* were present in 13% of the hens sampled in this study. The results of this study clearly show that commercial table egg laying hens housed in cages on wire floors, without the presence of roosters were naturally colonized with *Salmonella* and *Campylobacter* in their internal organs, reproductive and intestinal tracts.

Key Words: campylobacter, *Salmonella*, lymphoid organs

T53 Real time PCR for the rapid detection of avian reoviruses. K. Guo*¹, J. Giambone¹, T. Dormitorio¹, and H. Wu², ¹Auburn University, Auburn, Alabama, ²Alabama State University, Montgomery.

Avian reoviruses (ARV) can cause tenosynovitis, malabsorption syndrome, and chronic respiratory disease in commercial chickens. In addition, different antigenic subtypes exist, which make choosing the proper vaccine problematic. Our objectives were to develop a rapid and highly specific detection method utilizing Real-time RT-PCR to detect ARV infections in the chickens and to use the test to differentiate avian reovirus isolates based on pathogenicity, point of origin, and antigenicity. The protocol consists of two sets of one-step real-time RT-PCRs, which are conducted on a Roche LightCycler. Sample viral RNAs were first screened for ARV with primers and probes from a highly conserved S3 gene. This procedure was fast, sensitive, and could rapidly detect ARVs from cell culture and embryonating eggs. Experiments are now under way to detect ARVs directly from clinical tissue specimens as well as testing positive ARV samples using specific primers and probes from the highly variable region of S1 gene. It is hoped that these reagents can group ARVs according to their pathogenicity, point of origin, and antigenicity. If so, the test will aid in the epidemiology of ARV infections as well as help in the development of improved vaccines.

Key Words: avian, reovirus, real time PCR

T54 Sequence analysis of the infectious bursal disease virus VP2 gene from commercial chickens from Nigeria. O. A. Fagbohun¹, J. J. Giambone², T. V. Dormitorio*², W. Hongzhuan³, and K. Guo², ¹Auburn University, Auburn, Alabama, ²Auburn University, Auburn, Alabama, ³Alabama State University, Montgomery.

Bursae of Fabricius harvested from Nigerian commercial chickens, which were suspected of having very virulent (vv) infectious bursal disease virus (IBDV) infection, were obtained. The birds had a history of clinical signs and lesions indicative of vvIBDV. The tissues were treated in phenol: chloroform: isoamyl alcohol (25:24:1, pH 6.6) and exported to the U.S with the permit number 44226. IBDV RNAs from phenol chloroform treated tissues were characterized using molecular techniques. Ten of the samples were randomly selected and infectious bursal disease viral RNAs were isolated from the tissues. The hypervariable regions of VP2-encoding genes of seven samples were amplified using nested reverse transcription polymerase chain reaction (nested RT-PCR) and their sequences determined using an automated machine. Their sequences were compared with that of IBDV reference strains in the GenBank database. Sequence analyses revealed that the IBDVs were very virulent, which agreed with the clinical picture as seen in Nigerian poultry. Sequences also showed 96.6% identity indicating that the viruses were from the same ancestor, which was probably imported into the country with poultry from nearby countries as had recently occurred with influenza virus.

Key Words: infectious bursal disease virus, RT-PCR, Nigeria

T55 Identification of *Salmonella enteritidis* genes essential for *in vitro* growth by TnAraOut mutagenesis. J. N. Kim*, G. W. Youm, and Y. M. Kwon, University of Arkansas, Fayetteville.

Human infection by egg-associated *Salmonella* is an important public health problem in the United States. Among other serotypes

implicated in human illness, *S. enteritidis* is the only serotype that routinely colonize the reproductive tract of hens, leading to subsequent production of contaminated eggs. In this study, we used TnAraOut transposon system to identify *S. enteritidis* genes essential for *in vitro* growth. Such genes are important for understanding essential cellular mechanisms for growth and have a great potential as targets for development of novel antibiotics. TnAraOut is a *mariner*-based transposon containing arabinose-inducible promoter facing outward. Our screening strategy was based on the assumption that a mutant harboring TnAraOut insertion in promoter region of an *in vitro* growth essential gene shows arabinose-dependent growth pattern. The first step was to generate random insertion mutants and identify the mutants that form pinpoint colonies on LB plates containing low concentration (0.001%) of arabinose. These colonies were then tested for the growth on LB plates containing low (0.001%) and high concentrations (0.1%) of arabinose to identify the mutants with arabinose-dependent growth phenotype. Among 811 mutants forming pinpoint colonies, we identified 13 mutants showing arabinose-dependent growth phenotype, including an insertion in *atpI*. We will continue screening to obtain a comprehensive collection of TnAraOut mutants with insertions in essential genes. They will be further characterized for arabinose-dependent growth kinetics, *in vitro* competition assay, and sequencing of insertion sites.

Key Words: *Salmonella enteritidis*, transposon mutagenesis, essential genes

T56 Evaluation of spray application of a *Lactobacillus* -based probiotic and ability to protect broiler chicks against *Salmonella enteritidis* infection. A. D. Wolfenden*, C. M. Pixley, J. P. Higgins, S. E. Higgins, A. Torres Rodriguez, G. Tellez, and B. M. Hargis, University of Arkansas, Fayetteville.

A commercially available *lactobacilli* -based probiotic (FM-B11™, Ivesco LLC), has shown efficacy against *Salmonella* when poultry are treated via drinking water (DW). In this study the probiotic (B11) was administered by spray application consistent with hatchery use. In two experiments, day-of-hatch chicks were challenged with *Salmonella enteritidis* (SE) by oral gavage (10⁴cfu/chick) alone, challenged with SE and treated by coarse spray application of B11 (25 mL per 100 chicks, 10⁷cfu/mL), or challenged with SE and treated with B11 continuously in the DW (10⁶cfu/mL). Chicks in hatchery trays were placed under bright halogen light for 2.5 min after spray application. At placement, chicks were held in brooder batteries (n=40) and provided free access to feed and water. At 5 days post-challenge, 20 chicks per pen were humanely killed and subjected to necropsy with aseptic removal and culture of cecal tonsils for presence or absence of SE by established protocol. In Exp. 1, near-simultaneous administration of B11 with spray and immediate placement of chicks did not significantly reduce SE recovery. However, B11 treatment, by either spray or DW application significantly (p<0.05) reduced SE recovery (55% and 50% respectively, controls 85%) when chicks were held for 8h prior to challenge and placement. Similarly, when B11 spray treatment and challenge occurred simultaneously, with placement 8h after treatment, a marked and significant reduction of SE recovery was noted after 5d (55% controls, 10% treated). In Exp. 2, when B11 spray treatment and challenge occurred simultaneously, with placement 8h after treatment, a significant reduction of SE recovery was noted in both the spray and DW application (80% controls, 15% spray, 15% DW). Taken together, these results suggest that spray application of B11, when performed in

the manner described above, can be effective for protection of chicks against Salmonella infection.

Key Words: *Salmonella*, probiotic, hatchery

T57 Methods to improve the economics of egg antibody production. D. L. Trott*¹, M. Yang¹, P. L. Utterback², K. W. Koelkebeck², and M. E. Cook¹, ¹University of Wisconsin, Madison, ²University of Illinois, Urbana.

The number of markets where egg antibody can be utilized is limited by egg cost. A series of experiments were conducted to: 1) attempt to decrease the cost of antibody production by using molted/spent hens; or 2) to increase egg antibody levels by modification of adjuvant or immunization schedule. In all experiments, hens received a standard immunization (i.m. injection of 1 ml over 4 sites of Freund complete adjuvant (FCA) containing 3mg/ml of phospholipase-A₂ (PLA₂) antigen on day 0 and with Freund incomplete adjuvant on day 7). Antibody titers to PLA₂ in yolk were determined by ELISA. Antibody titers in eggs from 58 week-old non-molted hens compared to 110 week-old molted hens did not differ. In a second molt experiment, antibody titers did not differ between molted and non-molted hens receiving the first immunization either before or after molting period (2x2 factorial). These experiments suggest that the cost of antibody production can be reduced by using molted/spent hens. In a study to increase egg antibody levels, *Escherichia coli* bacterin was added to FCA, and antibody titers were found to be decreased 50% relative to standard immunization (p<0.01). In a second study, adjuvant was modified by addition of *Staphylococcus aureus* bacterin to FCA, and immunization schedules (0,7d v 0,14,28d) were compared in a 2x2 factorial. Addition of *S. aureus* bacterin to FCA increased antibody titers 2.2-fold, and the modified immunization schedule (0,14,28d) increased titers 1.9-fold compared to 0,7d immunization. The combination of *S. aureus* bacterin and modified immunization schedule did not appear to be additive (2.3-fold above standard). In summary, molting did not change egg antibody titers; *E. coli* adjuvant decreased titers; and *S.*

aureus adjuvant or modified injection procedure increased titers. The economics of egg antibody production was improved by using spent laying hens and by enhancing egg antibody levels through use of adjuvant combinations or optimized immunization schedules.

Key Words: egg antibody, molt, immunization protocol

T58 Supplementation of the probiotic MitoMax enhances immune responses to coccidiosis in broiler chickens. S-H. Lee¹, H. Lillehoj*¹, D-W. Park¹, R. Dalloul¹, Y-H Hong¹, E. Lillehoj², and J. Lin³, ¹Animal and Natural Resources Institute, ARS, USDA, Beltsville, Maryland, ²University of Maryland, Baltimore, ³Imagilin Technology, Frederick, Maryland.

Avian coccidiosis is one of the most economically important diseases of the poultry industry. As the banning threats of chemotherapeutic control of coccidiosis increase, the industry looks for new ways to control the disease. As such, we investigated the role of the commercial probiotic MitoMax[®], containing *Pediococcus* and *Saccharomyces*, on coccidiosis using weight gain, oocyst shedding, splenocyte proliferation, and antibody responses as parameters. Day-old broiler chicks were fed regular (REG) or probiotic- diets supplemented with MitoMax at 0.01% (MM 0.01), 0.1% (MM 0.1), or 1.0% (MM 1.0) of diet, and challenged two weeks later with 5,000 oocysts of either *Eimeria acervulina* (EA) or *Eimeria tenella* (ET). All groups fed the MitoMax-supplemented diets and infected with EA or ET showed lower oocyst shedding with no significant effect on weight gains. Splenocytes of chickens fed 0.1% MitoMax and tested 10 days post infection showed increased (P < 0.05) proliferation rates compared with those of birds fed the other diets. Further, MM 0.1 birds infected with EA exhibited higher (P < 0.05) serum *Eimeria*-specific antibodies than other groups. These results demonstrate that MitoMax may enhance the resistance of birds against coccidiosis when included at 0.1% of the broiler diet.

Key Words: probiotic, broiler, coccidiosis