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339P Determination of maternally derived antibodies in progeny of parents vaccinated with a live attenuated *Mycoplasma gallisepticum* vaccine. Jeffrey D. Evans*, Scott L. Branton, Spencer A. Leigh, and Stephanie D. Collier, *USDA-ARS Poultry Research Unit, Mississippi State, MS.*

Following a challenge with a virulent strain of *Mycoplasma gallisepticum* (MG), breeder chickens may pass maternal antibodies to their offspring via the egg. While their presence in the offspring is only short-lived, the antibodies may play an important role in protecting the developing embryo and young chick. Live-attenuated vaccines (LAVs) for MG are commonly applied to layer pullets within the commercial egg layer industry to protect against losses associated with challenges by virulent field strains of MG and certain MG LAVs elicit an easily detectable immune response in the vaccinated host. To evaluate the potential for the passage of maternal antibodies from hens vaccinated with an MG LAV to progeny, MG-free pullets and cockerels were obtained from a commercial source and were vaccinated at 10 wks of age with an MG LAV. They were maintained separately in an open house facility through 15 wks of age. At this time, the breeders were placed in a common breeder facility where they remained through study termination. Serum plate agglutination (SPA) assays utilizing MG specific antigen were utilized to assess the presence of IgM antibody in all study-related sera. At 5 wk intervals between 30 and 50 wk of age and at 60 wk of age, a random subsample ($n = 20$) of the parent birds were bled from the wing vein and eggs were collected and incubated for 22 d. At hatch, progeny were immediately sampled (<1 d of age) via the jugular vein. All (100%) parents tested during wk 30–50 were positive for MG-related IgM, but at wk 60 only 95% were SPA positive. Progeny testing per period averaged 117 ± 18.25 chicks per sampling and demonstrated SPA positive rates of 74.5, 94.2, 81.3, 92.8, 90.6, and 88.8% for wk 30, 35, 40, 45, 50, and 60, respectively. Results indicate both the immune response of the hens vaccinated with the MG LAV and the transmission of the associated maternally derived IgM antibody to the progeny.

Key Words: *Mycoplasma gallisepticum*, vaccine, egg transmission, maternal antibody

340P Comparison of accuracy, cost, and starting cells counts of two methods for *Histomonas meleagridis* in vitro cell screen assays. Caitlin E. Harris*, Miguel A. Barrios, Anna P. Kenyon, and Robert B. Beckstead, *University of Georgia, Athens, GA.*

Histomonas meleagridis is an anaerobic protozoa and the causative agent of blackhead disease. Current methods used to grow *H. meleagridis* for in vitro experiments include 96-well plates or tissue culture flasks. Therefore, the objective was to determine which method had decreased error and higher consistency. Furthermore, cost analysis and initial starting cell counts were studied. For the well method, there were 4 plates used, 3 replications per treatment, and the total volume (Dwyer's media + treatment) per well was 300 μ L. Treatments used for this method were 20,000, 15,000, 10,000, 8,000, 4,000, and 2,000 cells. Plates were incubated at 42°C for 8, 16, 24, and 32 h. For the flask method, each flask represented a replication. Three replications were used per treatment and the total volume (Dwyer's media + treatment) per flask was 11 mL. Treatments for this method were 200,000, 150,000, 100,000, 60,000, 20,000, and 2,000 cells. Flasks were incubated at 42°C for 8, 16, 24, and 32 h. The range of cell numbers counted at each period was 0 to 5 for the well method and 10 to 30 for the flask method. Loading error

was a concern for the well method because if the hemocytometer was loaded incorrectly, cell counts were less accurate than compared with the flask method. Consistency was increased using the flask method because the same cells per replication were counted for each period compared with the well method where there were multiple plates used for each replication. Optimal starting cells counts were 20,000 cells per well and 100,000 – 200,000 cells per flask. Due to limited space in the 96-well plates, inoculating with 20,000 cells per well would likely expend the nutrients in the media before cells were to be counted. A range of 100,000 – 200,000 cells per flask makes the flask method more ideal because there is an improved margin of error. Cost analysis revealed that the well method reduced overall cost. Further research may include testing alternative products that may be used to prevent blackhead disease in turkeys.

Key Words: blackhead disease, *Histomonas meleagridis*, in vitro cell screen assay, turkey, broiler

341P Effects of essential oils on lipopolysaccharide-induced acute-phase response in broiler chickens. Encun Du*, Changwu Li, and Yuming Guo, *China Agricultural University, Beijing, China.*

One experiment was carried out to evaluate the modulatory effects of essential oils (EO) on lipopolysaccharide (LPS)-induced acute-phase response in broiler chickens. The EO used in the present study was a combination of thymol and carvacrol. One hundred and 40 4 broiler chickens (7-d old) with similar weight were randomly allocated to 4 treatments following a 2×2 factorial arrangement to study the effects of EO addition (with or without EO at 60mg/kg corn-soybean basal diet), LPS injection (with or without LPS injection) and their interactions. Each treatment group consisted of 6 replicate cages with 6 birds per cage. On d 15, 17, 19, and 21, birds were injected intraperitoneally with LPS at 1mg/kg of BW, or the same amount of sterile saline. One bird from each replicate was euthanized for sampling at 8 h after the final injection on d 21. RNA was extracted from spleen for RT-PCR. Data were analyzed using the GLM procedure of SAS software. During d 7 to 21 and d 14 to 21, repeated LPS injections significantly decreased BW gain and feed intake ($P < 0.05$), but didn't affect feed conversion ratio ($P > 0.10$). The relative weight of spleen ($P < 0.05$) and liver ($P < 0.10$) was increased after LPS injections. Besides, LPS injections enhanced the gene expression of IL-1 β in spleen ($P < 0.05$). Dietary supplementation of EO had no influence on growth performance and lymphoid organ weight of broiler chickens ($P > 0.10$). However, gene expression of TNF- α and TLR-4 was downregulated ($P < 0.05$) in spleen with the addition of EO, and the gene expression of anti-inflammatory cytokine IL-4 was upregulated significantly ($P < 0.05$). In conclusion, immune-modulatory effects of EO against LPS challenge was significant, and the TLR-4 and downstream signaling pathways might be associated with the immune-modulatory effects of EO.

Key Words: broiler, lipopolysaccharide, essential oil, spleen, inflammation

342P Effects of essential oils on growth performance and intestinal functionality during a coccidial vaccine challenge in broiler chickens. Encun Du*¹, Todd J. Applegate², Yuming Guo¹, G. Raj Murugesan³, Qian Zhang², Xi Chen², and Susan D. Eicher⁴, ¹China Agricultural University, Beijing, China, ²Purdue University, West Lafayette, IN, ³Biomim America Inc., San Antonio, TX, ⁴USDA-ARS, West Lafayette, IN.

This study was conducted to evaluate the modulatory effects of essential oils (EO) on growth performance and intestinal functionality during a coccidial vaccine challenge in broiler chickens. The EO blend used in the present study contained oregano oil, anise oil and citrus oil. A total of 168 broiler chickens were randomly distributed to 4 treatments, following a 2×2 factorial arrangement to study the effects of EO addition (with or without EO at 125mg/kg basal diet), coccidial vaccine challenge (with or without a $20 \times$ recommended dosage of coccidial vaccine challenge) and their interactions. Each treatment group consisted of 6 replicate cages (7 birds per cage). On d 14, birds were orally administered with mixed *Eimeria* vaccine or water. On d 21, one bird from each replicate was euthanized. During the first wk post challenge, BW gain ($P < 0.05$) and feed intake ($P = 0.055$) were reduced, and feed:gain ratio was increased ($P < 0.05$) in challenged birds, as was reflected in jejunal morphology as evidenced by shorter villi, deeper crypt and decreased goblet cells ($P < 0.05$) on d 21. Besides, the activity of jejunum mucosal sucrase was inhibited in challenged birds ($P < 0.05$). Coccidial challenge also upregulated the gene expression of inflammatory cytokines (IFN- γ and IL-1 β) and downregulated the gene expression of occludin ($P < 0.05$). However, the expression of inflammation related genes were not influenced by coccidial vaccine challenge in cecal tonsils ($P > 0.10$). In the present study, dietary supplementation of EO did not improve growth performance, jejunal histological parameters, mucosal disaccharides activity, or intestinal lesions ($P > 0.10$). Further, EO addition had no effect on gene expression of IFN- γ , IL-1 β and occludin ($P > 0.10$) in the jejunum. However, EO decreased oocyte numbers in the excreta and alleviated bloody diarrhea on d 21 ($P < 0.05$). In conclusion, EO did not completely alleviate the retarded growth performance and the impaired intestinal functionality caused by coccidial vaccine challenge in the present study.

Key Words: broiler, essential oil, coccidial, intestine, inflammation

343P Effect of feeding a live yeast and a combination of organic acids and essential oils on *Campylobacter* colonization in broilers. Marta I. Gracia¹, Oscar Casabuena¹, Fernando Sánchez², Julie Mayot³, and Pedro Medel^{1*}, ¹Imasde Agroalimentaria, S.L., Madrid, Spain, ²Explotaciones Avícolas Redondo, Pantoja, Toledo, Spain, ³FIA, Paris, France.

An experiment was conducted within the EU-FP7 project CAMPYBRO to evaluate the effect of a live yeast (*S. cerevisiae*, Product A) and a blend of organic acids and essential oils (Product B) added to the feed on *Campylobacter* counts in broilers. There were 3 treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1 + Product A at 50 g/t and T3: T1 + Product B at 5,000 g/t. A total of 126 one-day-old Ross 308 broilers (half male and half female) were divided into the experimental treatments. At 14 d of age, all broilers were orally gavaged with 100 μ L of a solution containing 1×10^5 cfu/mL of ST-45 *C. jejuni* strain. On d 21, 35 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data expressed as log₁₀ cfu/g ceca content were first tested for normality and then analyzed by GLM procedure of SPSS v.19.0. No significant differences in the *Campylobacter* counts were observed between treatments at any of the times evaluated: 21 (7.40, 7.77 and 7.32 log₁₀ cfu/g; $P = 0.67$), 35 (7.38, 7.13 and 7.03 log₁₀ cfu/g; $P = 0.74$) and 42 d of age (7.71, 7.44 and 7.83 log₁₀ cfu/g; $P = 0.40$), for control, Product A and Product B treatments, respectively. It is concluded that the live yeast and the combination of organic acids and essential oils tested did not affect *Campylobacter jejuni* population under the experimental model assayed.

Key Words: *S. cerevisiae*, organic acids, essential oils, *Campylobacter jejuni*, broiler

344P Peritoneal exudate cells as a potential source of primary dendritic cells. Christine N. Vuong^{*1}, Wen-Ko Chou², and Luc R. Berghman^{1,2}, ¹Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, ²Department of Poultry Science, Texas A&M University, College Station, TX 77843.

Dendritic cells (DCs) are important antigen presenting cells and are the least characterized immune cell within the chicken. To obtain chicken DCs, current protocols require the isolation of bone marrow myeloid progenitor cells and the induction of dendritic cell differentiation with GM-CSF and IL-4 cytokines. Chicken peritoneal exudate cells (PECs) have traditionally been a source of various immune cells for ex vivo studies, primarily heterophils and macrophages. In this study, we observe the presence of dendritic cells within the PEC as an alternative method to isolate and study chicken primary DCs. Chickens were intraperitoneally injected with Sephadex and their immune cells isolated from flushed peritoneal cavities 42 h post-injection. From the pool of PECs, macrophages were removed by plastic adherence and the non-adherent portion screened for the presence of DCs. We found cells positive for expression of CD205 (a dendritic cell marker) via ICC of fixed PECs and RT-PCR. This suggests PECs can be used as a source of CD205⁺ dendritic cells for study in the chicken system.

Key Words: dendritic cell, CD205, peritoneal exudate cell

345P Withdrawn.

346P Dietary live yeast supplementation attenuates lipopolysaccharide-induced inflammation in broilers. W. W. Wang^{*1}, W. L. Ren¹, Z. Li¹, Y. M. Guo¹, B. Zhang², and R. D'inca³, ¹College of Animal Science and Technology, China Agricultural University, Beijing, China, ²Phileo Lesaffre Animal Care, Beijing, China, ³Phileo Lesaffre Animal Care, Lille, France.

The experiment with 3×2 factorial arrangement was carried out to evaluate the effects of supplemental live yeast (LY, *Saccharomyces cerevisiae*) on inflammatory responses induced by lipopolysaccharide (LPS) in broilers. The 2 treatments are dietary supplementation with live yeast at 0, 0.05% or 0.50% and immunological challenge with injection of saline or LPS at 1.5 mg/kg of body weight, respectively, on every other day between 21 and 27 d of age. Each treatment has 8 replicates of 10 birds. Samples were respectively obtained at 3 and 8 h post the first challenge (PFC) and last challenge (PLC). Results showed that dietary LY did not affect ($P > 0.05$) relative spleen weight and serum IgA and IgM levels regardless of immunological status, but tended to elevate ($P = 0.083$) serum IgG level at 3 h PFC. Supplemental LY at 0.05% elevated ($P = 0.037$) serum α -acidglycoprotein level in unchallenged birds and LY at 0.50% alleviated ($P < 0.05$) LPS-induced increase ($P < 0.05$) in serum lysozyme activity at 3 h PFC. Splenic TLR4 expression of unchallenged birds was enhanced ($P < 0.05$) by supplemental LY at 0.05%, which also reduced ($P < 0.05$) splenic IL-1 β expression 8 h after challenge when LPS ballooned ($P < 0.05$) it. Supplemental LY at 0.05% lowered ($P < 0.05$) splenic IL-1 β and NF- κ B expression at 8 h PLC when elevated ($P < 0.05$) by LPS challenge. Comparatively, supplemental LY at 0.50% relieved ($P < 0.05$) LPS-induced splenic TLR4 overexpression ($P < 0.05$) at 3 h PFC, enhanced ($P < 0.05$) LPS-induced upregulation ($P < 0.05$) of splenic IL-10 expression 3 h after challenge, and reduced ($P < 0.05$) splenic IL-1 β and TLR4 expression at 8 h PFC when elevated ($P < 0.05$) by LPS challenge. In conclusion, supplemental LY at 0.05% boosted humoral immunity of broilers, while supplemental LY at 0.50% attenuated the LPS-induced inflammation probably through suppressing TLR4 pathway and upregulating IL-10 expression.

Key Words: live yeast, lipopolysaccharide, inflammation, TLR4, IL-10

347P Effects of live yeast on immune response and intestinal morphological structure in lipopolysaccharide-challenged broilers. W. W. Wang^{*1}, W. L. Ren¹, Z. Li¹, Y. M. Guo¹, B. Zhang², and R. D'inca³, ¹College of Animal Science and Technology, China Agricultural University, Beijing, China, ²Phileo Lesaffre Animal Care, Beijing, China, ³Phileo Lesaffre Animal Care, Lille, France.

The experiment with 3 × 2 factorial arrangement was carried out to evaluate the effects of supplemental live yeast (LY, *Saccharomyces cerevisiae*) on immune response and intestinal morphological structure in lipopolysaccharide (LPS)-challenged broilers. The 2 treatments are dietary supplementation with live yeast at 0, 0.05% or 0.50% and immunological challenge with injection of saline or LPS at 1.5 mg/kg of body weight, respectively, on every other day between 21 and 27 d of age. Each treatment has 8 replicates of 10 birds. Serum samples were obtained at 3 h post the first and last injection, whole blood and intestine samples were collected at 24 h post the last injection. Results showed that LY addition did not affect ($P > 0.05$) growth performance, lymphocytes proliferation and monocytes phagocytosis regardless of immune status. LPS tended to reduce ($P = 0.071$) serum anti-NDV titers after the first injection, but it was enhanced ($P = 0.024$) by dietary supplemental LY at 0.05%. LPS tended to lower ($P < 0.10$) jejunal villus height (VH), ileal villus surface area and reduced ($P = 0.019$) ileal VH. Supplemental LY at 0.50% reduced ($P \leq 0.05$) the crypt depth (CD) and increased ($P \leq 0.05$) the ratio of VH/CD of jejunum and ileum, while LY at 0.05% elevated ($P \leq 0.05$) ileal villus width and villus surface area, and tended to relieve ($P = 0.097$) LPS-induced increase ($P = 0.024$) in ileal secretory IgA level and reduce ($P = 0.08$) ileal *Escherichia coli*, along with a attenuation ($P = 0.001$) of LPS-induced increase ($P < 0.05$) in serum diamine oxidase activity post the first injection. In conclusion, supplemental LY at 0.05% improved humoral immunity and LY at 0.50% relieved LPS-induced injuries of intestinal morphological structure in broilers.

Key Words: live yeast, broiler, immunity, intestinal morphological structure, lipopolysaccharide

348P Development of a dry medium selective for the isolation of *Histomonas meleagridis*. Miguel A. Barrios*, Anna P. Kenyon, Caitlin E. Harris, and Robert B. Beckstead, *The University of Georgia, Athens, GA.*

Blackhead disease results in mortality rates of 100 and 30% in turkeys and chickens, respectively. Blackhead disease is caused by *Histomonas meleagridis*, an anaerobic protozoan parasite. *H. meleagridis* is currently studied in laboratory settings using Dwyer's medium. Blackhead disease outbreaks are unpredictable and the harvesting of *H. meleagridis* strains from the field would be a significant resource for researchers to study the epidemiology of blackhead disease. Therefore, the objective of this study was to develop a dry medium, which would allow storage at warm temperatures over a long period. Dwyer's medium consists of M199 cell medium, sodium bicarbonate and rice powder, which are all dry compounds. Dwyer's medium also contains horse serum. To prepare a complete dry medium, horse serum was dried down using a speed vacuum. All these compounds were combined, weighed, and placed in cell culture flasks. To test the viability of the fresh dry media, 10 flasks of dry media were kept at 25°C and 60°C overnight, and fresh liquid media served as the control. To test the longevity of the dry media, flasks were stored at 25°C, 37°C, and 42°C for 1, 3, and 6 mo. Fresh media was the control. Dry media was rehydrated with tap water. Each flask was inoculated with 100,000 cells of an *H. meleagridis* strain obtained from a field outbreak. Cells were counted after 24, 48, and 72 h using a Neubauer hemocytometer. When comparing fresh dry and liquid media at different temperatures, all treatments performed similarly ($P > 0.05$).

After the dry media was stored for 1 mo, all dry media had similar cell counts, while fresh liquid media had nearly double the amount of cells ($P < 0.05$). When dry media was examined at 3 mo, all dry media performed alike; furthermore, all dry media had similar counts as the control after 72 h. Taken together these results show that this dry medium may be used as an epidemiological tool to obtain *H. meleagridis* samples from the field. Further work may be necessary to establish the longevity of dry media at cool temperatures.

Key Words: blackhead disease, *Histomonas meleagridis*, Dwyer's media, turkeys, in vitro

349P Effect of feeding polyphenols on *Campylobacter* counts in broilers. Patricia Vázquez, Carlos Millán, Silvia Porras, Jaime Sánchez, and Pedro Medel*, *Imasde Agroalimentaria, S.L., Madrid, Spain.*

An experiment was conducted to evaluate the effect of a blend of polyphenols (PPh; 1% hydroxybenzoic acids) added to the feed on cecal *Campylobacter* counts in broilers. There were 2 treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives) and T2: T1 + PPh at 4 kg/t from 0 to 21 d and 8 kg/t from 21 to 42d. A total of 60 one-day-old Ross 308 broilers (half male and half female) were divided into 2 floor pens with wood shavings as litter material, 1 pen/treatment. At 7 d of age, 3 randomly selected broilers per pen were orally gavaged with 100 µL of a solution containing 1×10^5 cfu/mL of ST-45 *C. jejuni* strain. On d 21 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data expressed as log₁₀ cfu/g ceca content were first tested for normality and then analyzed by GLM procedure of SPSS v.19.0. *Campylobacter* counts were significantly reduced by PPh supplementation at 21 (9.36 vs 8.45 log₁₀ cfu/g; $P = 0.013$) and 42 d of age (9.48 vs 6.53 log₁₀ cfu/g; $P = 0.001$). It is concluded that supplementation of broiler diets with a blend of polyphenols is effective reducing *Campylobacter jejuni* at cecal level.

Key Words: polyphenol, *Campylobacter jejuni*, broiler

350P The effect of the incorporation of two cysteines in hemagglutinin transmembrane on the virological features and immunity of H9N2 influenza viruses. Chunyi Xue^{*1}, Kang Liu¹, Yang Wang¹, Qiliang Liu¹, George Dacai Liu², and Yongchang Cao¹, ¹State Key Laboratory of Biocontrol, Life Sciences School, Sun Yat-sen University, Guangzhou, Guangdong, China, ²Firstline Biopharmaceuticals Corporation, Redmond, WA.

H9N2 influenza viruses circulating in chickens are low pathogenic. They could lead to the outbreak of avian influenza, which has great effect on China's poultry industry. Vaccination is the main defense against influenza virus, but current vaccines would lose their effectiveness when vaccine strains are mismatched with circulating strains. Comparison of hemagglutinin (HA) amino acid sequences showed that H3 subtype HA contains 2 particular cysteines in transmembrane (TM) domain, while H9 subtype HA has no cysteines in the corresponding sites. Our early study showed that the TM cysteines in H3 HA have profound effects on the virological features and immunity of H3N2 viruses. In present study, 2 recombinant H9N2 mutant viruses were generated by the mutations of 2 HA TM cysteines or the replacement of HA TM domain of H9 with that of H3 in the background of A/Chicken/Guangdong/69/2009[H9N2] using reverse genetics. Further characterization revealed that the introduction of cysteines in HA TM did not affect the virus assembly and growth, but the recombinant H9N2 mutant viruses exhibited smaller plaque sizes, decreased growth rate in cells, reduced fusion activity, enhanced thermal and acidic resistances, and decreased virulence in

embryonated eggs. BALB/c mice and SPF chickens were immunized with H9N2 recombinant virus inactivated vaccine, and the results showed that the mutant virus vaccine induced higher levels of antibody titer, and provided better protective efficacy when the animals were challenged with different branches of H9N2 viruses. The results demonstrated that HA TM is closely related to the virological features and immunity of H9N2 viruses. These findings would help the development of broad-spectrum H9N2 influenza vaccines.

Key Words: H9N2 influenza virus, hemagglutinin, transmembrane domain, cysteine, immunity

351P Intestinal luminal interleukin-10 during *Eimeria* infection in chickens. Maria K. Arendt*, Jordan Sand, and Mark E. Cook, *University of Wisconsin-Madison, Madison, WI.*

Previous data showed intestinal mucosa interleukin-10 (IL-10) mRNA is upregulated after a coccidia infection. While the role of intraepithelial IL-10 during coccidia infection is not fully understood, the presence of IL-10 receptors on the apical surface of enterocytes suggest a function of luminally secreted IL-10. A study was conducted to measure luminal IL-10 during coccidia infection and the role of feeding an anti-IL-10 antibody on luminal levels. Chicks were fed a diet with or without anti-IL-10 (0.341g/kg feed as egg yolk powder starting at d 1 of age) and with or without 10× dose of Advent coccidiosis vaccine (*Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*) at 3 d of age (2 × 2 factorial arrangement of treatments). Ten pens of 10 chicks were assigned to each of the 4 treatments in battery brooders with raised wire floor. Intestinal luminal samples were collected from the duodenum, jejunum, ileum, and cecum on d 3, 5, 7, 10, 13, 16, 19, and 22. Diluted samples (1:5 in phosphate buffered saline) were centrifuged, analyzed for total protein, and the IL-10 was determined using a capture ELISA. At d 5, dietary anti-IL-10 decreased the amount of IL-10 in the lumen of the ileum from 0.093 to 0.042 µg IL-10/mg protein ($P < 0.05$). On d 10, luminal IL-10 was lower in the jejunum of the uninfected chicks (0.514 µg IL-10/mg protein) relative to the infected chicks (1.209 µg IL-10/mg protein, $P < 0.05$). In conclusion, IL-10 is present in the intestinal lumen, and IL-10 luminal secretion can be affected by coccidia infection and oral antibody to interleukin-10.

Key Words: coccidia, *Eimeria*, interleukin-10

352P Immunomodulatory effect of levamisole hydrochloride and Mentofin in Newcastle disease-vaccinated commercial broilers. Oluwaseun O. Esan*, Omolade A. Oladele¹, Adebayo A. Ehinmowo¹, John O. Abiola¹, Fidelis Gberindyer², and Adebowale Adebisi¹, ¹*University of Ibadan, Ibadan, Oyo State, Nigeria.*, ²*University of Agriculture, Makurdi, Benue State, Nigeria.*

Newcastle disease (ND) is a major constraint to profitable poultry production in Nigeria with outbreaks sometimes occurring in vaccinated flocks. Immunostimulants such as levamisole, vitamin E and selenium have been incorporated into poultry production to enhance vaccinal response. The efficacy of Mentofin, a herbal product with claims of immunomodulation and its synergy with levamisole, were assessed. One hundred 1-d-old Abor Acre broilers were randomly divided into 5 groups of 20 each, reared in open-sided cages and fed ad libitum. Group A was the unvaccinated control; Groups B to E were vaccinated with ND vaccine (LaSota) at 14 and 42 d old. In addition, Group C was administered levamisole HCl at 150mg/kg body weight in drinking water for 3 consecutive days post-vaccination, Group D – Mentofin at 0.25mL/L and Group E – levamisole HCl and Mentofin. Each group was bled at 1, 14, 25, 32, 39 and 56 d-old. ND antibody titers were determined using

ELISA. Mean titers per group at each sampling time were calculated and significant differences were determined using ANOVA and Duncan's multiple range test at $P < 0.05$. Maternal antibody titer of 76.55 ± 2.35 at day-old declined to 3.83 ± 0.37 at 56 d-old in the control Group A. Peak antibody titers in Groups C, D and E (62.3 ± 4.51 , 60.2 ± 3.84 and 64.9 ± 5.58 , respectively) at 14 d post primary vaccination were significantly higher than that of Groups B (42.3 ± 4.28). Also, at 14 d post secondary vaccination, peak titer in group E (77.9 ± 3.14) was significantly higher than in Groups A, B, C and D (3.83 ± 0.37 , 44.0 ± 3.20 , 71.1 ± 3.48 and 70.3 ± 3.25 , respectively). However, values in Groups C and D were significantly higher than that of Group B. This study shows that Mentofin and levamisole HCl administration in drinking water significantly enhanced humoral response to live Newcastle disease vaccination. However, a combination of both caused a small but significant increase above the individual treatments.

Key Words: antibody response, broiler, levamisole, immunomodulatory, Newcastle disease vaccination

353P Validation of a chicken ileal explant culture model for measurement of mucosal inflammation induced by lipopolysaccharide. Qian Zhang*, Susan D. Eicher^{1,2}, Kolapo Ajuwon¹, and Todd J. Applegate¹, ¹*Department of Animal Sciences, Purdue University, West Lafayette, IN.*, ²*Livestock Behavior Research Unit, Agricultural Research Service, United States Department of Agriculture, West Lafayette, IN.*

Gut mucosa holds a single layer of epithelial cells and the largest mass of lymphoid tissue in the body. While epithelial cell culture is widely used to assess intestinal barrier functions, it has limitations for studying cellular interactions with other cells, in particular those of the immune system. In this study, a chicken ileal explant culture model was validated for investigating short-term mucosal inflammation in an ex vivo environment. Initially, ileal explants from broilers at 21 d of age were cultured ex vivo up to 6 h. Explants cultured for a maximum of 2 h remained over 90% viable, based on lactate dehydrogenase (LDH) activity. Morphologically, explant cultured for 2 h display normal outlook compared with those cultured longer, further confirming that short-term culture for 2 h duration is an acceptable model for studying ex vivo regulation of inflammation. Subsequently, LPS dose-related responses were determined for explants cultured for 2 h. Results from LDH activity assay showed that the viability of explants was decreased ($P < 0.05$) at LPS dose higher than 50 µg/mL. A significant ($P < 0.05$) nitric oxide release was observed at LPS concentrations of 10 and 20 µg/mL. In addition, the highest inflammatory response was detected at 20 µg/mL LPS based on expression of TLR-4, iNOS, TNF-α, IL-1β, IL-8, MUC2, IgA and PIgR. These results demonstrate the potential usefulness of this intestinal explant culture model for short-term study of cellular interactions as well as evaluating biological effects of nutrients in an inflammatory state.

Key Words: chicken, intestinal explant culture, inflammation, lipopolysaccharide

354P Effect of type of cereal and oat hulls addition on cecal morphology and *Campylobacter jejuni* colonization of broilers orally infected. Jaime Sánchez¹, Carlos Millán¹, Fernando Sánchez², Y. Carre³, and Pedro Medel^{1*}, ¹*Imasde Agroalimentaria, S.L., Madrid, Spain.*, ²*Explotaciones Avícolas Redondo, Pantoja, Toledo, Spain.*, ³*CIDEF, Rennes, France.*

An experiment was conducted within the EU-FP7 project CAMPYBRO to evaluate the effect of type of cereal and oat hulls (OH) addition in

cecal morphology and *C. jejuni* colonization of broilers orally infected. There were 5 treatments, based on the main cereal of the diet (corn [C], wheat [W] or barley [B]), and the inclusion of 5% of OH in the C and W diets. There were 2 diets (0–21 and 21–42d), and treatments were applied from 1 to 42d. A total of 180 one-d-old Ross 308 broilers (half male and half female) were divided into cages (3 birds/cage) and experimental treatments (12 cages/treatment). At 14 d of age, all broilers were orally gavaged with 100 μ L of a solution containing 1×10^5 cfu/mL of ST-45 *C. jejuni* strain. On d 21, 35 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined. At 35 and 42d, gastrointestinal tract (GIT) and ceca weights, and the pH at ceca were taken. Data were analyzed by GLM procedure of SPSS. Age increased ceca weights, but not when expressed in relation to BW or GIT. There were no significant differences for *Campylobacter* counts between treatments at any of the periods studied. Therefore, neither the type of cereal nor the inclusion of 5% OH caused a significant variation on *C. jejuni* counts at the ceca of the birds. B and W-OH based diets showed the largest ceca expressed as weight (18.7, 11.6, 14.3, 14.8 and 18.0g for B, C, C-OH, W and W-OH diets, respectively, $P < 0.001$), percentage of GIT (8.98, 6.73, 7.97, 8.21 and 9.63% for B, C, C-OH, W and W-OH diets, respectively, $P < 0.001$), percentage of BW (0.93, 0.60, 0.74, 0.73 and 0.93% for B, C, C-OH, W and W-OH diets, respectively, $P < 0.001$), or percentage of empty BW (1.04, 0.65, 0.82, 0.80 and 1.03% for B, C, C-OH, W and W-OH diets, respectively, $P < 0.001$), showing the C-based diets the smallest values, and the rest of treatments intermediate values. Dietary treatment did not affect pH at ceca. It is concluded that neither the type of cereal nor the OH addition modifies the cecal pH or *Campylobacter* counts in birds orally infected but changed the ceca morphology.

Key Words: *Campylobacter jejuni*, broiler, oat hulls, cereal

355P Withdrawn.

356P The effect of β -glucans on performance and response of broiler chicks during coccidiosis. Chris P. Ott^{*1}, Mike E. Persia¹, Rob L. Payne², and Rami A. Dalloul¹, ¹Animal & Poultry Sciences, Virginia Tech, Blacksburg, VA, ²Evonik Industries, Kennesaw, GA.

Coccidiosis is a costly parasitic disease to the poultry industry with multiple prevention methods being explored to control its impact. This experiment evaluated the feeding effects of β -glucans alone (BG) or with zinc (BGZn), on performance and responses of 28-d-old broiler chickens during a coccidiosis challenge. Cobb 500 male broilers ($n = 1280$) were assigned to 1 of 8 treatment groups (8 replicate pens; 20 birds/pen) in a 2×4 factorial arrangement, including non-infected and *Eimeria*-infected birds fed for 28 d a control diet, control + BG (150 g/MT Algamune 50), control + BGZn (100 g/MT Algamune 50Zn), and control + 0.01% Salinomycin (Sal). On d15, all birds received either a sham inoculation or a mixed *Eimeria* inoculum. Birds and feed were weighed weekly on a per pen basis to evaluate body weight gain (BWG), feed intake (FI), and feed conversion ratios (FCR). Lesion scores were assessed 6 d post infection (d21) on 3 birds per pen. Performance data were subjected to ANOVA and differences were established using the LS-MEANS statement with significance reported at $P \leq 0.05$. There were few lesion score differences among the dietary treatments in the infected groups, suggesting that diets had little direct effects on lesions. The cocci challenge main effect resulted in a significant reduction in 0–28 d BW and FI. Dietary treatment resulted in little effect on BWG, but Sal addition resulted in increased FI. A significant interaction between challenge and diet resulted in higher FCR in the cocci-challenged birds supplemented with Sal and BGZn in comparison to the other challenged

groups, likely due to reduced mortality in the challenged Sal and BGZn groups. Overall mortality was high (20%) and increased with the challenge but reduced with the Sal treatment. The BGZn treatment resulted in mortality intermediate to but not significantly different from any of the other treatments. The high mortality in this experiment complicates the interpretation, but the overall results suggest that Sal and BGZn had positive effects on performance regardless of the cocci challenge.

Key Words: broiler, performance, β -glucan, coccidiosis, *Eimeria*

357P Evidence of horizontal transmission of *Mycoplasma gallisepticum* from in ovo-vaccinated layer chickens. Katie E. Collins^{*1}, Scott L. Branton², Jeff D. Evans², Sharon K. Womack¹, and Edgar D. Peebles¹, ¹Department of Poultry Science Mississippi State University, Mississippi State, MS, ²USDA-ARS, Mississippi State, MS.

Mycoplasma gallisepticum (MG) is known to be transmitted from infected birds or fomites. This study was performed to determine the transmissibility of a live attenuated MG vaccine from in ovo-vaccinated chicks. Eggs from an MG clean flock were incubated together for 18 d, at which point all live embryonated eggs were either not injected (NI) or administered a 50 μ L injection volume of a dilution (using Poulvac Marek's diluent) of a live attenuated strain-F MG vaccine (Poulvac Myco F) of a high ($1 \times$ resuspended vaccine), medium (10^{-2} dilution), low (10^{-4} dilution), or low-low dose (10^{-6} dilution). NI eggs were hatched in a separate incubator. Ten NI and 1 in ovo-vaccinated chick at one of the 4 doses (low-low, low, medium, high) were each placed in 32 Horsfall-Bauer units located in 2 rooms. Room was considered as a blocking factor with 4 replicate units per room per injection treatment. Mortality was removed from each unit daily. At 6 wk of age (woa), birds that had been injected in ovo were removed and bled for Serum Plate Agglutination (SPA) and ELISA testing for the presence of antibodies against MG. At 12 woa, the remaining NI birds were bled for SPA and ELISA testing. Additional NI chicks were raised on a separate MG clean location and tested SPA and ELISA negative. The percentages of SPA and ELISA positive NI birds from each unit were analyzed with the General Linear Model procedure (SAS 9.4). No NI birds died and out of the in ovo-vaccinated chicks, 1 high, 2 medium, 6 low, and all 8 low-low dose chicks survived to 6 woa. The in ovo-vaccinated birds that survived to 6 woa were both SPA and ELISA positive except for 5 birds in the low-low dose. Percentages of SPA and ELISA positive NI birds did not differ by treatment (SPA $P = 0.7832$; ELISA $P = 0.8689$). SPA positive NI birds were 36.3, 18.8, 18.8, and 20.0% and ELISA positive were 27.5, 18.8, 16.3, and 13.8% in the low-low, low, medium, and high doses, respectively. These findings indicate that in ovo vaccination with a live attenuated MG vaccine yielded a viable population, which could be horizontally transmitted to other chicks.

Key Words: in ovo, vaccine, *Mycoplasma gallisepticum*, layer, embryo

358P Response of broiler chickens challenged with *Salmonella* Enteritidis or Minnesota supplemented with mannan oligosaccharide. Mariana C. Lourenço^{*}, Antonio L. Kraieski, Ricardo M. Hayashi, Alexandre M. de Souza, Dany Mesa, and Elizabeth Santin, Federal University of Paraná, Curitiba, Paraná, Brazil.

The control of *Salmonella* occurrence and spread is essential to ensure the safety of chicken products. Biosecurity practices are the most important methods for preventing *Salmonella* in commercial flocks. In addition, the use of prebiotics may help in this control acting directly on agglutination of bacteria type 1 fimbriae preventing it from connecting the GTI cells and also by immune modulation effects. This trial evaluated the effect of mannan oligosaccharide (MOS) in broilers challenged

orally with 10^6 cfu/mL of *Salmonella* Enteritidis (SE) at 8 d or Minnesota (SM) at 10 d. Several 160-d-old male Cobb broilers were divided in Trial A: 1) SE, birds challenged with SE; 2) SE+MOS, birds challenged with SE supplemented with MOS. Trial B: 3) SM, birds challenged with SM; 4) SM+MOS, birds challenged with SM supplemented with MOS. In both trial, the MOS used was 400g/t, Actigen (Alltech Inc.) since the first day of bird life and weight gain, *Salmonella* recover, ileum and cecum histology and ileum and liver IL10 e IL12 mRNA expression at 0 and 16 h after challenge (AC) were evaluated. At the time of challenge (0h AC), birds supplemented with MOS showed highest score of lymphocyte infiltration on ileum compared with non-supplemented birds, but it was not observed in the cecum mucosa in both trial. In the Trial A, MOS supplementation at 16h AC, reduced the isolation of SE in the liver and cecum, the lymphocyte infiltration on cecum mucosae and the IL12 mRNA expression on liver and increased the weight gain in SE-challenged broilers compared with group not supplemented. In trial B, MOS did not affect any parameters but the ileum mRNA IL10 and IL12 expression was reduced in the group supplemented compared with group SM challenge non-supplemented. The results showed that MOS supplementation increase the lymphocytes infiltration in ileum in birds not challenged and it was effective to control SE, but not have the same response against SM.

Key Words: cytokine, IL10, IL12, immunity, intestinal mucosa

359P Recent discoveries on flax biologicals with unique anti-parasitic properties: A natural approach to control coccidiosis in poultry. Andrew Olkowski* and Bernard Laarveld, *University of Saskatchewan, Saskatoon, SK Canada.*

Recently we discovered rather unique anti-parasitic properties associated with compounds naturally present in flax seeds. One group of the flax biologicals showing anti-parasitic properties were isolated from seeds or flax oil and was identified as small cyclic peptides termed cyclolinopeptides (CLPs). CLPs are isolated from flax seeds or oil using solvent extraction process, and purified using hydrophobic silica based solid phase column. In vitro experiments, where coccidian oocysts were incubated in water at RT overnight, showed that CLPs were effective in preventing sporulation and killing sporulated oocysts at a concentration of about 20 to 40 μ g/mL. In some aspects, the effects of CPLs on *Eimeria* oocysts closely resemble injury consistent with disruption of the oocyst shell, and thus CLPs appear to possess attributes of natural ionophores. Additional anti-parasitic properties were discovered in association with a nano-emulsion obtained from flax seeds using sub-critical water extraction, followed by extracted seeds sonication in water. These flax biologicals showed a very strong anti-coccidial effect in vitro on several *Eimeria* spp. isolated from chickens, pigeons, cattle, and sheep. In vitro experiments, where coccidian oocysts were incubated in water at RT overnight, showed that nano-emulsions was effective in preventing 98% of sporulation and killing sporulated oocysts at a concentration of

about 5 to 10%. Similar anti-parasitic effects were also observed with regard to a genus of nematodes in the family Capillariidae in chickens and pigeons. Further studies in vivo confirmed the high potential of these flax biologicals to control coccidiosis and capillariasis in birds. The anti-parasitic compounds naturally present in flax may provide a highly desirable alternative approach to control coccidiosis and other intestinal parasites in poultry.

Key Words: flax extract, anti-parasite properties, coccidiosis, capillariasis

360P Differences in cellular and humoral primary and secondary immune responses to protein antigen administered with or without adjuvant. Gisela Erf*, Hyeonmin Jang, Kristen Byrne, Olfat Alaamri, Christopher Lyle, Daniel Falcon, and Robert Dienglewicz, *University of Arkansas, Fayetteville, AR.*

While antibody responses to antigen can be assessed by sampling of blood, information on in vivo cellular immune responses to antigen is limited, due in part to MHC-restrictions of T cells and their need to carry out effector functions at the antigen location. The objective of this study was to monitor and assess the primary and secondary humoral and cellular responses to test-antigen (T-Ag; mouse IgG) administered intramuscularly (i.m.) with or without adjuvant. Specifically, 7-wk-old male Light-brown Leghorn chickens were injected into the breast muscle with vehicle (PBS; 0.1mL), T-Ag (0.26 μ g) or T-Ag (0.26 μ g) mixed with 15% Alum adjuvant. Antibody production (IgM, IgG) to T-Ag was monitored for 4 wk following both the primary and secondary i.m. immunizations. To monitor in vivo cell-mediated immune-activity to T-Ag in the same individuals, we used the pulp (dermis) of growing feathers (GF) as the test-site. This dermal test-site has the advantage of being a defined unit of tissue which can easily be removed for ex vivo analysis using minimally invasive procedures. To examine the cellular effector response to T-Ag, T-Ag was injected into 20 GF/bird (10 μ L/GF) on Day 10 or Day 5 post-primary or -secondary immunization, respectively. GF were collected before injection (0h) and at 0.25, 1, 2, 3, 4, 5 and 7d post-injection. For each bird, one GF/time point was used to prepare pulp cell-suspension, which were then immunofluorescently stained with chicken-leukocyte-specific monoclonal antibodies. Analysis of GF pulp cell suspensions by flow cytometry revealed temporal, qualitative and quantitative differences ($P < 0.05$) in leukocyte-infiltration profiles between immunization treatments and innate as well as primary/memory effector-responses to T-Ag. ELISA also revealed treatment differences and temporal, qualitative, and quantitative differences ($P < 0.05$) in the primary and secondary humoral responses to T-Ag. Minimally invasive, non-terminal procedures such as sampling of injected GF and blood provides unique insight into cellular and humoral immune activities in the same individual over time.

Key Words: humoral, cellular, primary, memory, adjuvant

237 Effect of particle size and feed presentation on gastrointestinal morphology in infected broilers with *C. jejuni*. Oscar Casabuena¹, Véronique Elgosi², Mark L. den Hartog³, Marta I. Gracia¹, and Pedro Medel^{*1}, ¹*Imasde Agroalimentaria, S.L., Madrid, Spain*, ²*FLA, Paris, France*, ³*NEPLUVI, Houten, the Netherlands*.

An experiment was conducted within the EU-FP7 project CAMPYBRO for evaluating the effect of particle size (PS) of wheat (W) and feed presentation (FP) on gastrointestinal morphology in broilers in orally infected birds with *C. jejuni*. There were 4 treatments factorially arranged with 2 W screen sizes (Ø2 vs. 5mm) and 2 FP (mash [M] vs. pellets [P]). A total of 144 one-day-old Ross 308 broilers were divided into cages (3 birds/cage) and experimental treatments (12 cages/treatment). At 14 d, all broilers were orally gavaged with 100 µL of a solution containing 1×10^5 cfu/mL of ST-45 *C. jejuni* strain. At 35 and 42 d, performance parameters, weight of GIT, empty BW (EBW), proventriculus (PRO) and gizzard (GIZ), and pH at PRO and GIZ were taken. Data were analyzed by GLM procedure of SPSS. Birds fed P diets were heavier during the whole period (728 vs. 848, 1557 vs. 2112 and 2162 vs. 2770g for M and P diets at 21, 35, and 42 d, respectively; $P < 0.001$). These higher weights were due to a higher feed intake and growth in birds fed P diets ($P < 0.01$). P decreased the GIT weight expressed as % of BW (8.27 vs. 7.74% for M and P diets, respectively; $P < 0.05$). P increased PRO weight (8.7 vs. 10.1 g for M and P diets, respectively; $P < 0.05$) but decreased its relative importance to EBW (0.44 vs. 0.40% for M and P diets, respectively; $P < 0.05$). Also, P decreased the relative importance of the GIZ expressed as weight (50.7 vs. 40.4 g for M and P diets, respectively; $P < 0.05$) or as % of GIT, BW or EBW ($P < 0.05$). On the other hand, PS increased the GIZ as weight (41.7 vs. 49.4g for Ø2 or 5mm, respectively; $P < 0.05$) or as % of GIT, BW or EBW ($P < 0.066$). P of fine size diets decreased the ceca weight and the opposite was observed for 5-mm diets (1.17, 1.08, 1.08 and 1.23 g for Ø2 M, Ø2 pellet, Ø5 M and Ø5 pellet, respectively; $P = 0.048$). The same interaction was found for the empty GIZ weight ($P = 0.047$). P increased the pH at PRO (3.87 vs. 4.29 for M and P diets, respectively; $P < 0.05$) and GIZ (3.53 vs. 4.13 for M and P diets, respectively; $P < 0.05$). It is concluded that feed presentation affected performance and GIT morphology much more than particle size.

Key Words: Campylobacter jejuni, pelleting, particle size, broiler, gastrointestinal tract

238 Effect of feed presentation and whole wheat addition on gastrointestinal morphology in orally infected broilers with *C. jejuni*. Carlos Millán¹, Ángel Martín², Györgyi Molnár³, Jaime Sánchez¹, and Pedro Medel^{*1}, ¹*Imasde Agroalimentaria, S.L., Madrid, Spain*, ²*Propollo, Madrid, Spain*, ³*BTT, Budapest, Hungary*.

An experiment was conducted within the EU-FP7 project CAMPYBRO for evaluating the effect of feed presentation (FP) and whole wheat (WW) addition on gastrointestinal (GIT) morphology in orally infected birds with *C. jejuni*. There were 6 treatments factorially arranged with 2 FP (Mash [M] vs Pellets [P]), and 3 levels of WW from 0 to 21/21–42d: 0/0, 7.5/15%, 15/30%. A total of 216 one-day-old Ross 308 broilers were divided into cages (3 birds/cage) and experimental treatments (12 cages/treatment). At 14 d, all broilers were orally gavaged with 100 µL of a solution containing 1×10^5 cfu/mL of ST-45 *C. jejuni* strain. At 35 and 42d, performance parameters and weight of GIT, empty BW (EBW), proventriculus (PRO), gizzard (GIZ), and pH at PRO and GIZ were taken. Data were analyzed by GLM procedure of SPSS. Birds fed P diets were heavier and presented better feed conversion for the whole trial (2123 vs 2770g at 42d and 1.93

vs 1.68g/g for M and P diets, respectively; $P < 0.001$). WW addition did not modify BW of birds at any dosage. The higher BW of birds fed P were due to a higher feed intake ($P < 0.05$). P decreased the GIT relative to BW (8.47 vs. 7.78% for M and P diets, respectively; $P < 0.05$). P increased PRO weight (8.0 vs. 12.2g for M and P diets, respectively; $P < 0.05$) and as GIT % ($P < 0.05$). Also, P decreased the relative importance of GIZ as %GIT, BW or EBW ($P < 0.05$). M diets showed lower pH values at the GIZ (3.04 vs. 3.52 for M and P diets, respectively; $P < 0.05$). WW inclusion decreased the PRO weight (13.3, 8.8 and 8.8 for 0/0, 7.5/15 and 15/30%ww, respectively; $P < 0.05$), and relative to GIT and BW ($P < 0.05$). On the contrary, WW inclusion increased GIZ weight (41.7, 58.2 and 53.9 for 0/0, 7.5/15 and 15/30%ww, respectively; $P < 0.05$) and relative to GIT, BW or EBW ($P < 0.05$). Also, WW inclusion reduced pH at GIZ and % of fresh digesta in the PRO (3.48, 3.07 and 3.29, and 26.1, 8.0 and 12.2% for 0/0, 7.5/15 and 15/30%ww, respectively; $P < 0.05$). WW inclusion decreased PRO size in P but not in M diets ($P < 0.05$). It is concluded that P improved performance and affected GIT morphology, and WW addition did not impair productivity.

Key Words: pellets, whole wheat, Campylobacter jejuni, broilers

239 Effects of feeding XPC to broilers challenged with infectious laryngotracheitis virus (ILT) following different vaccination methods. Donald R. McIntyre^{*1}, John K. Rosenberger², Milos Markis², and Jonathan N. Broomhead¹, ¹*Diamond V, Cedar Rapids, IA*, ²*AviServe LLC, Newark, DE*.

A study was designed to compare the effects of feeding XPC (1.25 kg/mt) to Cobb broiler chicks challenged with infectious laryngotracheitis virus (ILT) at 29 d of age. Fertile hatching eggs ($n = 250$) were sourced from a commercial hatchery and assigned to 1 of 4 groups: 1) Control with no ILT vaccination; 2) embryos vaccinated in ovo at 18 d of incubation with a full dose of rHVT ILT + SB-1 using Vectormune HVT LT (CEVA); 3) chicks given ocular vaccine at 14 d of age using LT-IVAX* (Merck) and 4) chicks vaccinated by oral gavage at 14 d with Laryngo-Vac CEO (Zoetis). Splitting each group with and without the addition of feed XPC (Diamond V) resulted in 8 treatments ($n = 27$ chicks/trt.), with each tagged bird representing an experimental unit. Chicks were reared to 29 d according to assigned treatments, and then each bird was challenged intratracheally with $10^{3.5}$ EID₅₀ dose of pathogenic ILT (USDA). Five and 10 d post-ILT challenge, tracheal swabs were collected from each bird and ILT re-isolations completed by means of inoculation of 9 d to 11 d SPF embryos via the chorioallantoic route (3 embryos per swab). If virus was re-isolated, the source bird was considered susceptible to challenge. Combining results for 5 d and 10 d post-challenge showed that recombinant vaccine given in ovo achieved 70% protection; when XPC was added to the feed, 86% of birds were protected. Ocular vaccination with LT-Ivax protected 67% of birds; adding XPC in the feed increased protection to 85%. Birds gavaged with CEO vaccine showed similar protection with and without XPC (92 and 93%, respectively). Control groups challenged without vaccination indicated that 61% of control birds were susceptible to challenge; incidence was 45% in birds fed XPC.

Key Words: laryngotracheitis, vaccine, broiler, XPC

240 Influence of garlic (*Allium sativum*) feed-inclusion on hemoprotozoan infections and cellular immunity in turkey poults. Omolade A. Oladele*, Oluwaseun O. Esan, Olasupo Olasemi, and Oluwatoshin S. Oyeboode, Department of Veterinary Medicine, *University of Ibadan, Ibadan, Oyo State, Nigeria*.

Suboptimal performance due to diseases is a major attribute of poultry production in the tropics. In lieu of the use of antibiotic as growth promoters, garlic (*Allium sativum*), with its reported antibiotic and immunomodulatory properties, was considered a sustainable alternative. Its effect on natural hemoprotozoan infection and cellular immunity of turkey was studied. One hundred 1-d-old poults were divided into groups A and B of 50 each, reared in open-sided cages and fed ad libitum with Group A, having 0.125% garlic meal feed-inclusion. Each group was subdivided into 2 (A1, A2; B1, B2) as replicates. Five poults/replicate were bled at 2, 4, 6 and 8 weeks-old and screened for hemoprotozoans. Levels of parasitemia and severity of infections were determined. At 8 wk old *Staphylococcus aureus* antigen in polyethylene glycol-PEG (0.1 mL; 0.75% antigen) was administered to subgroups A1 and B1 subcutaneously while 0.1 mL PEG was administered to subgroups A2 and B2 and repeated at 9 week-old. At 10 week-old, all poults were challenged with 0.75% *S. aureus* antigen in PBS (0.1 mL) subdermally into right footpads and PBS only into left footpads. Footpad thickness was measured up till 72 h post-challenge (pc), delayed footpad reaction (DFR) in each poult at each sampling time and mean DFR/group were calculated. Comparison of means was via ANOVA and DMRT at $P < 0.05$. Severity of infection was cumulatively scored 3 and 63 for *Plasmodium gallinaceum*, 0 and 6 for *P. juxtanucleare*, 1 and 5 for *P. relictum*, and 84 and 87 for *Hemoproteus columbae* in Groups A and B, respectively. At 24, 48, and 72 h pc, mean DFR for subgroup A1 were significantly higher than for other subgroups. While DFR increased to 3.47 ± 0.27 mm by 72 h pc in subgroup A1, subgroup B1 peaked (2.26 ± 0.19 mm) at 48 h pc. This study shows that garlic feed-inclusion substantially reduced severity of natural *Plasmodium* species infections and possibly enhances cellular immunity as evidenced by the observed prolongation of DTH. The apparent efficacy indicates that this could be beneficial for the control of some disease etiologies.

Key Words: cellular immunity, garlic, *Plasmodium* species, turkey poults

241 Susceptibility of broiler chickens to coccidiosis when fed subclinical doses of deoxynivalenol and fumonisins: Special emphasis on the immunological response and the mycotoxin interaction. Bertrand Grenier^{1,2}, Ilse Dohnal², Revathi Shanmugasundaram³, Susan D. Eicher⁴, Ramesh K. Selvaraj³, Gerd Schatzmayr², and Todd J. Applegate¹, ¹Department of Animal Sciences, Purdue University, West Lafayette, IN, ²Biomin Research Center, Tulln, Austria, ³Department of Animal Sciences, Ohio Agricultural Research and Development Center, Wooster, OH, ⁴Livestock Behavior Research Unit, Agricultural Research Service, USDA, West Lafayette, IN.

This study reports the interaction in the intestinal tract of chickens of 2 mycotoxins, deoxynivalenol (DON) and fumonisins (FB) with *Eimeria* spp., responsible for coccidiosis. Male broilers were assigned to 4 diets (12 pens/diet, 7 birds/pen), from hatch to 20 d, containing no mycotoxins, 1.5 mg DON/kg, 20 mg FB/kg, or both toxins. At 14 d, 6 pens of birds per diet were challenged with a 25 \times -recommended dose of coccidial vaccine, and samples were collected 6 d later. The lesions observed in the intestinal tract of challenged birds fed mycotoxins were more frequent than in challenged birds on control feed. Higher numbers of oocysts were also found in the mucosa and the feces of challenged birds fed mycotoxins (+164% and +45% respectively, $P < 0.05$). Combination of DON and FB reduced the apparent nitrogen digestibility in challenged birds (–14%, $P < 0.05$). Upregulation of cytokines following coccidial infection was higher in the jejunum of birds fed mycotoxins (IL-1 β , IL-6, IL-8, and IL-10 – 1.6 to 3.7 fold higher, $P < 0.05$). Further work done revealed that this higher intestinal immune response might be associated with the higher percentage of lymphocytes T CD4⁺CD25⁺,

also called Tregs, observed in the cecal tonsils of challenged birds fed mycotoxins (+67%, $P < 0.05$). Recruitment of more Tregs would help birds to control the inflammatory response. Interestingly, the increase of the biomarker of FB exposure (sphinganine/sphingosine ratio in serum and liver) suggested a higher absorption of FB in challenged birds. To determine the type of interaction between DON and FB, a 2-way factorial ANOVA was applied with significant interactions considered as antagonistic or synergistic effects, and no interaction as additive. All 3 types of interactions were seen, thus emphasizing the importance of endpoints when studying interactions. In conclusion, subclinical doses of DON and FB showed little effects in unchallenged chickens, but seem to pose a serious risk in the presence of intestinal pathogens.

Key Words: mycotoxin, coccidiosis, challenge, interaction, intestinal immune response

242 Effect of dietary vanadium and vitamin C on egg quality and antioxidant status in laying hens. J. P. Wang*, K. R. He, Y. H. Luo, S. P. Bai, Q. F. Zeng, Z. W. Su, Y. Xuan, and K. Y. Zhang, Sichuan Agricultural University, Chengdu, Sichuan, China.

This paper assessed the effect of dietary vanadium (V) and vitamin C (VC) on production performance, egg quality and antioxidant status in laying hens. A total of 360 laying hens (31-wk-old) were randomly allotted into a 3 \times 3 factorial arrangement treatments (4 replicates and 10 chicks per replicate) with 3 levels of dietary vanadium (0, 5 and 10 mg/kg) and 3 levels of vitamin C (0, 50, and 100 mg/kg) for 12 wks. The effect of V and VC did not alter egg production, egg weight, ADFI, or FCR during 1 to 12 wk. Albumen height and Haugh unit value were linearly decreased ($P < 0.001$) by addition of V, whereas the effect of 100 mg/kg VC were observed to counteract ($P < 0.05$) this effect in V containing treatments during 1 to 12 wk. Hens fed vanadium containing diet laid lighter (linear effect, $P < 0.05$) colored eggs (higher lightness value, lower redness and yellowness value), and the VC exerted no influence on it during 1 to 12 wk. The serum superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH-Px) activities, ability to inhibit hydroxyl radical, were significantly decreased, and the malondialdehyde (MDA) and vanadium contents were increased ($P < 0.05$) by effect of V during 4, 8 and 12wk. The effect of VC alone and the interactive effect between VC and V were shown to increase serum ($P < 0.05$) SOD activity in 4wk and decrease MDA levels in 12 wk. The result indicate that vanadium decreased the egg quality and caused the oxidative stress at level of 5 mg/kg and 10 mg/kg, and the addition of 100 mg/kg vitamin C can alleviate its egg quality reduction effect and can mitigate the oxidative stress to some extent.

Key Words: vanadium, vitamin C, productive performance, antioxidative status

243 The effect of *B. subtilis* and *B. licheniformis* on *C. perfringens* in vitro and in vivo. Anée B. Kehlet¹, Dorthe Sandvang¹, and Frank L. Jin², ¹Chr. Hansen A/S, Hørsholm, Denmark, ²Chr. Hansen Inc., Milwaukee, WI.

A stable intestinal flora is fundamental for optimal broiler performance. It not only helps the bird utilize nutrients but also aids in managing challenges such as pathogen overgrowth. Two studies were carried out to evaluate the effect of *B. licheniformis* (BL) and *B. subtilis* (BS) on *C. perfringens* (CP) both in vitro and in vivo. A suspension of BL and BS were streaked on agar plates and incubated overnight. After 24 h, overnight cultures of CP type A or C were streaked vertical to the Bacillus cultures and incubated overnight. The plates were evaluated by size of inhibition zone (IZ). A total of 1980 male broilers were allocated to

1 of 5 treatments; uCON: Neg. control; iCON: CP challenged control; BLBS: BL+BS (1.28E+06 cfu/g feed) + CP; BL: BL (1.60E+06 cfu/g) + CP; 1/2BLBS: BL+BS (6.40E+05 cfu/g) + CP, for 46 d with 12 reps/treat and 33 birds/pen. D17 birds were orally challenged with 1 mL/bird of 8.0E+08 cfu/mL CP. Performance was recorded and NE lesions determined at d 21 (0–3 score). Data were analyzed by ANOVA. The in vitro analysis showed that BS had no inhibitory effect on CP whereas BL showed an IZ < 10 mm against both CP type A and B. At d21 BLBS, BL, 1/2BLBS had a significant lower scores (1.306–1.472) than iCON (2.583). At d21 BW was significantly higher in probiotic treated groups (780.2–788.8 g) compared with iCON (725.3 g). The same trend was seen d46 but not significantly (2097.4 g compared with 2115.6–2170.0 g). No significant differences in FCR were seen between the challenged birds although BLBS (2.339) and BL (2.365) had lower FCR than iCON (2.467) and 1/2BLBS (2.420). Probiotic treatment groups showed numerically lower morbidity (22.17–23.20) at d 35 compared with iCON (45.23). At d 46 mortality was numerically diminished in BLBS (10.18%) and BL (12.03) compared with iCON (17.9) and 1/2BLBS (17.9). Above results show that BL have an inhibitory effect against CP in vitro and that both BL alone and in combination with BS improves performance, reduce mortality and diminish the severity of lesions in CP challenged broilers.

Key Words: broiler, *C. perfringens*, *Bacillus*, probiotic

244 Effect of dietary oleoresin mix on the performance of broilers experimentally challenged with avian influenza virus. Alberto Casarin-Valverde, Prashant K. Mishra*, Rodrigo Garcia-Ortega, and Pablo A. Sánchez, *Grupo Nutec, El Marqués, Querétaro, México.*

Influenzavirus A type H5N2 is of grave importance in aviculture, vaccination is a useful but not definitive solution, as the recombination rate of this *Orthomyxoviridae* is particularly high, therefore substances that regulate the immune system become relevant to breeders. Hence, a 2-phase experiment with artificially inoculated broilers (inoculated with avian influenza virus) was conducted to measure the effect of dietary oleoresins on the zootechnical and immunological parameters. A total of 144 Ross-308 birds were housed in isolation units during a full rearing period (49 d). The study consisted of 3 treatments: a negative control (– supplementation + viral challenge), an experimental group (+ supplemented with capsicum and curcuma oleoresin) and a positive control (– supplemented +viral challenge). Birds were challenged with 2 mL of a strain H5N2 virus suspension via ocular inoculation at d 33, isolated from an outbreak in the state of Puebla (Mexico) on 2012. The challenge was assessed by immunological parameters such as antibody titer (HI), serum viral load, and α -glycoproteins (AGP). Results were analyzed through a one-way ANOVA test and a Tukey's range test to find differences in the mean values of different treatments. The test showed that dietary oleoresins have a statistically significant ($P < 0.05$) effect on the conversion index (± 0.2), as well as in the final weight of the birds (± 300 g). Similarly the antibody titer of the experimental-challenge disease (AI) was maintained at protective values up to the end of the test, while the titer of a disease (NC) vaccinated-against but not challenged were maintained at protective levels; the serum viral load of the experimental group was lowest ($10\times$ lower); there was no clear effect on the mortality and morbidity. Altogether, these results suggest that the oleoresin mix has an immunomodulation effect, aiding the animal in the efficient and well-directed immune response that is translated in lower energy expenditure. Such thrift is observable in a maintaining the zootechnical parameters at levels similar to an uninfected state.

Key Words: avian influenza, oleoresin, immunomodulation, broiler.

245 Effect of calcium level and a direct-fed microbial on performance of broilers experiencing naturally occurring necrotic enteritis. Alamanda Calvert*, Marie Schirmacher¹, Christa Honaker¹, Michael Persia¹, and Audrey McElroy², ¹Virginia Tech, Blacksburg, VA, ²Texas A&M, College Station, TX.

Necrotic enteritis (NE) is an economically significant disease for broiler producers. Evaluation of dietary approaches to maintain intestinal integrity is essential for prevention of this intestinal disease. The objective was to evaluate if a direct-fed microbial, containing 3 isolates of *Bacillus subtilis*, could mediate the effects of NE on bird performance when birds were fed different dietary Ca levels. Nine dietary treatments were arranged in a 3 \times 3 factorial using 8 replicate pens of 36 chicks. The factors were dietary Ca (0.60, 0.75, and 0.90%) and feed additive (0.5lb/ton Sporulin, 50g/ton BMD, or no additive). Broilers were vaccinated with a live coccidia vaccine on d 0 and raised on used litter. The flock developed a natural NE occurrence starting at 10d. Pen BW and feed weights were measured at 0, 9, 18, and 28d and corresponding performance characteristics evaluated. Tibia ash and D-xylose plasma levels were analyzed on 9 and 18d from one bird per pen. Treatment and LSmean (Tukey adjusted) differences were determined at $P < 0.05$ in Glimmix (SAS 9.4). Increased percent NE mortality of 16% was seen for the 0.9% Ca diets compared with 6% for the 0.6% Ca diets from 10 to 28d. An interaction between Ca and additive was seen for BW, ADG and feed conversion. Increased 28d BW of 1.57kg was seen in the 0.6% Ca and Sporulin diet compared with 1.46kg in the 0.9% Ca and no additive diet, with similar differences for 0–28 and 10–28d ADG. Feed conversion from 0 to 9d was 1.18 in the 0.9% Ca and Sporulin diet, which was better than all BMD and all 0.75% Ca diets. From 0 to 28 and 19–28d, lower feed intake was seen for the 0.9% Ca verses 0.6% Ca diet. Bone ash weight was higher in the 0.9% Ca compared with the 0.6% Ca diets at 9d and percent bone ash was higher in the 0.75% Ca compared with 0.9% Ca diets at 18d. No differences were seen for absorption of D-xylose. Decreasing the dietary level of Ca to 0.6% resulted in reduced mortality from NE, and with the inclusion of Sporulin, resulted in heavier BW.

Key Words: broiler, necrotic enteritis, calcium, probiotic

246 Probiotics and application methods affect intestinal gene expression of broilers during coccidiosis. Chasity M. Pender*, Wael Abdelrahman², Michaela Mohn², Miranda M. Ritzi³, and Rami A. Dalloul³, ¹Biomim America Inc., San Antonio, TX, ²Biomim Holding GmbH, Herzogenburg, Austria, ³Virginia Tech, Blacksburg, VA.

Coccidiosis, an inherent risk in the poultry industry, consistently inflicts devastating economic losses. This study evaluated the effects of probiotic applications on intestinal gene expression in broilers during coccidiosis. Male broilers (n = 1008) were assigned to 1 of 6 treatments, including non-infected negative (NEG) and *Eimeria*-infected positive (POS) controls, salinomycin (SAL), intermittent high dose water-applied probiotic (WPI), continuous low dose water-applied probiotic (WPC), and feed-added probiotic (FSP). On d 15, all birds except those in NEG were challenged with a mixed *Eimeria* inoculum. On d 15, 21, and 28, small intestinal sections were collected to evaluate gene expression by qRT-PCR. Differences among treatments were tested using Tukey HSD following ANOVA with significance reported at $P \leq 0.05$. On d 15, interleukin (IL)-13 was downregulated in the jejunum of all treated groups and lower in FSP than WPC. Jejunal inducible nitric oxide synthase (iNOS) was upregulated in all probiotic groups compared with POS and SAL. Ileal IL-1 β expression was decreased in WPI and FSP compared with POS and SAL. Expression was decreased in the cecal tonsils for interferon (IFN)- γ in WPC and LPS-induced TNF α -factor

(LITAF) in WPI and FSP compared with POS and SAL. On d 21, iNOS was upregulated in the jejunum of challenged groups with WPC and FSP having higher levels than POS and SAL, and in the ileum of challenged groups except for SAL and FSP, which were similar to NEG. IFN- γ was higher in the ileum of WPC but lower in the cecal tonsils of FSP compared with SAL. Expression was decreased in the cecal tonsils for IL-12 and IL-13 in all probiotic groups, and LITAF in WPI and FSP compared with POS. On d 28, IL-12 was upregulated in the jejunum of POS, SAL, and FSP compared with NEG, while WPC and WPI were similar. In the jejunum, IL-13 was increased for all challenged groups except WPI, higher for LITAF in POS and iNOS in POS and FSP compared with NEG, while other treated groups were intermediate. Probiotic supplementation without anticoccidials modulates intestinal gene expression, which may help alleviate the effects of coccidiosis.

Key Words: poultry, cytokine, Eimeria, immunity

247 Early mucosal permeability model for studying spondylolisthesis (kinky back) in broilers. J. C. Bielke¹, V. A. Kuttappan¹, E. A. Vicuña¹, R. W. Moore², A. S. Al-Ogaili¹, J. D. Latorre¹, A. D. Wolfenden¹, B. M. Hargis¹, G. Tellez¹, and L. R. Bielke^{*1}, ¹Department of Poultry Science, University of Arkansas, Fayetteville, AR, ²Poultry Diagnostic Laboratory, Texas A&M Veterinary Medical Diagnostic Laboratory, Center, TX.

Spondylolisthesis (kinky back) lameness is related to abscess of the free thoracic vertebrae (FTV) in heavy broilers, and has been linked to *Enterococcus cecorum* (EC), a common GIT bacteria. Furthermore, enteric inflammation and mucosal permeability are often associated with translocation of bacteria into circulation, which could promote development of abscess in FTV. Kinky back incidence occurs at a rate of 3 to 9% in affected flocks, and can manifest as early as 6 weeks of age. We evaluated the effect of early induction of mucosal permeability on serum fluorescein isothiocyanate-dextran (FITC-d, d 11) levels, total aerobic bacterial translocation to liver on d11 (BTL), and EC recovery from FTV at 15 d of age. Dexamethasone (DEX; 0.57mg/kg of feed d 4–15), rye based diet (RBD; d 7–15), and 15% dried distillers grain with solubles (DDGS; d 1–15) were evaluated as inducers of mucosal permeability and EC was administered on d 11. A negative control (CON, no EC) and EC only control (ECC) were included. In Exp 1, serum levels of FITC-d (d 11) were higher ($P < 0.05$) in all inflammation groups than CON and ECC, and only RBD resulted in higher BTL. Incidence of FTV EC recovery on d 15 was 75, 50, and 55% for DEX, RBD, and DDGS,

respectively, which was significantly higher than CON (10%) and ECC (15%). In Exp 2, serum FITC-d was increased DEX and RBD treated birds, plus both exhibited increased BTL when individually compared with ECC. Recovery of EC in FTV reflected BTL results, with DEX and RBD both resulting in +2 Log₁₀ increase ($P < 0.05$) over ECC group. These studies suggest early enteric inflammation models may increase gut leakage of EC to FTV and markers such as serum FITC-d and EC FTV recovery in young broilers may be a useful research model for studying methods to prevent spondylolisthesis in broilers.

Key Words: kinky back, enteric inflammation, serum FITC-d, dexamethasone, DDGS

248 Genetic and genome analyses of bacteria cultured from lame broilers with osteomyelitis. Adnan Al-Rubaye*, Sura Zaki, Robert Wideman, and Douglas Rhoads, *University of Arkansas, Fayetteville, AR.*

Lameness is a significant problem in the poultry industry resulting in millions of dollars in lost revenue annually. In commercial broilers, the most common cause of lameness is bacterial chondronecrosis with osteomyelitis (BCO). We are using a wire flooring model to induce lameness attributable to BCO. We used 16S ribosomal DNA sequencing to determine that *Staphylococcus* spp. were the main species associated with BCO. *Staphylococcus agnetis*, which previously had not been isolated from poultry, was the principal species isolated from the majority of the bone lesion samples. *Staphylococcus* spp. also were isolated from the blood of apparently healthy broilers. Administering *S. agnetis* in the drinking water to broilers reared on wire flooring increased the incidence of BCO 3-fold when compared with broilers drinking tap water ($P = 0.001$). We found that the minimum effective dose of *Staphylococcus agnetis* to induce BCO in broilers grown on wire flooring experiment is 10^5 cfu/mL. Our results conclusively demonstrate bacterial translocation across the intestinal epithelium into the blood. More severe lesions were found in the proximal femoral and tibial heads in the lame birds that received *S. agnetis* in the water compared with the control group. We sequenced and assembled a draft of the *S. agnetis* genome for further investigation of genetic diversity, toxins, and pathogenicity determinants, for this poorly characterized species. Isolating pathogenic bacterial species, defining their likely route of transmission to broilers, and genomic analyses will contribute substantially to the development of measures for mitigating BCO losses in poultry.

Key Words: broiler, lameness, bacteria, genome, leg