M132 Effects of dietary beta-glucan on the T helper cytokine balance in the intestine of broiler chicks. C. M. Cox*[1], L. H. Stuard[1], S. Kim[1], A. P. McElroy[1], M. Bedford[2], and R. A. Dalloul[1], [1]*Virginia Tech*, *Blacksburg*, [2]*AB Vista Feed Ingredients*, *Marlborough, UK*.

Immunomodulators like β-glucans have attracted considerable attention as potential alternatives to the prophylactic use of antibiotics. Despite increasing research, little is known about their regulatory influence on immune function in poultry. Two studies were conducted to evaluate the effects of a yeast-derived β-glucan (Auxoferm YGT) on gene expression of T helper cytokines in the intestine. Day old chicks were fed a diet containing 0, 0.02, or 0.1% β-glucan. For the first study, small intestinal sections were collected on d 7 and d 14 to evaluate gene expression by quantitative real-time PCR. On d 7, interleukin (IL)-18 expression was upregulated in the jejunum but decreased on d 14 in the duodenum of the 0.02% β-glucan birds. Expression of IL-18 also decreased on d14 in the ileum of both β-glucan groups when compared with control. On d 7, IL-4 expression was downregulated in both β-glucan treated groups in the duodenum and in the 0.1% treated group in the jejunum and ileum. In contrast, IL-4 was upregulated in the duodenum of treated birds and in the ileum of 0.1% fed birds on d 14. Similarly, IL-13 was downregulated in all intestinal sections of 0.1% β-glucan fed birds on d 7. The second study included a mixed *Eimeria* infection on d 8 and samples were collected on d 10, 14, and 21 post-hatch. Despite the fact that no significant differences were seen among treatment groups, IL-18 expression was consistently upregulated in the *Eimeria* challenged birds due to β-glucan exposure. IL-4 expression was downregulated in the non-challenged birds fed the 0.1% β-glucan diet. Mucin-1 expression was significantly decreased due to 0.1% β-glucan supplementation. On d 14, mucin-2 expression was decreased due to the *Eimeria* infection in the 0.1% β-glucan fed birds. Though not significant, there was a tendency for birds fed 0.1% β-glucan to express lower levels of IL-13 than the control birds. Taken together, the data provided from these trials strongly suggest that β-glucans downregulate T helper type 2 cytokines and thus favor a T helper type 1 cell response.

Key Words: β-glucan, poultry, cytokines

M133 Effect of capsicum and turmeric oleoresins with betaine on the performance of broilers challenged with coccidiosis. V. Brito[1], C. Moynat*[2], A. Casarin[3], M. Forat[3], and D. Bravo[1], [1]*Euronutec*, *Queretaro, Mexico*, [2]*Pancosma*, *Geneva, Switzerland*, [3]*Instituto Internacional de Investigacion Animal*, *Mexico*.

During coccidiosis, vaccination acquired immunity is insufficient to keep broiler performance. Studies showed that betaine (BE), capsicum (CA) and turmeric (TU) positively impact innate immunity. The combination of these 3 products should positively affect immunity and therefore improve performance of birds infected with coccidiosis. The objective was to evaluate the efficiency of a mixture of CA and TU oleoresins (PF = Proflora / XT 6986) combined with 2 levels of BE on performance of vaccinated broilers challenged with coccidiosis. Day-old broilers were allotted to 4 treatments and challenged with *Eimeria* spp. at d 14 (48 birds * 10 cages/treatment). The treatments were set as follow, doses in ppm, with bacitracin (BA), Nicarbazin (NI), salinomycin (SA), nitrofuran (NO). Starter Diet (d 1 to 14): T1 = 55 BA + 125 NI; T2 = 50 PF; T3 = 50 PF; T4 = 50 PF. Grower diet (d 15 to 42): T1 = 55 BA + 65 SA + 50 NO; T2 = 50 PF; T3 = 50 PF + 500 BE; T4 = 50 PF + 1000 BE. Finisher (d 43 to 52): T1 = 55 BA + 65 SA + 50 NO; T2 = 100 PF; T3 = 100 PF + 500 BE; T4 = 100 PF + 1000 BE. All birds except in T1 were vaccinated against coccidiosis at d 1. BW, BWG, FCR were recorded. Data were analyzed using GLM procedure of SAS. Before the challenge, there was no difference between treatments in FCR ($P = 0.23$). After the challenge, FCR of T1 was lower than T4 (−3.4%, $P = 0.02$) and tended to be lower than T2 (−2.6%, $P = 0.08$). FCR was similar between T1 and T3 (2.50 kg/kg, $P = 0.93$). No difference between treatments was observed in terms of BWG ($P = 0.65$) and final BW ($P = 0.68$). In spite of different modes of action, anticoccidials or vaccine combined to PF and BE lead to similar performance. These results show that a mixture of capsicum and turmeric oleoresins with 500 ppm of BE can be used associated to vaccination to maintain broiler performance in case of coccidiosis infection.

Key Words: essential oils, betaine, coccidiosis

M134 Excess dietary amino acids reduce splenic pro-inflammatory cytokine mRNA abundance and increase anti-inflammatory cytokine mRNA abundance during an acute phase response. A. Diaz[1], N. Hamel[1], K. Martorana[1], R. Angel[2], and B. D. Humphrey*[1], [1]*California Polytechnic State University*, *San Luis Obispo,* [2]*University of Maryland*, *College Park*.

The objective of this experiment was to determine the effect of dietary amino acid levels on the catabolic response to infection. Catabolic responses to infection are coordinated through the pleiotropic effects of cytokines, thus mRNA abundance of interleukin (IL)-1β, IL-6, IL-18, IL-4, IL-13 and transforming growth factor (TGF)-β4 were quantified in the spleen. Male Cobb 500 hatchlings were raised in pens (n = 15/pen) for 14 d and were fed a diet that met NRC requirements. On d 14, birds were fed 1 of 2 diets (n = 20/diet) that contained adequate (A) or excess (E) amino acid levels. The E diet contained excess Phe (+0.43%), Trp (+0.14%), Thr (+0.30%) and Arg (+0.35%). On d 21, half of the pens per dietary treatment (n = 10) were either not injected or injected with 1 mg/kg BW of *E. coli* lipopolysaccharide (LPS). The spleen from one bird per pen was collected at 3, 12, 24, 48, 96 and 168 h post-injection for measurement of cytokine mRNA abundance using quantitative real-time PCR. IL-4 and IL-13 mRNA were not detected at any time point. At 3 h, LPS-injected chicks fed the E diet had 2.6-fold higher TGF-β4 mRNA abundance compared with LPS-injected chicks fed the A diet ($P < 0.05$). At 12 and 168 h, LPS-injected chicks fed the A diet had 2-fold and 7.8-fold higher IL-1β mRNA abundance compared with LPS-injected chicks fed the E diet, respectively ($P < 0.05$). At 24 h, LPS-injected chicks fed the E diet had 3-fold higher IL-18 mRNA abundance compared with LPS-injected chicks fed A diet ($P < 0.05$). At 168 h, LPS-injected chicks fed the A diet had 12.6-fold higher IL-6 mRNA compared with LPS-injected chicks fed the E diet ($P < 0.05$). Taken together, the changes in cytokine profiles in response to LPS-injected birds fed the E diet indicate that feeding specific amino acids in excess of their growth requirement may mitigate the catabolic response to LPS-injection.

Key Words: amino acid, cytokine, inflammation

M135 Effects of repeated intravenous lipopolysaccharide injection on hematological characteristics of chicken blood. O. T. Bowen, R. F. Wideman, R. L. Dienglewicz, and G. F. Erf*, *Department of Poultry Science, Division of Agriculture, University of Arkansas*, *Fayetteville*.

Lipopolysaccharide (LPS) is a cell-wall component of gram-negative bacteria and an important pathogen-associated molecular pattern

recognized by pattern-recognition receptors of innate immunity. LPS stimulates monocytes/macrophages via Toll-like receptor-4 to produce vasoactive factors including nitric oxide, a potent vasodilator. In previous in vivo studies we established that in chickens, intravenously (i.v.) administered LPS induced a transient pulmonary hypertensive response within 1 h, increased plasma nitric oxide (NO) reaching peak levels by 6 h, and resulted in greatly reduced monocyte levels in blood samples collected 1 h post-LPS injection. Examination of the effects of a repeat i.v. injection of LPS revealed a lack of response of the pulmonary vasculature to a second LPS injection that lasted 5 d post-primary LPS injection. The objective of this study was to examine in vivo effects of a second i.v. LPS injection on cells in the systemic circulation using the 6 h peak in plasma NO levels and the 1 h drop in monocytes as end-point measurements of in vivo LPS effects. The second i.v. LPS injection was administered at 1 (24 h), 2, 3, 4, 5, 6, or 7 d post-initial LPS injection. Additionally, LPS-specific antibody titers in the plasma were also monitored post-primary LPS injection. While the drop in monocyte concentrations at 1 h and the increase in plasma NO at 6 h were observed with all second LPS administrations, the LPS-stimulated rise in plasma NO levels was, however, attenuated when LPS was injected at 5- or 6-d post-initial LPS injection. This attenuation in the increase in plasma NO following a second LPS administration at 5- and 6-d post-primary LPS injection may be explained by the presence of LPS-specific antibodies which reached peak plasma levels 4- to 6-d post-primary LPS injection. Hence, it appears that unlike the pulmonary vasculature, cells in the systemic circulation continue to be responsive to LPS stimulation administered 24 h to 7 d post-primary LPS injection.

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Key Words: lipopolysaccharide, nitric oxide, chicken leukocytes

M136 Effects of dietary conjugated linoleic acid on macrophage functions in broilers immunosuppressed with cyclophosphamide. D. Liu*, F. Y. Long, Y. M. Guo, Z. Wang, and J. M. Yuan, *China Agriculture University*, *Beijing, China*.

This study was carried out to investigate the effects of conjugated linoleic acid (CLA) on macrophage functions in broilers immunosuppressed with cyclophosphamide (CY). The experiment was designed as a 3 × 2 factorial arrangement, i.e., 3 CLA levels (0, 1.0% and 2.0%) in the diet and with or without CY injection as an immunosuppressive challenge. Two hundred and 16 1-d-old male Arbor Acres broiler chickens were randomly allocated into 6 treatments with 6 replicates. CLA was the mixture of 2 CLA isomers (c9,t11-CLA:t10,c12-CLA = 20:80). CY was injected into the femoral muscle of broilers at a dose of 80 mg/kg of body weight for 3 consecutive days (14, 15 and 16). On d 21, the peritoneal exudate macrophages from 12 broilers per treatment were collected for in vitro culture. Phagocytic rate and phagocytic index of macrophages were detected through ingesting sheep red blood cells. The levels of nitric oxide (NO) and the cytokine interleukin (IL)-1 in the culture supernatants of macrophages were assayed by Griess reagent and bioassay method, respectively. Statistical analysis of all data was performed by 2-way ANOVA with SPSS. Individual treatment means were compared using Duncan′s multiple comparison when the significant ($P < 0.05$) interaction between the main effects was observed. The results showed that the immunosuppressive challenge with CY significantly decreased the phagocytic rate and index of macrophages ($P < 0.05$), and the addition of 2% CLA significantly increased the phagocytic rate and index of macrophages ($P < 0.05$). There was a significant interaction between CLA levels and immunosuppressive challenge on the secretion of NO and IL-1 of macrophages ($P < 0.05$). But 1% dietary CLA had no influence on phagocytic functions and the secretion of NO and cytokines of macrophages. These results suggest that the dietary supplementation of 2% CLA alleviates the suppressive effects of CY on macrophage phagocytic functions and has the effects of bidirectional regulation on the secretion of NO and IL-1 of macrophages in broilers.

Key Words: conjugated linoleic acid, macrophage function, broilers

M137 Broiler breeder feeding programs and trace minerals on cytokine gene expression response in progeny. N. M. Leandro[1,2], R. Ali[1], M. Koci[1], V. Moraes[1], M. J. Wineland[1], J. Brake[1], and E. O. Oviedo-Rondón*[1,3], [1]*North Carolina State University*, *Raleigh,* [2]*Universidade Federal de Goiás*, *Goiania, GO, Brasil*, [3]*Universidade Estadual Paulista, UNESP*, *Jaboticabal, SP, Brasil*.

This study examined how feed allocation programs during breeder pullet rearing and dietary trace mineral (TM) sources during lay could affect the immune response of broiler progeny. Cobb 500 breeders were fed according to 2 feed allocation programs, either sigmoid late fast (LF) or sigmoid late slow (LS) until peak of production. From 56 to 62 wk of age, breeders were fed corn-soybean diets with 5% DDGS with either inorganic TM or an organic source (Mintrex P) to replace 30% of Cu, Zn, and Mn. Total dietary levels of the TM evaluated were 25, 125, and 125 ppm, respectively. Fertile eggs were collected for 4 d, incubated, and placed in pedigree bags at 19 d of incubation. Thirty 6 chicks/treatment were identified with neck tags to track hen effects, and placed in 2 isolation rooms, each with 6 floor pens. Three broilers per treatment were placed in each pen for 12 broilers per pen total. All broilers were fed the same diet. At 7 d of age, La Sota Newcastle disease virus (NDV) vaccine was applied by ocular route in one room only. Whole blood cells were collected at 4 d after vaccination to assay for cytokine (interleukin (IL)-2, IL-4, and interferon-γ) gene expression and serum was collected at 14 d post vaccination to assay for humoral response to NDV. Data were analyzed as a 2 × 2 × 2 factorial design considering breeder feeding programs, TM sources in breeder diet, and broiler vaccination as main factors. Broiler vaccination increased gene expression of all cytokines evaluated. Broiler progeny from breeders fed diets with 30% organic TM increased IL-4 expression after NDV vaccination, while vaccination did not cause significant upregulation of this gene in broiler progeny when breeders were fed 100% inorganic. Expression of IL-2 was found to be increased following vaccination in broilers from LF breeders fed diets with 30% organic TM. However, there was no significant change in IL-2 expression post vaccination in broilers from LS breeders fed the same diet. It was concluded that breeder feeding programs during rearing and dietary TM source during egg production influences the type and magnitude of cytokine expression in broiler progeny.

Key Words: breeder effects, trace minerals, cytokines

M138 Copy number variants in two genetically distinct chicken lines. X. Li*[1], W. Chou[1], S. J. Lamont[2], R. Croomjmas[3], and H. Zhou[1], [1]*Texas A&M University*, *College Station,* [2]*Iowa State University*, *Ames,* [3]*Wageningen University*, *PO Box 338, 6700 AH, Wageningen, the Netherlands*.

Genomic copy number variation (CNV) is another important source of genetic variation besides single nucleotide polymorphisms and microsatellites. In humans, CNVs are associated with Mendelian disease and complex traits. The high-throughput array has provided a powerful tool to discover copy number variation at the genome level. Two genetically distinct highly inbred chicken lines (Fayoumi and Leghorn G-B1) were used in this study. Previous study has shown that Fayoumi is resistant,

while Leghorn is susceptible, to avian influenza virus (AIV) infection. The Agilent 244K chicken CGH array was utilized to identify the potential CNVs associated with host response to pathogen. Six biological replicates from each line, in total, 12 biological replicates, were used. Red Jungle fowl was used as a reference to normalize the microarray data. There were 241 and 269 CNVs identified in Fayoumi and Leghorn, respectively, of which 116 and 119 were located in known chicken QTLs based on chicken QTLdb database. The CNVs identified in this study have also generated strong candidate CNVs potentially associated with host response to AIV infection in chickens.

Key Words: copy number variation, host response, avian influenza virus

M139 Phage display selection and characterization of a single-chain antibody (scFv) against chicken CD40. D. Abi-Ghanem*[1], C-H. Chen[1], L. Njongmeta[1], J. Bray[1], W. Mwangi[1], S. D. Waghela[1], J. L. McReynolds[2], and L. R. Berghman[1], [1]*Texas A&M University*, *College Station*, [2]*U.S. Department of Agriculture, Agricultural Research Service*, *College Station, TX*.

CD40, an integral membrane glycoprotein of the tumor necrosis factor-receptor super family, is mainly expressed on antigen-presenting cells (APCs), including B-cells, macrophages, and dendritic cells. The interaction between CD40 and its ligand CD154 (CD40L) mediates specific $CD4^+$ T-cell help to APCs in response to T-cell dependent antigens, and provides crucial signals for antigen-specific T-cell priming and expansion, as well as heightened antibody production and immunoglobulin class switching in B-cells. In contrast to the extensive characterization of mammalian CD40 by use of agonistic anti-CD40 monoclonal antibodies, which can mimic $CD4^+$ T-cell help to APCs, investigation of chicken CD40 (cCD40) has been limited. In this study, we describe the production of a dimeric single-chain antibody fragment (scFv) against cCD40. An immune antibody library against cCD40 was constructed by phage display. Following 3 rounds of panning against cCD40, specific, likely high-affinity antibodies were obtained. Soluble anti-cCD40 scFv (~35 KDa) was purified by nickel affinity chromatography and characterized by immunoblotting. This scFv recognized cCD40 in ELISA, and agglutinated chicken DT40 B-cells in vitro. We are currently investigating the biological activities of this scFv, particularly the induction of nitric oxide synthesis in chicken HD11 macrophages and proliferative stimulation of serum-starved chicken DT40 B-cells. These activities will evaluate the extent to which the anti-cCD40 scFv can mimic the effects of CD40L, providing the signals needed to induce activation of chicken APCs in vitro. Such an agonistic anti-cCD40 scFv may therefore constitute a powerful tool to study the role of CD40 in the chicken immune system.

Key Words: chicken CD40, single-chain antibody fragment, co-stimulation

M140 Functional characterization of the avian macrophage migration inhibitory factor (MIF). S. Kim*[1], K. B. Miska[2], M. C. Jenkins[2], R. H. Fetterer[2], C. M. Cox[1], L. H. Stuard[1], and R. A. Dalloul[1], [1]*Animal & Poultry Sciences, Virginia Tech*, *Blacksburg,* [2]*Animal Parasitic Diseases Laboratory, ARS, USDA*, *Beltsville, MD*.

Macrophage migration inhibitory factor (MIF) is recognized as a soluble factor produced by sensitized T lymphocytes and inhibits the random migration of macrophages. Recent research shows a more prominent role of MIF as a multi-functional cytokine mediating both innate and adaptive immune responses. This study describes the cloning and functional characterization of avian MIF in an effort to better understand its function and potential in poultry health applications. The full-length avian MIF gene was amplified from stimulated chicken lymphocytes and cloned into a prokaryotic expression vector. The confirmed 115 amino acid sequence of avian MIF has 71% identity with human and murine MIF. The bacterially expressed avian recombinant MIF (rChMIF) was purified, the endotoxins removed, and a 4 h-chemotactic assay performed using a 48-well chemotaxis chamber. Diff-Quick staining results showed sharply decreased migration of macrophages in the presence of 10 ng/ml rChMIF. Further, the expression of various cytokines was measured in peripheral blood mononuclear cells (PBMCs) or splenocytes using quantitative real-time PCR (qRT-PCR). Isolated PBMCs or splenocytes were cultured in the presence or absence of rChMIF, with or without lipopolysaccharide (LPS) or Concanavalin A (Con A) for 6 or 12 h. qRT-PCR analysis revealed that rChMIF alone did not induce transcription of interleukin (IL)-1β or induced-nitric oxide synthase (iNOS). However, the presence of rChMIF enhanced levels of IL-1β and iNOS during PBMCs stimulation with LPS. Similarly, there was no effect of rChMIF alone on splenocytes; however, the Con A-stimulated lymphocytes showed enhanced interferon (IFN)-γ and IL-2 transcripts in the presence of rChMIF. Interestingly, addition of rChMIF to the stimulated PBMCs, in the presence of lymphocytes, showed anti-inflammatory function of rChMIF. To our knowledge, this study represents the first report for the functional characterization of avian MIF, which inhibits migration of macrophages similarly to mammalian MIF, and it also mediates inflammatory responses during antigenic stimulations.

Key Words: MIF, avian immunity, real-time PCR

M141 US Veterinary Immune Reagent Network. H. Lillehoj*[1], S.-H. Lee[1], D.-K. Kim[1], M.-S. Park[1], D. Tompkins[2], C. Baldwin[2], J. LaBresh[3], and B. Wagner[4], [1]*USDA-ARS*, *Beltsville, MD,* [2]*University of Massachusetts*, *Amherst,* [3]*Kingfisher Biotech*, *St. Paul, MN,* [4]*Cornell University*, *Ithaca, NY*.

To advance veterinary immunology and animal disease research, a CSREES-funded NRI consortium grant (#2005–01812) was established in 2005 to develop immunological reagents specific for poultry, ruminants, swine, equine and aquaculture species (http://www.umass.edu/vetimm). Immunological reagents to be developed through this grant include monoclonal antibodies (mAb) and polyclonal antibodies that identify the major leukocyte subpopulations (T and B lymphocytes, NK cells, neutrophils, macrophages, and dendritic cells) for many animal species including fish. In addition, recombinant cytokines and chemokines as well as antibodies to them and to their receptors, will be developed and these immune reagents will be valuable in research to understand the major components of immune system which are involved in inflammation, innate and adaptive immunity. These immunological reagents will be used to (1) evaluate changes associated with diseases and vaccination, and (2) manipulate various lymphocyte subpopulations to evaluate their roles in protective immunity as well as in immunopathology. In this report, progress in poultry immune reagent development will be discussed.

This project is funded by USDA-CSREES proposal 2005-01812 and was carried out as part of the US Veterinary Immune Reagent Network. For commercially available immune reagents from this consortium, go to http://kingfisherbiotech.com

Key Words: poultry, immune reagent, diseases