Immunology and Pathology: Poultry Immunology and Diseases

648 Testosterone exposure alters embryonic bursal gene expression in chicken lines selected for differential antibody response. R. L. Taylor, Jr.*[1], T. Burks[1], C. Timmerman[2], P. B. Siegel[3], and C. M. Ashwell[2], [1]*University of New Hampshire*, *Durham,* [2]*North Carolina State University*, *Raleigh,* [3]*Virginia Tech*, *Blacksburg*.

Chicken B cells mature in the bursa of Fabricius (BF) microenvironment producing antibody diversity through their V-gene repertoire. Testosterone propionate (TP) exposure on d 3 of incubation severely impairs BF development, elevates IgM, lowers IgG and causes bursal epithelial cell proliferation. From a common founder population, White Leghorn lines selected for high (HAS) or low (LAS) antibody produced 5 d after a 0.25% SRBC suspension (0.1 mL) injected intravenously, have a several fold difference in anti-SRBC antibody titer. Eggs from the 34th selected generation were dipped in a 2% TP ethanol solution or ethanol alone for treatment and control groups, respectively. We examined gene expression in bursal tissue collected from 4 embryos of each line and treatment (HAS, HAS TP, LAS, LAS TP) at 15, 18, and 21 d of incubation. Tissue samples were held in RNALater at −80 C until RNA was extracted followed by reverse transcription to cDNA. Individual cDNA samples, labeled indirectly with Cy3 or Cy5 fluorescent dyes, were hybridized including a dye swap to a 320 gene focused microarray. Each gene, represented by a 70-mer oligonucleotide, was spotted 12 times in a single array enhancing sensitivity to detect sample group differences. Fluorescent intensity data were transformed via log2, normalized by weighted regression, and analyzed by a mixed-model ANOVA. Pathway, process and networks were evaluated using the Metacore database. Growth hormone (GH), growth hormone receptor (GHR), thyroid hormone receptor α, and hemoglobin rho expression was higher in Line LAS controls than Line HAS controls. TP treatment in Line LAS elevated GH and GHR genes as well as interleukin(IL)-1 whereas Line HAS TP embryos had higher fibroblast growth factor 1 and endothelin receptor expression. Network analysis identified biomarkers associated with pathways for immune response IL-1, immune response NK2g, and developmental growth hormone signaling. Real-time PCR confirmed gene expression differences found on the microarray.

Key Words: immune response, microarray, bursa of Fabricius

649 Limiting dilution studies to detect avian influenza viruses from questionable allantoic fluid samples. T. V. Dormitorio* and J. J. Giambrone, *Auburn University*, *Auburn, AL*.

Detection of avian influenza viruses (AIV) from wild birds can be complicated when there is the presence of other hemagglutinating agents, including some avian adenoviruses, mycoplasma, Newcastle disease virus (NDV), and other paramyxoviruses (APMV). Moreover, dual isolations of influenza virus and APMVs are not unusual. Limiting dilution studies were conducted using fecal swab samples collected from wild birds, which were determined to be AIV positive by real-time RT-PCR (rRT-PCR), but had high crossing point (Cp) values (>32). The NVSL failed to isolate AIV from 2 of these samples, but instead they reported the presence of APMV-1 and APMV-4. Results showed that AIV positive samples, with high Cp, had either AIV, APMV, or both. The effect of egg passage on AIV detection by rRT-PCR had lowered Cp, indicating that there was an increase in the detectable virus. When there was a mixed infection, several dilutions and passages were needed to separate the viruses. Moreover, the presence of APMVs may have interfered with AIV detection, and thereby produced false positive AIV rRT-PCR results.

Key Words: avian influenza virus, avian paramyxovirus, real-time RTPCR

650 Development and characterization of mouse monoclonal antibodies reactive with chicken CD80. S.-H. Lee*[1], H. Lillehoj[1], M.-S. Park[1], K.-W. Lee[1], C. Baldwin[2], D. Tompkins[2], B. Wagner[3], U. Babu[4], and E. Del Cacho[5], [1]*Animal and Natural Resources Institute, ARS-USDA*, *Beltsville, MD,* [2]*University of Massachusetts*, *Amherst,* [3]*Cornell University*, *Ithaca, NY,* [4]*Food and Drug Administration*, *Laurel, MD,* [5]*University of Zaragoza*, *Zaragoza, Spain*.

The CD80 (B7.1) is a molecule found on monocytes providing a signal necessary for T cell activation and interferon-γ production. The characteristics of this molecule have been studied in human, swine, ovine, feline, and canine. However, information about CD80 and its antibodies (Abs) in chicken has not been reported. To develop immune reagents for chicken CD80 (chCD80), we have immunized mice with recombinant chCD80, and hybridomas producing monoclonal Abs against chCD80 were produced. Recombinant chCD80/IgG4 fusion protein was expressed in mammalian cells and chCD80 was purified and used to immunize mice. In this study, 158 hybridomas were screened and 3 mAbs with high binding specificity against chCD80-transfected cells were selected by flow cytometric analysis. Western blot showed a 80 kDa protein against chCD80. Monoclonal Abs to chCD80 showed staining of chicken macrophage cell line (HD11), bursa, and spleen. Taken together, mouse monoclonal antibodies specific for chicken CD80 have been developed and these immune reagents will be useful tools to analyze CD80 activity during infections and to do basic and applied research for poultry.

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Key Words: chickens, CD80, monoclonal antibody

651 Suppressive properties of chicken CD25$^+$ cells during lipopolysaccharide injection. R. Shanmugasundaram[1,2] and R. K. Selvaraj*[1,2], [1]*Ohio Agricultural Research and Development Center*, *Wooster,* [2]*The Ohio State University*, *Wooster*.

Suppressive properties of splenic chicken CD25$^+$ cells were evaluated during in vivo lipopolysaccharide (LPS) injection. One-week-old chickens were injected with 0 or 100 μg of LPS/kg body weight. Spleen CD4+8–25+ cell percentage increased from 8% to 24% at 4 d post-LPS injection. Spleen CD4+8–25+ cell percentage decreased to 11% at 5 d post-LPS challenge, but remained greater than the 0 μg LPS control group until 12 d post-LPS injection. Suppressive properties of CD25$^+$ cells were determined by naïve cell proliferation suppression assay. At CD25$^+$:T responder cell ratio of 1:1, CD4+8–25+ cells collected at 5 and 12 d after 100μg LPS injection were suppressive while CD4+8–25+ cells collected at 2 d after 100 μg LPS injection were not suppressive. CD4+8–25+ cells collected at 5d after LPS injection had a greater suppressive potential in the 100 μg LPS group than CD4+8–25+ cells from the 0μg LPS group. At 5 d post LPS injection, CD4+8–25+ cells from 100 μg LPS injection were suppressive at CD25$^+$:T responder ratio of 0.25:1, while those from 0 μg LPS injection were suppressive only at Treg:T responder ratio of 1:1, but not at 0.25:1. It could be concluded that in vivo LPS treatment alters the suppressive properties of chicken CD25$^+$ cells.

Key Words: T regulatory, suppression, LPS

652 Expression profile of cytokines in cecal tonsils of broiler chicks challenged with *Clostridium perfringens*. Y. O. Fasina*[1], H. S. Lillehoj[2], M. S. Park[2], and D. E. Conner[1], [1]*Auburn University*, *Auburn, AL*, [2]*USDA-ARS-ANRI-APDL*, *Beltsville, MD*.

Necrotic enteritis is an economically important enteric disease of poultry that is caused by *Clostridium perfringens* (CP). Understanding the role of cytokines in modulating intestinal innate immune response to CP will facilitate the design of effective non-antibiotic control measures such as vaccines. An experiment was conducted to determine the effect of CP infection on the expression of selected T helper type 1 (Th1 - inflammation-inducing) and T helper type 2 (Th2 - antibody-inducing) cytokines in the cecal tonsils of broiler chicks. Chicks (400) obtained from a commercial hatchery were randomly assigned to 4 treatments. Treatment 1 (CX) consisted of chicks fed unmedicated corn-soybean meal (SBM) diet. Treatment 2 (MX) consisted of chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was added at 0.055g/kg. Treatments 3 (PCX) and 4 (PMX) consisted of chicks fed diets similar to those given to CX and MX, respectively, and were additionally challenged with 3.5 mL of CP inoculum (10^8 CFU/mL) on d 14, 15, and 16 of experiment. At 1 and 7 d post-challenge, intestinal CP levels were estimated. Cecal tonsils were also collected and subjected to quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) to determine the expression levels of cytokine genes. From results, PCX and PCM (3.04 to 3.68 $\log_{10}$ CFU/mL) had higher CP levels ($P < 0.05$) compared with control CX and MX (1.12 to 1.70 $\log_{10}$ CFU/mL), thus confirming an established infection in PCX and PCM chicks. RT-PCR results showed upregulation of genes of several Th1/proinflammatory cytokines (interleukin [IL]-1β, IL-12, and lipopolysaccharide-induced TNF-associated factor], and to a lesser extent Th2 cytokines such as IL-13 in PCX and PCM ($P < 0.05$), compared with control CX and MX. There was no difference in anti-inflammatory IL-10 among treatments. It was concluded that cytokine response to CP infection in the cecal tonsil is more of a Th1 type which promotes cell-mediated immunity.

Key Words: *Clostridium perfringens*, cytokines, broiler chickens

653 Gel spray as a viable method to apply a coccidia vaccine to chickens. G. F. Mathis*[1], E. H. Lee[2], T. Cosstick[2], and B. Lumpkins[1], [1]*Southern Poultry Research, Inc.*, *Athens, GA,* [2]*Vetech Laboratories, Inc.*, *Guelph, Ontario, Canada*.

On day of hatch, chicks are routinely administered uniformly low doses of live coccidia oocysts to aid in the development of immunity against coccidiosis. Two studies were conducted to examine the novel approach of using a spray gel to orally deliver the oocysts to the chicks through mutual preening. In both studies, chicks were vaccinated with Immucox, a coccidia vaccine. In study 1, on d 21 chicks were individually weighed and challenged with field isolates of *Eimeria* (*E. acervulina* (EA), *E. maxima* (EM), *E. tenella* (ET), or *E. necatrix* (EN)). Individual bird weights and coccidia lesion scores were recorded on d 26 (6 d post challenge). EA, EM, ET, and EN lesion scores were significantly lower (greater than 1.5 score reduction for each species) and weight gain during the challenge period was significantly better than non-vaccinated challenged and equal to the non-vaccinated, non-challenged controls. Study 2 examined performance of non-vaccinated non-challenged, non-vaccinated challenged and Gel sprayed challenged. To represent a natural coccidiosis challenge, on d 28 all birds in the challenge groups were orally dosed with a mixture of EA, EM, and ET. Feed conversion and weight gain were determined on d 21 and at market weight (d 42). On d 21, weight gain was not significantly different among treatments. There was significantly higher feed conversion in the gel spray treatment birds than the non-vaccinated treatment birds. On d 42, gel spray treatment birds had significantly better feed conversion (1.859) and average live weight (1.985 kg) than non-vaccinated challenged (1.954 and 1.857 kg, respectively). However, the gel sprayed birds had significantly lower performance than the non-vaccinated non-challenged birds. Efficacy of the gel spray vaccination was indicated by the reduction in lesion scores and improvements in performance when compared with those of the non-vaccinated challenged control.

Key Words: coccidia, vaccine, chicken

654 A mixture of capsicum and turmeric oleoresins improve performance of vaccinated broilers challenged or not with coccidiosis. V. Brito*[1], C. Moynat[2], A. Casarin[3], M. Forat[3], and D. Bravo[2], [1]*Euronutec*, *Querétaro, Mexico*, [2]*Pancosma*, *Geneva, Switzerland*, [3]*Instituto Internacional de Investigacion Animal*, *Mexico*.

Capsicum (CA) and turmeric (TU) positively impact innate immunity. Their combination should improve performance of birds. Vaccines induce acquired immunity and are efficient in case of challenge. So combining CA, TU and vaccination should improve performance of birds infected by *Eimeria*. The objective was to evaluate the effect of a mixture of CA and TU oleoresins (PF = Proflora/XT 6986) on performance of vaccinated broilers challenged with coccidiosis, and non-challenged non-vaccinated birds. One-day-old broilers were allotted to the following treatments (48 birds × 10 cages/treatment), doses in ppm, with Bacitracin (BA), Nicarbazin (NI), Salinomycin (SA). T1b and T1a acted as positive controls either challenged or not (T1a = 125 NI + 55 BA from d1 to 28, 125 NI + 55 BA from d29 to 52; T1b = T1a + challenge to Eimeria spp. at d14). T2a tested the effect of PF on non vaccinated and non challenged birds (T2a = 50 PF from d1 to 42, 100 PF from d42 to 52). T2b tested the effect of PF on vaccinated and challenged birds (T2b = T2a + coccidiosis vaccine at d1 and challenge to *Eimeria* sp. at d14). BW, BWG and FCR were recorded. Data were analyzed using GLM procedure of SAS. Pre-challenge, T2a and T2b performed better ($P < 0.01$) than T1a and T1b for BWG (+8.2%), FCR (−5.5%) and BW at d14 (+6.9%), showing the positive effect of stimulating innate immunity on performance. T2a and T2b had similar ($P > 0.1$) BWG, FCR and BW, confirming that vaccination was not affecting performance without challenge. After d14, T2a and T2b exhibited same BWG, FCR and final BW ($P > 0.7$), as well as T1a and T1b ($P > 0.5$). This demonstrated that PF with vaccination can maintain the performance of challenged birds at the same level as non challenged birds. Finally, BW in T2a and T2b was 3.7% higher than in T1a and T1b ($P < 0.03$), suggesting that using PF during the whole growing period improved final BW of birds.

Key Words: essential oils, coccidiosis, vaccination

655 Cinnamaldehyde and a blend of capsicum and turmeric oleoresins improve performance of vaccinated broilers subject to coccidiosis. C. Moynat*[1], V. Brito[2], A. Casarin[3], M. Forat[3], and D. Bravo[1], [1]*Pancosma*, *Geneva, Switzerland*, [2]*Euronutec*, *Queretaro, Mexico*, [3]*Instituto Internacional de Investigacion Animal*, *Mexico*.

Cinnamaldehyde (CI), capsicum (CA) and turmeric (TU) positively impact innate immunity. The combination of these 3 products should positively affect immunity and improve performance of birds infected by *Eimeria*. The objective was to evaluate the effect of a mixture of CA and TU oleoresins (PF1 = Proflora / XT 6986) and a product with CI (PF2 = Proflora Plus / XT 6987) on performance of vaccinated broilers challenged with coccidiosis. Broilers vaccinated against coccidiosis at d 1 were allotted to 5 treatments and challenged at d 14 with *Eimeria* sp. (40 birds * 12 cages/treatment). The treatments were set as follow,

doses expressed in ppm, with Bacitracin (BA), Nicarbazin (NI), Salinomycin (SA): T1 = un-supplemented. For starter diet (d 1 to 14): T2 = 55 BA; T3 = 100 PF1; T4 = 100 PF1 + 10 PF2; T5 = 10 PF2. For grower diet (d 15 to 42): T2 = 55 BA + 50 NI + 30 SA; T3 = 100 PF1 + 50 NI + 30 SA; T4 = 100 PF1 + 5 PF2 + 30 SA; T5 = 100 PF1 + 10 PF2 + 30 SA. For finisher diet (d 43 to 52): T2 = 55 BA + 50 NI + 30 SA; T3 = 100 PF1 + 50 NI + 30 SA; T4 = 100 PF1 + 2.5 PF2 + 30 SA; T5 = 100 PF1 + 5 PF2 + 30 SA. BW, BWG and FCR were recorded. Data were analyzed using GLM procedure of SAS. Pre-challenge, there was no difference between treatments in BW, BWG, FCR ($P > 0.1$). Post-challenge, FCR of T1 was deteriorated (+4.3%, $P < 0.01$) showing the positive impact of supplementations on vaccinated birds subject to coccidiosis. No difference between supplementations was observed for FCR ($P > 0.1$). Final BW of T1 was lower ($P < 0.01$) than T2 (−4.2%), T3 (−4.8%) and T4 (−16.4%), and tended to be lower than T5 ($P = 0.08$). A higher inclusion of PF2 post-challenge is not beneficial for the supplementation program. These results show that a mixture of CA and TU oleoresins alone or combined with adequate doses of CI can be used with vaccination to maintain broiler performance.

Key Words: essential oils, cocciodiosis, vaccination

656 Ileal and cecal microbial populations and coccidia infection in broilers given probiotics and essential oil blends. M. E. Hume*[1], E. O. Oviedo-Rondón[2], N. A. Barbosa[2,3], N. K. Sakomura[3], M. C. Jenkins[4], and S. E. Dowd[5], [1]*USDA, ARS, FFSRU*, *College Station, TX*, [2]*Department of Poultry Science, North Carolina State University*, *Raleigh*, [3]*Universidade Estadual Paulista*, *UNESP-Jaboticabal, Brazil*, [4]*Animal Parasitic Diseases Laboratory, USDA, ARS*, *Beltsville, MD*, [5]*Research and Testing Laboratories, Medical Biofilm Research Institute*, *Lubbock, TX*.

A protective digestive microflora helps prevent and reduce broiler infection and colonization by enteropathogens. Some feed additives (FA) promote healthy and protective microbial populations (MP) and could be used as alternatives to antibiotic growth promoters. In the current experiment, broilers fed corn-soybean meal diets with inclusion of 5% DDGS and supplemented with essential oil (EO) blends and probiotics were infected with mixed *Eimeria* spp. to determine effects of these FA and *Eimeria* infection on ileal and cecal MP. The 8 treatments (Trt) included 4 controls: Uninfected-Unmedicated (UU), Unmedicated-Infected (UI), BMD+Coban as positive control (PC), and ionophore (Coban) as negative control (NC). The 4 Trt included FA: 2 probiotics, BC30, and Calsporin; and 2 EO, Crina POULTRY Plus (CPP) and Crina PoultryAF (CPF). Day-old male Ross broilers were raised to 14 d and inoculated at 15 d with *E. acervulina*, *E. maxima*, and *E. tenella*. Ileal and cecal samples were collected at 14 and 22 d (7 d post-infection). Digesta DNA was PCR-amplified at the 16S rDNA V3 region and analyzed by denaturing gradient gel electrophoresis (DGGE) to generate % similarity coefficients (%SC) on band pattern dendrograms. Coccidia infection, probiotic, and EO changed MP from those seen in UU ilea. Treatment with CPF greatly altered cecal MP. UU ilea MP had about 60%SC to those in other treatment groups. While CPF cecal MP at pre-challenge had 77.7%SC with other groups, there was a decreased to 54%SC at post-challenge. There were expected changes in pre- and post-challenge ileal and cecal MP, respectively, with CPF having the greatest treatment effect on digestive MP.

Key Words: coccidia, probiotic, essential oil

657 Effect of microbial-nutrition interaction on chicken immune system after the early administration of probiotic with organic acids in young chicks. J. C. Rodriguez-Lecompte*[1], J. Brady[1], G. Camelo-Jaimes[2], S. Sharif[3], G. Crow[1], G. O. Ramirez-Yañez[1], W. Guenter[1], and J. D. House[1], [1]*University of Manitoba*, *Winnipeg, Manitoba, Canada*, [2]*ADYZYME*, *Bogota, Cundinamarca, Colombia*, [3]*University of Guelph*, *Guelph, Ontario, Canada*.

The present study was conducted to characterize the effect of *Lactobacillus acidophilus*, *Streptococcus faecium*, and *Saccharomyces cerevisiae* and organic acids (e.g., sorbic acid and citric acid) on intestinal morphology, immunological parameters, and nutritional associated-genes. One-day old chicks were randomly allocated to one of 3 treatments: treatment 1 (T1) consisting of chicks not receiving either probiotic or organic acids; chicks in treatment 2 (T2) orally received probiotics and organic acids for 7 consecutive d; and treatment 3 (T3) chicks receiving probiotics and organic acids for 14 consecutive d. The study lasted 21 d. On d 11 and 21 (4 and 7 d after finishing T2 and T3 respectively), intestinal sections from duodenum (distal), jejunum (proximal to Meckel's diverticulum), ileum (proximal to ileo-cecal junction), and cecal tonsils were collected and analyzed by histology, reverse transcriptase-polymerase chain reaction (RT-PCR), and quantitative RT-PCR. T2 and T3 decreased ($P < 0.05$) villous height and width, crypt depth in the jejunum area during the first 7 d. At d 21, T3 affected increasing crypt depth and the number of goblet cells/mm2 in the jejunum and ileum area ($P < 0.05$). Regardless of the treatment at d 0, 11 and 21, there was only avian β-defensin (AvBD)3 mRNA expression in the crop, proventriculus, duodenum, jejunum, ileum, cecal tonsil and bursa; however, cathelicidin B1 was present in the bursa in all treatments after d 7. Interestingly, AvAng4 was not affected either by treatment or time. In the ileum area, only mRNA levels of interleukin (IL)-10 and IL-12 were affected by T2 and T3 at d 22 ($P < 0.05$). In cecal tonsils, IL-6 and IL-12 were affected by T2 and T3 at d 11 ($P < 0.05$); however, there was a significant downregulation of IL-12 and upregulation of interferon-γ at d 22 ($P < 0.05$). GHR and GHSR mRNA expression was detected in all the groups at d 11 and d 22. In conclusion, probiotic and organic acid effects on chick's intestine include triggering sensor molecules of the innate immune system, which may produce antimicrobial proteins and peptides.

Key Words: probiotic, defensin, cytokines

658 Probiotic, prebiotic and yeast supplementation in broiler diets from 1 to 42 days of age: 2. Immune response and slaughter traits. H. M. Safaa*[1], S. A. Riad[1], F. R. Mohamed[1], S. S. Siam[2], and H. A. El-Minshawy[3], [1]*Animal Production Department, Faculty of Agriculture, Cairo University*, *Giza 12613, Giza, Egypt*, [2]*Breeding Department, Animal Production Research Institute*, *Dokki, Giza, Egypt*, [3]*Ministry of Agriculture*, *Dokki, Giza, Egypt*.

A total of 630 Arbor Acres broiler chicks at one-day old was used to study the effect of probiotic, prebiotic and/or yeast supplementation on the immune response and slaughter traits. Chicks were divided randomly into 6 treatments and housed at deep litter in an open house system. The 6 treatments, each replicated 3 times (35 chicks/replicate), were as follows: T1 (control; chicks fed corn-soy basal diet), and the other treatments diets were supplemented with 1g probiotic/kg diet as *Lactobacillus acidophilus* (T2), 1g yeast/kg diet as *Saccharomyces cerevisiae* ($5x10^{12}$ CFU/g; T3), 1g prebiotic/kg diet as mannan-oligosaccharide (T4), 1g probiotic+1g prebiotic/kg diet (T5), or 1g yeast+1g prebiotic/kg diet (T6). Basal diet contained 23.1% CP and 3,103 kcal AME/kg for the starter diet (0–21 d) and 20.0% CP and 3,207 kcal AME/kg for the finisher diet (21–42 d). Results indicated that, all biological additives increased the total count of white blood cells, the antibody titer against sheep red blood cells and the relative weights of immune organs (bursa, spleen and thymus) at 42 d. However, heterophils/lymphocytes ratio was

not affected by any treatment. In addition, carcass, giblets and edible relative weights at 42 d of age were improved by using the biological feed additives. For all traits, the best values were obtained in T6 followed by T5. It could be recommended from this study to supplement the biological additives to broiler diet from 0 to 42 d of age as mentioned above because it improves the broiler immunity and slaughter traits.

Key Words: probiotic, prebiotic, yeast, broiler immune response, slaughter traits