Growing broilers on wire flooring provides an excellent experimental model for exposing susceptibility to lameness attributable to bacterial chondronecrosis with osteomyelitis. Two independent experiments (E1, E2) were designed to compare the susceptibilities of broilers from 4 commercial crosses (W, X, Y, Z). The standard crosses (W, Y) grow rapidly at an early age, whereas high-yield crosses (X, Z) initially tend to grow more slowly. Chicks were obtained from a commercial hatchery for E1 or were hatched at the Univ. of Arkansas Poultry Research Hatchery for E2. Males and females were reared together (E1; n = 360/cross) or separately (E2; n = 390/cross) in 3 × 3 m pens on litter (Litter) or wire flooring (Wire). Necropsies revealed lesions that are pathognomonic for BCO in ≥ 94% of the birds that became lame. The SigmaStat Z-test was used to compare cumulative lameness incidences at 8 wk of age. For birds reared on Litter, lameness incidences were low and did not differ between crosses or sexes (range: 2.2% to 4.6%; P ≥ 0.6). When males were reared on Wire their lameness incidences (by cross) were: E1 = 52%X, 42%Y, 69%Z, and 44%bc; E2 = 31%W, 19%X, 49%Y, and 25%Zbc. For females reared on Wire the lameness incidences were: E1 = 40%Wb, 30%Xc, 49%c, and 28%Zc; E2 = 16%W, 15%X, 16%Y, and 15%Z (ns). Accordingly the hierarchical ranking for BCO susceptibility by broiler cross was X ≤ Z ≤ W.

Key Words: lameness, broiler, osteomyelitis, bone, growth


Bacterial chondronecrosis with osteomyelitis (BCO) is recognized as an important cause of lameness in commercial broilers. Relatively little is known about the microbial communities associated with BCO. This study was conducted to increase our understanding of the microbial factors associated with BCO using a culture-independent approach. Using high-throughput Illumina sequencing of the hyper-variable region V6 in the 16S rRNA gene, we characterized the bacterial communities in 97 femoral or tibial heads from broilers carefully selected to represent diverse variations in age, line, lesion type, floor type, clinical status and bone type. Our in-depth survey based on 14 million assembled sequence reads revealed that complex bacterial communities existed in all samples, including normal bones from healthy birds. Overall, Proteobacteria (mean 90.9%) is the most common phylum, followed by Firmicutes (6.1%) and Actinobacteria (2.6%), accounting for more than 99% of all reads. The statistical analysis using analysis of similarity (ANOSIM) suggested that there are distinct bacterial communities in different bone types (femur vs. tibia), lesion types [normal, FHS (proximal femoral head separation or ephiphyseolysis), FHN (proximal femoral head necrosis), THN (tibial head necrosis), and THNc (tibial head necrosis severe or caseous)], and individual birds. Interestingly, there were general trends that the diversity within a bacterial community (α-diversity), highest in normal bones and at 1 week of age, decreased with progression of necrosis and aging, respectively. These observations suggest that there are selection processes toward certain bacterial subgroups in association with the development of BCO and aging. Understanding the microbial species associated with BCO will provide valuable insights for development of strategies to mitigate lameness in broiler chickens.

Key Words: microbiota, pyrosequencing, necrotic enteritis, Clostridium, lesion

P454 16S pyrosequencing of the intestinal microbiota of broiler chickens challenged with Clostridium perfringens. J. A. Benson, L. Romero, G. F. Mathis, and E. A. Galbraith.

The intestinal microbiota of poultry has major impacts on bird health and growth. Enteric diseases are costly to producers due to increased mortality and decreased growth performance. A strong understanding of the microbiota will enable producers to maintain a healthy gut balance to maximize growth performance and disease resistance. In this study we compared the intestinal microbiota of broilers subjected to a necrotic enteritis disease challenge to unchallenged birds to understand the effects of this enteric disease on the intestinal microbiota. Day-old chicks were randomly assigned to challenged or control treatments in one of 14 floor pens (50 chicks/pen). On d 19, 20, and 21 challenged birds were dosed with a broth culture of Clostridium perfringens at 1 × 10⁸ to 1 × 10⁹ cfu administered in the feed. On d 22, 5 birds per pen were examined for intestinal lesions. Cecum and jejunum samples from 8 birds per treatment were collected for microbiota profiling on d 11, 21, and 42. DNA was isolated from each sample and DNA from 2 birds was pooled before 16S rDNA pyrosequencing. Lesions were observed in 22 challenged and 1 unchallenged bird. Sequence data indicated vastly different microbial communities in the cecum and jejunum. Several genera of the order Clostridiales were more abundant in the ceca (P < 0.001), while the jejunum had higher levels of genera within the Lactobacillales (P < 0.001). A progression of the microbiota with age was also observed. In the jejunum, proportions of Enterococcus decreased (P = 0.05), while Lactobacillus trended higher (P = 0.06) with age. Major shifts in the microbiota due to challenge were not observed and very few C. perfringens sequences were detected. This finding suggests that the necrotic enteritis symptoms observed in the challenged birds may have resulted from toxins present in the inoculum rather than active infection. The thorough characterization of broiler chicken microbiota in the jejunum and ceca and its progression with age in this study did not reveal major impacts of necrotic enteritis challenge on the microbiota.

Key Words: microbiota, pyrosequencing, necrotic enteritis, Clostridium, lesion

P455 Effect of olive leaf as medicinal plant supplementation on immune system and heart related ascites indices in broilers reared under normal and cold temperature conditions. S. Varmaghani, S. Rahimi, M. A. Karimi Torshizi, H. Lotfollahian, and M. Hassanzadeh.

The intestinal microbiota of poultry has major impacts on bird health and growth. Enteric diseases are costly to producers due to increased mortality and decreased growth performance. A strong understanding of the microbiota will enable producers to maintain a healthy gut balance to maximize growth performance and disease resistance. In this study we compared the intestinal microbiota of broilers subjected to a necrotic enteritis disease challenge to unchallenged birds to understand the effects of this enteric disease on the intestinal microbiota. Day-old chicks were randomly assigned to challenged or control treatments in one of 14 floor pens (50 chicks/pen). On d 19, 20, and 21 challenged birds were dosed with a broth culture of Clostridium perfringens at 1 × 10⁸ to 1 × 10⁹ cfu administered in the feed. On d 22, 5 birds per pen were examined for intestinal lesions. Cecum and jejunum samples from 8 birds per treatment were collected for microbiota profiling on d 11, 21, and 42. DNA was isolated from each sample and DNA from 2 birds was pooled before 16S rDNA pyrosequencing. Lesions were observed in 22 challenged and 1 unchallenged bird. Sequence data indicated vastly different microbial communities in the cecum and jejunum. Several genera of the order Clostridiales were more abundant in the ceca (P < 0.001), while the jejunum had higher levels of genera within the Lactobacillales (P < 0.001). A progression of the microbiota with age was also observed. In the jejunum, proportions of Enterococcus decreased (P = 0.05), while Lactobacillus trended higher (P = 0.06) with age. Major shifts in the microbiota due to challenge were not observed and very few C. perfringens sequences were detected. This finding suggests that the necrotic enteritis symptoms observed in the challenged birds may have resulted from toxins present in the inoculum rather than active infection. The thorough characterization of broiler chicken microbiota in the jejunum and ceca and its progression with age in this study did not reveal major impacts of necrotic enteritis challenge on the microbiota.

Key Words: microbiota, pyrosequencing, necrotic enteritis, Clostridium, lesion

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This research was conducted to evaluate the effects of dried olive leaf (Olea europaea) on immune system and heart related ascites indices in male broilers under normal and cold temperature conditions. For the normal temperature conditions, 500 d-old male broilers divided into 5 experimental groups: control group; antihypertensive medicine group (propranolol, 4 mg/kg body weight); and 3 groups with olive leaf supplementation at 5, 10, or 15 g/kg diet. The experiments were performed in 4 replicates of 25 birds per pen. The same grouping with another 500 birds was used for the cold temperature conditions. Growth performance, immune system and ascites indices (right ventricle [RV] and total ventricle [TV]) weight and RV/TV were evaluated. At the end of the experiment on d 42, 40 birds per group were randomly selected, sacrificed, and used for weight of bursa fabricius and spleen, and RV/TV determination. Under both temperature conditions, weight gain and feed conversion ratio were similar among all groups, except the propranolol-treated groups. Relative weight of bursa Fabricius and spleen were unaffected by treatments. Ascites-related mortality decreased linearly with increasing olive leaf supplementation under both conditions (P < 0.05). Heterophils percentage and heterophil:lymphocyte ratios between treatments were significantly different (P < 0.01) under both temperature conditions. Increasing olive leaf supplementation was associated with a linear decrease in RV/TV under the normal and a linear decrease in RV, TV, and RV/TV under cold temperature conditions (P < 0.001). We conclude that dietary olive leaf supplementation at a dose of 10 g/kg decreases ascites incidence without impairing broiler performance under normal and cold ambient temperatures.

**Key Words:** olive leaf, immune system, heart, ascites, broiler

P456  **Endothelin-1 expression in the lung arteries of pulmonary hypertensive and non-pulmonary hypertensive chickens.** L. C. Monroy* and A. Hernandez, Universidad Nacional de Colombia, Bogota, Cundinamarca, Colombia.

Genetically predisposed chickens develop pulmonary arterial hypertension (PAH) when subjected to hypobaric hypoxia and/or low ambient temperatures. Hypobaric hypoxia generates endothelial dysfunction, which results in an imbalance between vasodilators and vasoconstrictors. Within the latter, endothelin-1 (ET-1), a potent endogenous agent, induces contraction of pulmonary vascular muscle cells, following its liberation from endothelial cells. To find possible differences in ET-1 expression in the muscle layer of lung arteries in pulmonary hypertensive chickens (PHC) and non-pulmonary hypertensive chickens (NPHC), 2 groups of broilers were exposed to either relative normoxia (n = 145) or hypobaric hypoxia (n = 145) at 310 and 2638 m above sea level from d 0 to 33 of the growing period, respectively. Broilers were classified as PHC when their cardiac mass index (CI) values were over 26, and as NPHC when those values were under 26. The CI = right ventricular weight / total ventricular mass weight multiplied by 100. The expression of ET-1 protein (mouse monoclonal anti-endothelin1, Thermo scientific MA3-005) in the muscular tunic of pulmonary arteries was evaluated by immunohistochemistry (IHC) Supersensitive Polymar-HRP IHC Detection System (Biogenex). Ten blood vessels with 2 to 8 muscle layers in the middle tunic per animal (4 birds/group) were analyzed under the light microscope using an automated software (Image-Proplus 7), as to calculate the percentage of cells expressing ET-1. Incidence of PAH was 22 and 0% in birds kept under hypobaric hypoxia and relative normoxia, respectively. ET-1 expression was higher in broilers exposed to hypoxia (P < 0.01) than birds under relative normoxia. PHC had higher ET-1 expression than healthy chickens subjected to hypoxia (P < 0.01). Hypoxic PHC develop a mechanism which involves endothelial ET-1 expression. ET-1 goes to the muscle cells and provokes contraction as a part of the well-known compensatory mechanism of augmenting blood perfusion.

**Key Words:** chickens, pulmonary hypertension, hypoxia, endothelin 1, immunohistochemistry

P457  **Effects of different pre-lay Mycoplasma gallisepticum vaccines in conjunction with an F-strain M. gallisepticum challenge overlay on the blood characteristics of commercial layers.** R. Jacob*1, S. L. Branton2, and E. D. Peebles1, 1Mississippi State University, Mississippi State, 2USDA-ARS, Mississippi State, MS.

*Mycoplasma gallisepticum* (MG) is a major and economically significant pathogen of avian species. When administered during pre-lay, F-strain MG (FMG) can reduce egg production during lay, but the ts-11 strain of MG (ts-11MG) does not exert this effect. Two trials were conducted to determine the effects of pre-lay vaccinations of ts-11MG, MG-Bacterin, or their combination, in conjunction with an FMG challenge overlay after peak production on the blood characteristics of commercial layers. 160 mycoplasma-free Hy-Line W-36 layers were housed in negative pressure biological isolation units (4 units per treatment, 10 birds per unit) through 52 wk of age (woa). The following treatments were utilized at 10 woa: 1) Control (no vaccinations); 2) ts-11MG vaccine; 3) MG-Bacterin; and 4) ts-11MG vaccine and MG-Bacterin combination. Commercially available MG vaccines were used according to manufacturer’s recommended dosages for all vaccinations. At 45 woa, half of the birds were challenged with a laboratory stock of the 99th passage of FMG. Parameters measured in both trials were whole blood hematocrit, serum protein, serum cholesterol, serum triglycerides, and serum calcium. Results were considered significant at P ≤ 0.05. Significant age × treatment interactions were observed for serum cholesterol levels before and after the FMG challenge and for serum protein levels after the FMG challenge. Serum protein levels were significantly reduced in the control birds during 52 woa, irrespective of the FMG challenge. Therefore, it is evident that although the use of the ts-11MG vaccine alone or in combination with MG-Bacterin may influence circulating cholesterol and protein concentrations when administered during prelay, it remains effective in protecting against the adverse effect of a post peak challenge of FMG on egg production.

**Key Words:** poultry, Mycoplasma gallisepticum, vaccine, mycoplasmosis