**Immunology**

**P301  The effect of IgY and vaccine on reducing C. jejuni in intestine of broilers.** G. Sajadi1, S. Rahimi*2, P. Khaki1, and H. Ebrahimi1, 1Tarbiat Modares University, Tehran, Tehran, Iran, 2Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran.

The purpose of this study was to determine the effect of C. jejuni specific IgY and vaccine on BW, FCR, FI and reducing C. jejuni (C.J) in GI tract of broilers. Ten TETRA-SL hens were hyper immunized with C.J whole cell antigens obtained by ultrasonication and administrated at protein concentration of 500 µg/mL after centrifugation. Primary immunization was performed with 250 µg of the antigen prepared in equal volume of Freund’s complete adjuvant and saline. Booster injection was done twice in each 14 d, using incomplete Freund’s adjuvant. Bleedings were performed 20 d after first injection and eggs were collected. The presence of anti-C.J IgY in the yolk and serum was monitored by HI test. The results indicated that high titer of IgY may be obtained 21 and 28 d after first immunization in serum and egg yolk, respectively. In phase 2 of experiment, 210 d-old female broilers were randomly assigned to 7 groups and 3 replications of 10 birds. The experimental groups identified by: C, A, B, C.J, C.J A, C.J B, and C.J V. At d 21 the birds from 4 groups were gavaged with 1 mL of C.J 1 × 10⁶ cfu/mL. The groups that supplemented with antibody (A, C.J A) received 15 mL of yolk antibody (Ab) in 3.84 mL of drinking water from d 1 to d 42. The yolk powder treated groups (B, C.J B) received yolk powder, 0.4% in feed, from d 1 to d 42. One group received treatment vaccine of C.J (C.J V) in 1 and 14 d of age. The control group (C) did not treat with C.J, Ab and vaccine. Ab alone and vaccine treated groups had significantly lower cecal concentration of C.J. Ab and vaccine treated groups had a lower isolation of C.J from the liver (P < 0.05). There was no significant difference (P > 0.05) in BW, FI, FCR and mortality rate between the experimental groups.

**Key Words:** C. jejuni, IgY, vaccine, powder yolk, broiler

**P302  Evaluation of bacterial and viral toll-like receptor ligands stimulation of chicken thrombocyte inflammatory response.** F. Fereidoon*1 and T. R. Scott, Clemson University, Clemson, SC.

Thrombocytes have been well known for their role in homeostasis, initiation of wound repair, and, like mammalian platelets, are capable of producing several bio-reactive proteins. Thrombocytes have been found to express several toll-like receptors (TLRs) that detect the presence of bacterial or viral pathogens and signal the release of certain pro-inflammatory cytokines and mediators. We investigated the response of chicken thrombocytes when stimulated with bacterial and viral TLR ligands. Based on results from preliminary studies, the purpose of this study is to detect any differential responsiveness to bacterial and viral ligands. A series of completely randomized designed experiments with 2 × 5 factorial arrangements of treatments was used where treatments were 2 response times (10 and 60 min) and 5 TLR ligands. Thrombocytes were isolated from chicken blood and stimulated with no ligand, bacterial ligands [Lipopolysaccharide (LPS), and Lipoteichoic acid (LTA)], and viral ligands [Polyinosinic-polycytidylic acid (Poly I:C)] and thymidine homopolymer phosphorothioate ODN (Poly(dT)). Relative quantification of IL-6 and iNOS gene expression was performed using RT-PCR. Microscopy was used for detection of intracellular IL-6 and NO, and releases of these products were examined by the B9 cell bioassay and Griess Reagent assay, respectively. Only LPS significantly increased gene expression of the IL-6 at 60 min. Although gene expression of iNOS did not significantly increase due to ligand exposure, constitutive expression of iNOS was observed compared with GAPDH. Viral ligand stimulation led to significant increases of NO release into thrombocyte supernatants at both time points. IL-6 was observed only in LPS stimulated thrombocyte supernatants at 60 min. Differential responsiveness was observed due to different TLR ligands in thrombocytes, which appear to be specialized innate immune cells.

**Key Words:** thrombocyte, nitric oxide, cytokine, toll-like receptor ligand, innate immunity

**P303  Propolis, bee pollen and mannann oligosaccharides supplemented continuously or intermittently enhanced antioxidants enzymes, immunity and lymphoid organs of broiler chicks.** Y. A. Attia*,1,3, M. S. Ibrahim2, A. E. Abd-Al-Hamid3, M. A. Al-Harthi1, and A. S. El-Naggar1, 1Arid Land Agriculture Department, King Abdulaziz University, Jedda, Saudi Arabia, 2Department of Animal and Poultry Production, Faculty of Agriculture, Damahour University, Damanhour, Behria, Egypt, 3Department of Microbiology, Faculty of Veterinary Medicine, Damahour University, Damanhour, Behria, Egypt.

The aim of this research was to compare the effect of bee pollen (BP) and/or propolis (Pro) as an alternative to well-known growth promotors mannan oligosaccharides (MOS) when given continuously or intermittently on immune responses, antioxidants enzymes, weight and morphology of lymphoid organs of broilers. Thus, a total of 324 unsexed one-day-old Arbor Acres broilers were randomly distributed into 9 treatment groups, each replicated 6 times of 6 birds per replicate. The chicks were kept in wire cages and fed the same basal diet and were submitted to the following treatments: control without supplementation (control), or supplemented with BP at 300 mg, Pro at 300 mg, BP+Pro at 300 mg and MOS at 0.5 g/l water. Each supplemented group was subdivided into 2 subgroups in which the additives were administrated continuously or intermittently. Thus, there were 4 additives each given by 2 administration ways plus the control group (un-supplemented). In the continuous supplemented groups, supplemental treatments were given from one till 36 d of age, and in the intermittent supplemented groups, the administration was only 3 d before, on the day of and day after vaccination. In conclusion, BP or Pro administrated either by continuous or intermittent way was equally potent for improving immunity, antioxidants enzymes and was similar or even better than MOS. All supplements given either continuously or intermittently resulted in a significant higher thymus and bursa percentages than the control group. Combining BP with Pro resulted in further increase in thymus percentage compared with control group. Furthermore, diameter of small and large follicle of Fabricius bursa, thymus and splenic lymphoblastic were increased. Thus, according to these findings, either BP or Pro is adequate when given by intermittent way which indicated considerable saving (40%) of supplementation cost.

**Key Words:** broiler, immunity, bee pollen, propolis, mannan oligosaccharides

**P304  L-Arginine requirement of broiler chickens challenged with infectious bursal disease vaccine.** J. Z. Tan*1, Y. M. Guo1, T. J. Applegate2, E. C. Du1, and X. Zhao1, 1China Agricultural University, Beijing, China, 2Purdue University, West Lafayette, IN.
The aim of present study was to determine the l-arginine (Arg) requirement of broiler chicks under infectious bursal disease vaccine (IBDV) caused immunosuppression, and investigate the effect of dietary Arg supplementation on immune responses of broilers challenge with IBDV. Five hundred one day old female Ross 308 broilers were equally assigned into 10 groups in a 5 × 2 factorial arrangement (n = 5 cages/treatment; 10 birds/cage). There were 5 dietary Arg concentrations (0.99, 1.39, 1.76, 2.13, and 2.53%) and with or without immune challenge (intramuscular inoculation of IBDV or saline at 14 d of age). At 21 d of age, blood samples were collected from each bird for peripheral blood mononuclear cells (PBMC) and serum isolation. The IBDV inoculation significantly suppressed (P < 0.05) the serum IgA content and mitogen-stimulated peripheral blood mononuclear cell (PBMC) proliferation, indicating that IBDV inoculation caused an immunosuppression. Increasing dietary Arg concentration significantly (P < 0.05) enhanced these immune indices. The Arg requirement of IBDV inoculated broilers for minimum FCR in quadratic model (1.89%) was higher (P < 0.05) than that of control broilers (1.60%; P = 0.034). The broken-line analysis suggested that the Arg requirements of IBDV inoculated broilers for optimal immune status (IgA: 1.65%; PBMC proliferation (stimulated by LPS): 1.74%) is higher (P < 0.05) than control broilers (IgA: 1.24%; PBMC proliferation (stimulated by LPS): 1.31%; P ≤ 0.04). These results indicate that Arg supplementation is required to get the optimal growth performance for immunosuppressed broilers, and dietary Arg supplementation has beneficial effects in attenuating the immunosuppressive effects of IBDV inoculation.

**Key Words:** broiler, arginine, infectious bursal disease, immunosuppression, requirement

**P305 Sandwich ELISA for detection of dengue non-structural glycoprotein antigen using chicken immunoglobulin Y (IgY).** A. Ganguly and H. H. Sunwoo*, University of Alberta, Edmonton, AB, Canada.

This study describes the production of IgY polyclonal egg yolk antibodies against the non-structural protein (NS). The objective of this study was to develop a quantitative detection system for dengue viral protein, targeting NS to determine the presence and/or degree of infection. Since, the NS is a 46 to 50 kDa glycoprotein expressed in infected mammalian cells and its template mRNA is the most abundant subgenomic RNA, it is a suitable candidate for developing antibodies for diagnostic applications. The NS contains several antigenic sites, which are targeted by both the humoral immune response and cytotoxic T lymphocytes. In this study we have prepared full length dengue viral NS expressed in E. coli and purified. The full length NS was used for chicken polyclonal IgY antibodies for development of hetero-sandwich enzyme-linked immunosorbent assay (ELISA) for early diagnostics of dengue. The immunization of chickens with NS induced a relatively strong immune response. The titer of specific IgY antibodies was monitored by ELISA measurement using NS every week during the immunization period. It increased continuously to a maximum of 0.827 nm against NS at 4 weeks after the first booster. Using the high titer of IgY allowed us to develop the sensitive sandwich ELISA, showing the detection limit of the viral antigen at 9.9 pg/mL. This study describes the production of 2 mouse monoclonal (P148.L1 and P148.L2) and chicken IgY polyclonal antibodies against the most abundant dengue NS. The most important finding was the use of inexpensive polyclonal IgY antibody to increase the sensitivity of the detection system for dengue viral protein at picogram levels. Furthermore, the immunossay method of detecting dengue NS antigen developed could be an effective and sensitive dengue quantitative detection system that can be used during any future dengue outbreak.

**Key Words:** dengue virus, non-structural protein, monoclonal antibody, chicken IgY antibody, ELISA

**P306 The hematology of polymicrobial bacteremias in commercial hens.** P. Cotter*, Cotter Laboratory, Arlington, MA.

A polymicrobial bacteremia (PB) exists when multiple species of bacteria occur in an animal’s blood. Examination of Wright’s stained peripheral blood films of apparently healthy commercial hens indicated a high incidence of PB. The objective is to provide a microscopic description of the hematological consequence of PB. The study population was 17 wk commercial hens housed in conventional, enriched, or aviary systems. Wright’s stained samples were examined using (40x and 100x objectives) by an Olympus CX41 light microscope equipped with an Infinity-2 1.4 Megapixel CCD USB 2.0 Camera. PB was established by finding either free existing or phagocytosed bacteria of at least 2 types. A differential white blood count was determined on 200 cells. A first heterophil/lymphocyte ratio (H/L) was computed by dividing the total number of heterophils by the number of small (resting) lymphocytes, a second H/L was computed by dividing total heterophils by total lymphocytes. Hens in which the H/L ratio by the first method was <0.4 were selected for a detailed microscopic study. These had cellular abnormalities affecting cytoplasm and nuclei, involving granulocytes and lymphocytes. Many granulocyte “ghosts,” in which the cell is barely visible, were found. Cell size variants, dwarfs and giants, were common. High numbers of reactive lymphocytes, plasmacytoid lymphocytes, and various types of Mott cells (plasmacytomas) were common. No hen with PB was without cyanophils, a recently described fourth granulocyte type distinct from heterophils, basophils, and eosinophils. Additional abnormalities were the irregular presence of karyolitic lymphocytes, and occasional karyolitic basophils. Reactive clusters (RC) in which groups of lymphoid and granulocytic cells are physically associated were a common feature. In conclusion, certain hens with an apparent low H/L may have a PB. In such cases, a standard “differential” count may not mirror their true “hemogram” without a more extensive consideration of hematological “character.”

**Key Words:** polymicrobial bacteremia, hematology, hematogram, cyanophil,

**P307 Dietary L-arginine supplementation alleviates infectious bursal disease vaccine-induced cellular immunosuppression in broiler chickens.** J. Z. Tan1, Y. M. Guo1, T. J. Applegate2, E. C. Du3, and X. Zhao1, 1China Agricultural University, Beijing, China, 2Purdue University, West Lafayette, IN.

Infectious bursal disease (IBD) is a highly contagious and immunosuppressive disease, which could result in secondary infections and suboptimal response to vaccinations. l-Arginine (Arg), the precursor of nitric oxide and polyamine, has been reported to enhance the immunity of animals. Thus, the object of this study was to evaluate the immunological effect of dietary Arg supplementation on the immunity of broiler chickens inoculated with infectious bursal disease vaccine (IBDV). Five hundred 1-d-old female Ross 308 broilers were equally assigned into 10 groups in a 5 × 2 factorial arrangement (n = 5 cages/treatment; 10 birds/cage). There were 5 dietary Arg concentrations (0.99, 1.39, 1.76, 2.13, and 2.53%) and with or without immune challenge (intramuscular inoculation of IBDV or saline at 14 d of age). At 2, 4 and 6 d post-inoculation (DPI), samples of blood was collected from...
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China, 3Jilin University, College of Veterinary Medicine, Changchun, Jilin, China. 1China Agricultural University, Beijing, China, 2USDA-ARS, West Lafayette, IN, 3Purdue University, West Lafayette, IN.

This study was conducted to evaluate whether dietary supplementation with l-arginine (Arg) could attenuate lipopolysaccharide (LPS)-induced systemic inflammatory response in broiler chickens. The experiment was designed as a 2 × 3 factorial arrangement (n = 8 cages/treatment; 6 birds/cage) with 3 dietary Arg concentrations (1.05, 1.42, and 1.90%) and 2 immune treatments (i.p. intraperitoneal injection of lipopolysaccharide (LPS) or saline). From 14 to 21 d of age, LPS (Escherichia coli 0111:B4) was injected 4 times at 48-h intervals (1 mg/kg of BW), after which spleen and cecal tonsil samples were collected. The LPS injection significantly decreased BW gain and feed intake (FI), increased feed conversion ratio (FCR) from 14 to 21 d of age (P < 0.05). Dietary Arg supplementation improved 14 to 21 d BW gain, feed intake, and FCR (P < 0.05). LPS injection increased (P < 0.05) mRNA expression of pro-inflammation cytokines (IL-1β and IL-6) in the spleen and cecal tonsils. The dietary Arg supplementation decreased (P < 0.05) the mRNA expression of IL-1β in the spleen and cecal tonsils. The only cytokine that had a significant Arg*LPS interaction was IL-1β in the cecal tonsils, with increasing dietary Arg concentration, IL-1β mRNA expression reduced in LPS challenged treatments (P < 0.005). LPS injection increased TLR-4 (P < 0.05) mRNA expression in the spleen and cecal tonsils, dietary Arg supplementation decreased (P < 0.05) the TLR-4 mRNA expression in spleen and cecal tonsils, which likely induced the measured decrease (P < 0.05) of NFκB mRNA expression in the cecal tonsils. Thus, these results suggest that dietary Arg supplementation modulates the inflammatory response partly through the suppression of LPS/TLR-4 pathway.

Key Words: arginine, broiler, inflammatory response, lipopolysaccharide

P308 Dietary L-arginine supplementation modulates lipopolysaccharide-induced systemic inflammatory response in broiler chickens. J. Z. Tan*,1, Y. M. Guo1, S. D. Eicher2, and T. J. Applegate1, 1China Agricultural University, Beijing, China, 2USDA-ARS, West Lafayette, IN.

This study was conducted to evaluate whether dietary supplementation with l-arginine (Arg) could attenuate lipopolysaccharide (LPS)-induced systemic inflammatory response through LPS/TLR-4 signaling pathway in broilers. The experiment was designed as a 2 × 3 factorial arrangement (n = 8 cages/treatment; 6 birds/cage) with 3 dietary Arg concentrations (1.05, 1.42, and 1.90%) and 2 immune treatments (i.e. intraperitoneal injection of lipopolysaccharide (LPS) or saline). From 14 to 21 d of age, LPS (Escherichia coli 0111:B4) was injected 4 times at 48-h intervals (1 mg/kg of BW), after which spleen and cecal tonsil samples were collected. The LPS injection significantly decreased BW gain and feed intake (FI), increased feed conversion ratio (FCR) from 14 to 21 d of age (P < 0.05). Dietary Arg supplementation improved 14 to 21 d BW gain, feed intake, and FCR (P < 0.05). LPS injection increased (P < 0.05) mRNA expression of pro-inflammation cytokines (IL-1β and IL-6) in the spleen and cecal tonsils. The dietary Arg supplementation decreased (P < 0.05) the mRNA expression of IL-1β in the spleen and cecal tonsils. The only cytokine that had a significant Arg*LPS interaction was IL-1β in the cecal tonsils, with increasing dietary Arg concentration, IL-1β mRNA expression reduced in LPS challenged treatments (P < 0.005). LPS injection increased TLR-4 (P < 0.05) mRNA expression in the spleen and cecal tonsils, dietary Arg supplementation decreased (P < 0.05) the TLR-4 mRNA expression in spleen and cecal tonsils, which likely induced the measured decrease (P < 0.05) of NFκB mRNA expression in the cecal tonsils. Thus, these results suggest that dietary Arg supplementation modulates the inflammatory response partly through the suppression of LPS/TLR-4 pathway.

Key Words: arginine, broiler, inflammatory response, lipopolysaccharide

P309 Duck toll-like receptor family. W. K. Elfeil*,1,3, R. R. Abouelmaatti5, W. Y. Han3, H. Abdien1, and X. K. Li1,2, 1Suez Canal University, Faculty of Veterinary Medicine, Ismailia, Egypt, 2Jilin University, Norman Bethune College of Medicine, Changchun, Jilin, China, 3Jilin University, College of Veterinary Medicine, Changchun, Jilin, China.

Duck shows different immune response to many microbes if compared with chicken, our work focused on identify the duck PRRs families and check either it functional genes or nonfunctional genes, then make analysis to its protein structures and trying to identifying the sites of mutation between duck and chicken as a universal bird model, so we can get more clear data about bird evolution, then trying to study the effect of this mutant sites on immune response. We cloned and expressed almost the complete duck TLRs family which reported as dTLR1–1/1–2/2–2/3–2/4/5/7/15/21 sequences in various duck tissues. We reported a degree of genetic variation in TLRs genes among the 3 different aves families (Anseriformes, Galliformes and Passeriformes); we also found patterns of positive selection acting on specific amino acid sites that could be linked to species-specific differences in pathogen-associated molecular pattern recognition. We also success to clone duck node like receptors include NOD1, NLR3, NLRX1 (to our knowledge this is the first actual report, not just prediction, for those NLR members in all birds) and NLR5 and from RLRs family we cloned LGP2, EIF4A3, and Dicer I type 3. And duck CLEC16A we also reported the in vivo and in vitro response of duck toll-like receptor to stimulation with LPS on peritoneal macrophage as and our result cleared its completely functional receptor and consider ortholog to the TLR4. This study provides evidence for the evolutionary patterns and implications of TLR polymorphism in new avian models species and extends the list of available avian immunogenetic genes.

Key Words: duck, toll-like receptor, cloning, expression


This study evaluated the effect of yeast-derived products on gut morphology and gene mRNA expression of toll-like receptors, C-type lectins, and cytokine profile in broiler chickens. Seventy one-day-old chickens were randomly assigned to 7 dietary treatments: Positive control containing antibiotic; Negative control (NC) without antibiotic; NC+10% of DDGS; NC+0.25% of yeast cell wall polysaccharides (CWP); NC+0.2% of a commercial product Maxi-Gen Plus containing nucleotides and CWP; NC+0.025% of nucleotides, and NC+0.05% of nucleotides. For intestinal morphology measurements, 5 birds per treatment were euthanized on d35 and approximately 5 cm long duodenum, jejunum, and ileum segments were collected. For gene mRNA expression analysis, cecal tonsil, and spleen were collected to assess the expression of toll-like receptors (TLR2 and TLR21), C-type lectins including monocyte mannose receptor (MMR), and mannose binding lectin (MBL), and cytokines (IL-10, IL-13, IL-12p35, and IFN-γ). The results of gut morphology demonstrated that diets containing 0.05% nucleotides and CWP significantly increased (P < 0.05) villus height in the jejunum of broiler chickens. The results for local innate immunity of broiler chickens in cecal tonsil showed that despite some changes in expression of the receptors and cytokines by dietary treatments, only the expression of TLR21 was significantly (P < 0.05) upregulated by diet containing 0.05% nucleotides. The results for the systemic innate immunity of broiler chickens in spleen demonstrated that diet containing nucleotides plus CWP upregulated the expression of TLR2, MBL, and IL-10. In addition, diet containing 0.05% of nucleotides upregulated the expression of TLR2 and MBL. In conclusion, diets containing CWP and 0.05% nucleotides increased villus height in the jejunum, and demonstrated immune-modulating effects by upregulation of receptors and cytokine involved in innate immunity of broiler chickens.

Key Words: yeast, gut morphology, cytokine, toll-like receptor, broiler chicken
P311 Functional phenotyping of chicken peritoneal exudate macrophages elicited with either Sephadex beads or egg yolk particles. W. K. Chou*, C. H. Chen, C. Vuong, D. Abi-Ghanem, and L. Berghman, Texas A&M University, College Station.

Mammalian macrophages can be polarized into 2 distinct phenotypic subtypes: classical (pro-inflammatory, M1) vs. alternative (anti-inflammatory, M2), based on their exposure to Th1- or Th2-driven stimuli, respectively. M1 macrophages display antimicrobial activity and M2 macrophages act as scavengers. They both catabolize arginine as a substrate, through inducible nitric oxide synthase (iNOS) and arginase I metabolism pathways, resp., to develop their ultimate characteristics. The polarization of elicited peritoneal exudate macrophages (PEMs) has been described recently in the murine system, but little attention has yet been paid to PEM polarization in the chicken. In this study, functional phenotyping of chicken PEMs elicited with 2 different stimuli (Sephadex beads vs. egg yolk) was performed based on arginase activity (whose expression level is higher in M2 macrophages), and nitrite oxide (NO) production (one of the main effector molecules of M1 macrophages). Peritoneal exudate cells were harvested 30 h or 42 h after a single i.p. injection of 50% egg yolk emulsion in PBS or 3% Sephadex suspension, respectively, and purified by the plastic adherence method. Both expressed similar arginase activity (2.11 vs. 2.50 µg urea, resp.), which was higher than in blood monocytes (0.93 vs. 0.94 µg urea, resp.). When the harvested PEMs were then further stimulated with LPS (a strong M1 polarizing stimulus) for 8 h in vitro, NO production and arginase activity were measured again. Sephadex-elicited PEMs produced 5-fold more NO than egg-yolk-elicited PEMs (5.85 vs. 0.97 µM). Arginase activity in Sephadex elicited PEMs had slightly declined (from 2.50 to 2.30 µg urea). However, the arginase activity in egg yolk elicited PEMs had increased from 2.10 to 3.10 µg urea. These results indicate that Sephadex-elicited PEMs, but not egg-yolk-elicited PEMs, have the potential to switch from the scavenger (M2) into the inflammatory phenotype (M1) when exposed to an M1 stimulus. Gene expression analysis of the polarization mechanisms will be conducted in the near future.

Key Words: functional phenotyping, PEM, arginase, nitrite oxide, phenotype switching

P312 Acquisition of immunity to the protozoan parasite E. adenoeides in turkey pouls and cellular responses to infection. U. Gadde, T. Rathinam, G. F. Erf, and H. D. Chapman*, University of Arkansas, Fayetteville.

Day-old turkey pouls were infected with 10^2 oocysts of Eimeria adenoeides and subsequently re-infected with 10^3 and 10^4 oocysts at 6 and 12 d of age respectively to simulate potential field exposure to infection. Numbers of new oocysts produced in the feaces peaked at 3 wk followed by a gradual decline. A second group of pouls were given an identical dosing regimen and challenged with 5 × 10^4 oocysts/poult at different times to evaluate the acquisition of immunity. Judged by weight gain and mortality, no protection had been acquired at 6 d of age, but partial protection was observed by 12 and 18 d of age. A third group of day-old pouls were also infected with 10^2 oocysts and subsequently re-infected with 10^3 and 10^4 oocysts at 6 and 12 d of age to evaluate cellular immune responses to infection. Sections of ceca from infected pouls showed a significantly higher leukocyte infiltration on d 6, 10, 12, 16, and 18 after infection than uninfected controls. The percent area occupied by CD4+ and CD8+ lymphocytes in the ceca, as assessed by immunohistochemistry, was significantly elevated in infected pouls on d 12, 16, and 18. The relative expression of chemokine CXCL2, and cytokines IL1β, IFNγ, IL10, IL13, IL2, IL12b, and IL18 was measured by real-time reverse-transcription PCR. The expression of CXCL2 and IL10 was found to be elevated on d 12, and IFNγ on d 10, 12, and 16. Expression of IL13 and IL18 was increased on d 10 and IL2 on d 10 and 16, and that of IL12b on da16 in infected pouls. Increase in the infiltration of leukocytes, percent area occupied by CD4+ and CD8+ lymphocytes, and changes in the relative expression of cytokines in the ceca characterize the dynamics of immune responses in turkey poult's infected with E. adenoeides.

Key Words: Eimeria, turkey, oocyst, cellular immunity, cytokine


Understanding the dynamics of Salmonella serovar contamination in poultry is important due to consumption of egg-containing products and its association to human salmonellosis. S. Enteritidis (SE) is shown to colonize the ovary of layers with subsequent egg-transmission. While enforcement actions target the eradication of SE from layers, there is a concern that other Salmonella serovars could occupy this niche and be a concern for egg-transmitted human salmonellosis. Thus, an understanding of ovarian susceptibility/resistance mechanisms is key in evaluating egg-contaminating potential across poultry-associated Salmonella serovars. Studies have identified S. Heidelberg (SH) as an egg-transmitted pathogen, albeit at a lower frequency than SE. Although varying serovar virulence cannot be ruled out, we hypothesize that the differential egg-contaminating potential of SE and SH is due to the stimulation of ovarian follicular and granulosa resistance mechanisms. In this study (n = 3), medium-to-large yolk-follicles were exposed to SE PT8 and SH (10^6 cfu/mL) for 2 h, and the transfer into the yolk was determined by plate counts. Further, granulosa cells were isolated from the preovulatory follicles, and anti-bacterial gene expression to Salmonella serovars at 5 and 24 h post infection was evaluated. Results from the Salmonella invasion assay showed a 2-fold decrease in SH transfer into the yolk as compared with SE, suggesting that SE is more pathogenic or the follicles are more susceptible to SE invasion. Gene expression, using chicken antibacterial response arrays showed a significant increase in pro-inflammatory chemokines and cytokines (CCL4, CCL5, IL1B, IL8), suggesting an antibacterial response to SH and not SE exposure. Further, there was also a significant increase in the lysozyme expression (90-fold) by granulosa cells on SH exposure. Our results suggest that a lower egg-contaminating potential of SH could be a result of increased anti-bacterial response by the ovarian granulosa cells and such a model could be useful for risk assessment of Salmonella serovars in relation to their egg contaminating potential.

Key Words: Salmonella, invasion, follicle, granulosa, egg


Mucosal immunity (IgA) is crucial for prevention of infection, but IgA responses to parenterally administered inactivated vaccines are often minimal. Recently, vectored vaccines have been investigated for potential mucosal effectiveness in controlling diseases affecting poultry including avian influenza and Salmonella. Presently, we have evaluated drinking water (DW) administration of inactivated antigens with a novel modified chitosan adjuvant (MCA), with and without sub-
P315  Proteomic analysis of macrophage activated with salmonella lipopolysaccharide. S. Makkar1,2, B. Packialakshmi1,2, R. Liyanage1, J. O. Lay1, and N. C. Rath2, 1University of Arkansas, Fayetteville, 2USDA-ARS, Fayetteville, AR.

Macrophages play pivotal role in immunity as phagocytes and accessory cells, produce various cytokines, chemokines, and growth factors which can perpetuate and resolve inflammation. Activation of these cells is initiated by many factors including bacterial lipopolysaccharides (LPS). To understand the proteomic changes associated with activation, we treated chicken macrophage HTC cells with and without Salmonella typhimurium LPS for 24 h. Equal amounts of soluble cell lysate proteins were subjected to high through put liquid chromatography tandem mass spectrometry (LC-MS/MS) approach after digesting cell lysates with trypsin (MudPIT method). The peptide mass fingerprints were searched in NCBI Gallus protein database to determine their identities. Our results showed 226 proteins in untreated control group and 311 in LPS treated group with 196 common to both. Thirty proteins were unique to control and 105 to LPS treated group. Based on the gene ontology classification associated with molecular functions, class and biological process, the LPS treatment increased the number of nucleic acid binding proteins, and proteins with catalytic functions. The newly expressed proteins found in LPS stimulated group included lyases, transcription factors, and proteins involved in transporter and transcription regulatory activities. LPS treatment decreased angiogenic and PDGF signaling pathway proteins. Overall, these results suggest that stimulation of transcription and carbohydrate metabolisms appear important in the activation of macrophages.

Key Words: LPS, macrophage activation, proteomics, gene ontology

P316  Effects of in ovo supplementation of probiotics on performance and immunocompetence of broiler chicks to an Eimeria challenge. C. M. Cox*,1, M. M. Ritzi1, T. D. Potter1, S. Kim1, M. Young2, and R. A. Dalloul1, 1Virginia Tech, Blacksburg, 2Star Labs/Forage Research Inc., Clarksdale, MO.

Coccidiosis is regarded as the parasitic disease with the greatest economic impact on the poultry industry due to reduced performance and increased mortality. This study investigated the effects of in ovo administration of probiotics (Primalac W/S) on hatchability, performance, and lesion scores in broiler chicks during a mixed Eimeria infection. At embryonic day (d) 18, 210 eggs were injected with either sterile water (Neg) or 1 × 10⁶ probiotic bacteria (Pro). On d 3 post-hatch, half of the chicks from each treatment group were challenged with a mixed inoculum of Eimeria acervulina (50,000 oocysts/bird), E. maxima (10,000 oocysts/bird) and E. tenella (2,500 oocysts/bird). Measurements were taken on day of hatch (DOH) and d 3, 9 and 15. On d 9, 24 birds per treatment were scored for intestinal Eimeria lesions. No differences were seen among groups for hatchability as well as for body weight (BW), or BW gain (BWG) before the Eimeria challenge. On d 9, the non-challenged birds with probiotic supplementation had higher BW and BWG than the non-supplemented controls while no differences were seen among the challenged groups. On d 13, probiotic supplemented birds had improved BW compared with the non-supplemented birds as well as increased BWG from d9 to d15. Birds receiving the probiotic had significantly lower mortality than non-treated birds. Additionally, gross lesion severity was reduced due to in ovo probiotic supplementation in all intestinal segments evaluated. These results suggest that in ovo supplementation of Primalac does not negatively affect hatchability and can improve performance and provide protection against a mixed Eimeria infection.

Key Words: probiotic, in ovo, performance, Eimeria, immunity