**Response of old laying hens to an Escherichia coli lipopolysaccharide challenge when fed diets with or without supplemental folic acid.** P. M. Munyaka,* G. Tactacan, M. Jing, K. O, J. D. House, and J. C. Rodriguez, University of Manitoba, Winnipeg, Manitoba, Canada.

The aim of the study was to investigate the effects of dietary folic acid (FA) supplementation in old laying hens challenged with *Escherichia coli* lipopolysaccharide (LPS). 48 Shaver White laying hens at 58 wk were fed 2 diets in a completely randomized design. The diets were wheat-soybean based, with either 0 or 4 mg supplemental FA per kg of diet. After 8 wk of feeding and at 66 wk, 6 hens from each dietary treatment were injected intravenously with either 8 mg/kg body weight of LPS or saline. Four h after injection, blood was collected and hens were euthanized to obtain spleen and cecal tonsils. T cell subsets (CD4+, and CD8+) in the blood and spleen, serum IgG, total protein, albumin, globulin, fibrinogen, and expression of immune-associated genes in the spleen and cecal tonsils were examined. T cell subsets in the blood and the spleen, serum IgG, total protein, albumin, globulin, and albumin:globulin ratio were not influenced by dietary FA supplementation. Compared with saline-injected hens, CD4+, CD8+, total proteins, albumin, globulin, and albumin:globulin ratio decreased (P < 0.05) in LPS-injected hens whereas CD4+:CD8+ ratio increased (P < 0.05). T cell subsets in the spleen, and fibrinogen were not influenced among treatment groups (P > 0.05). Gene expression in the spleen and cecal tonsils was not influenced by dietary FA supplementation except a reduction (P < 0.05) in the expression of IL-8 in the cecal tonsils. Expression of IL-8 in the spleen was affected by a diet x challenge interaction. Relative to saline-injected hens, expression of IL-1β, IFN-γ, and IL-10 increased in LPS-injected hens in the spleen and cecal tonsils, IL-8 increased in LPS-injected hens in the cecal tonsils only, while IL-4, IL-17, IL-18, and TLR-4 increased in the LPS-injected hens only in the spleen, however, IL-13 decreased in the cecal tonsils. In conclusion, the results show that FA may not enhance immune responses in old laying hens under acute LPS challenge.

**Key Words:** folic acid, lipopolysaccharide (LPS), supplementation, laying hen

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**Prebiotics and symbiotics supplementation to pullets differentially regulate toll-like receptors and cytokines in the intestine and systemically.** A. Yitbarek,* H. Echeverry, P. Munyaka, M. Alizadeh, W. K. Kim, and J. C. Rodriguez-Lecompte, Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada.

The present study was conducted to determine toll-like receptors (TLR) and cytokine profiles of chicken pullets fed yeast-derived carbohydrates (YDC) as a prebiotic or a blend of pre- and probiotics (*Lactobacillus acidophilus, L. casei*, *Streptococcus faecium, Bacillus subtilis* and *Saccharomyces cerevisiae*) as a symbiotic (SMB). Three hundred 1-d old Lohmann chicks were randomly assigned, in a randomized complete design, to one of 3 dietary treatments for 6 weeks. Treatments consisted of T1, (basal diet, neither YDC nor SMB); T2, (T1 + YDC); and T3, (T1 + SMB). Weekly feed intake (FI) and body weight (BW) were recorded. On d42, tissues from the ileum, cecal tonsil, and spleen were collected for gene expression profiles of TLR2, TLR4, TLR21, IL-4, IL-6, IL-10, IL-12p35, and IFN-γ. Performance and gene expression data (T1 as a control and β-actin as a housekeeping gene) was analyzed using the MIXED procedure of SAS and REST 2009, respectively. No significant difference in FI, BW and gain:feed was observed among treatments (P > 0.05). There was an upregulation of TLR2 and a downregulation of TLR4 and TLR21 in T2, and T3 in the ileum (P < 0.05). An upregulation of IFN-γ, IL-6 and IL-4 in the ileum in T3 was observed (P < 0.05). No difference in all TLRs was observed among treatments in the cecal tonsil (P > 0.05), while only IL-10 upregulated in both T2 and T3. Splenic TLRs profile showed a significant upregulation of TLR2 and TLR21 in T3 (P < 0.05). All cytokines, except IL-12p35, were upregulated in the spleen in T2 and T3 (P < 0.05). In conclusion, the effect of YDC and SMB was gut location dependent. While supplementation of YDC to pullets’ diet supports a more systemic response via T helper (Th)-1 cell associated pathways, supplementation of SMB supports a cytokine balanced pro-and anti-inflammatory effect via Th-1 and Th-2 cell associated pathways both locally and systemically.

**Key Words:** toll-like receptors, cytokines, prebiotics, symbiotics, pullets

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**Temporal and treatment changes in embryonic bursal gene expression after testosterone exposure in high and low antibody lines.** R. L. Taylor Jr.1,*1, T. A. Burks,1 P. B. Siegel2, and C. M. Ashwell3,1University of New Hampshire, Durham. 2Virginia Tech, Blacksburg. 3North Carolina State University, Raleigh.

The bursa of Fabricius (BF) provides the microenvironment for chicken B cell maturation. BF development is perturbed by exposure to testosterone propionate (TP) during the embryonic period. High (HAS) and low (LAS) antibody lines were produced through selection for antibody response 5 d post intravenous injection of 0.25% SRBC suspension (0.1 mL). The resulting lines have greater than a 5-fold difference in antibody titer. Parents from the 34th selected generation of both lines produced fertile eggs which were assigned to treatment or control groups. On d 3 of incubation, treatment eggs were dipped in a 2% TP ethanol solution whereas control eggs were dipped in ethanol alone. Four embryos of each line and treatment (HAS, HAS TP, LAS, LAS TP) were sampled for bursal tissue at 15, 18, and 21 d of incubation. Tissue was placed in RNALater at ~80 C. RNA was extracted from all samples. The RNA was reverse transcribed cDNA for individual samples. Indirectly labeled cDNA samples having either Cy3 or Cy5 fluorescent dyes, including a dye swap, were hybridized to a 320 gene microarray represented by 70-mer oligonucleotides. Each oligonucleotide is spotted 12 times within the array which increases sensitivity to detect sample differences. Transformed (log2) fluorescent intensity data was normalized through weighted regression and analyzed through mixed-model ANOVA. Comparisons of line, day, and treatment plus their interactions revealed significant differences among multiple genes. Immune related genes having differential expression included interleukin 13, interferon regulatory factor 5 and 6, neural cell adhesion molecule 1, and EPH receptor B2. Homeobox A3, fibroblast growth factor receptor 1, engrailed homeobox 1, and insulin-like growth factor binding protein 1 were some of the growth or developmental genes found to have modulated expression. Pathway analyses revealed biomarkers for immune responses and developmental signaling. Alteration of immune response and growth gene expression relates to physiological differences between the selected lines.

**Key Words:** bursa of Fabricius, microarray, immune response, hormone
A shared feature of herpesviruses is their ability to enter a latent state following an initially lytic infection. Marek's Disease virus serotype 1 (MDV-1), an avian herpesvirus, is considered oncogenic as it has the ability to transform latently infected lymphocytes. Numerous lymphoblastoid cell lines have been generated from tumors and lesions of MDV infected birds, which allows for an in vitro examination of the molecular mechanisms underlying MDV latency and transformation. Recent small RNA profiling studies have suggested that microRNA (miRNA), a type of small regulatory RNA, is involved in viral latency. To further our understanding of genes and pathways associated with MDV latency and reactivation the present study was undertaken to determine global transcriptome and miRNA changes upon viral reactivation induced by sodium butyrate in 3 MDV transformed cell lines, MSB1, RP2, and CU115. Integration of the transcriptome and miRNAome will provide a detailed view of the pathways and regulatory networks associated with MDV latency and reactivation. In the first 24 h after the addition of sodium butyrate, microarray analysis of transcriptional changes in cell lines MSB1, RP2, and CU115 identified 197, 139, and 118 differentially expressed genes, respectively. In addition, small RNA deep sequencing analysis has revealed more than 20 host miRNAs were differentially expressed within 24h of reactivation. Furthermore, the expression of miRNAs encoded by MDV was also altered upon reactivation, including mdv1-miR-M4 and mdv1-miR-M2–3p. Bioinformatic analyses found that many of the genes and miRNAs that are differentially expressed upon reactivation of MDV are involved in the regulation of the cell cycle, mitosis, DNA metabolism, and lymphocyte differentiation. Together these results suggest that herpesvirus reactivation is a complex process, involving multiple cellular pathways associated with cell proliferation and survival, as well as, miRNA-mediated gene regulation.

Key Words: MDV, latency, transformation, microRNA, microarray

Environmental pollution from mining activities can affect immune system of the host. The study investigated the effect of mine dust on immune response and pathomorphological alterations of immune organs in chicken. The experiment consisted of 5 treatment groups (A-E) with 225, one day old broiler chicks were distributed into 5 groups A, B, C, D, and E received a test immunomodulator (IMD; Immunotech) in water. All the chicks were administered ND, IBD and IB vaccinations and E received a test immunomodulator (IMD; Immunotech) in water. Further studies will focus on characterization of macrophage subpopulations in mammalian species is significant, their chicken counterparts are yet to be elucidated, requiring specific monoclonal antibodies (mAbs). In this communication, one of the subpopulations of chicken inflammatory macrophages was studied by using new mAbs raised against chicken peritoneal exudate macrophages (PEMs). Two mAbs that reacted strongly with PEMs were selected and designated as 3A8 and 4A5. To assess whether it is possible for new mAbs to recognize chicken inflammatory macrophages, 2 chicken macrophage-like cell lines, HD11 and MQ-NCSU, were treated with LPS (5µg/ml) to enhance levels of cell surface receptor expression. After 4 h of incubation, the expression of receptors recognized by our mAbs was measured by flow cytometry. Antigen expression levels were reported as median fluorescence intensity (MFI) ratios. The final MFI ratios were the value of each individual MFI divided by the MFI of unstained cells. Flow cytometric analysis showed that after stimulation, the subpopulations of MQ-NCSU cells recognized by 3A8 and 4A5 were slightly decreased (from 15.1 to 12.6% and 10.2 to 7.6%, respectively). However, the MFI ratios of 3A8 and 4A5 were increased from 4.248 to 7.203 and 1.732 to 2.738, respectively, suggesting the numbers of recognized antigen per MQ-NCSU cell by our 2 mAbs were upregulated during inflammatory response. In contrast to MQ-NCSU cells, no differences were observed in the MFI ratios in HD11 cells after LPS stimulation. We further challenged the HD11 cells with LPS up to 16 h, but no increase in either cell numbers or MFI ratios were observed. The results suggest that the antigens recognized by the 2 mAbs are specifically upregulated in MQ-NCSU cell line. Further studies will focus on characterization of the antigen specifically upregulated during the inflammatory response in MQ-NCSU cells.

Key Words: heterogeneity, MQ-NCSU, HD11, monoclonal antibodies, median fluorescence intensity

Macrophage heterogeneity, defined by cell surface receptors, is important in response to various kinds of stimuli. Although the recent progress in characterization of macrophage subpopulations in mammalian species is significant, their chicken counterparts are yet to be elucidated, requiring specific monoclonal antibodies (mAbs). In this communication, one of the subpopulations of chicken inflammatory macrophages was studied by using new mAbs raised against chicken peritoneal exudate macrophages (PEMs). Two mAbs that reacted strongly with PEMs were selected and designated as 3A8 and 4A5. To assess whether it is possible for new mAbs to recognize chicken inflammatory macrophages, 2 chicken macrophage-like cell lines, HD11 and MQ-NCSU, were treated with LPS (5µg/ml) to enhance levels of cell surface receptor expression. After 4 h of incubation, the expression of receptors recognized by our mAbs was measured by flow cytometry. Antigen expression levels were reported as median fluorescence intensity (MFI) ratios. The final MFI ratios were the value of each individual MFI divided by the MFI of unstained cells. Flow cytometric analysis showed that after stimulation, the subpopulations of MQ-NCSU cells recognized by 3A8 and 4A5 were slightly decreased (from 15.1 to 12.6% and 10.2 to 7.6%, respectively). However, the MFI ratios of 3A8 and 4A5 were increased from 4.248 to 7.203 and 1.732 to 2.738, respectively, suggesting the numbers of recognized antigen per MQ-NCSU cell by our 2 mAbs were upregulated during inflammatory response. In contrast to MQ-NCSU cells, no differences were observed in the MFI ratios in HD11 cells after LPS stimulation. We further challenged the HD11 cells with LPS up to 16 h, but no increase in either cell numbers or MFI ratios were observed. The results suggest that the antigens recognized by the 2 mAbs are specifically upregulated in MQ-NCSU cell line. Further studies will focus on characterization of the antigen specifically upregulated during the inflammatory response in MQ-NCSU cells.

Key Words: heterogeneity, MQ-NCSU, HD11, monoclonal antibodies, median fluorescence intensity

85 New monoclonal antibodies recognize macrophage cell surface receptors on activated MQ-NCSU chicken macrophage cell line, but not activated HD11 chicken macrophages. W. K. Chou*,1, C. H. Chen1, D. Abi-Ghanem1, O. B. Faulkner2, B. M. Hargis3, and L. R. Berghman1,1Texas A&M University, College Station, 3University of Arkansas, Fayetteville.

86 Effect of different levels of vitamin E and mannanoligosaccharide on productive performance and immune response of broiler chicks. H. S. El-Din*1 and A. El-Hamid2,2Poultry Diseases Dept., Fac. of Vet. Med., D Damanhour University, Damanhour, Behria, Egypt, 2Animal and Poultry Production Dept., Faculty of Agriculture, Damanhour University, Damanhour, Behria, Egypt.

A total 225, one day old broiler chicks were distributed into 5 groups in straight run design, each group contained 5 replicates of chicks per
each. The treatments were a control group without mannanoligosaccharide (MOS; 0.5 and 1.0 g/kg) and Vit E supplementation (40 and 80 mg/kg). The chicks were randomly housed in cage and provided water and feed ad-libitum. Chicks were vaccinated against Newcastle disease (ND) at 7th day and avian influenza (AI) at 10th day of age. Gumboro disease (IBD) at 13 d of age, and live vaccine of IBD and ND at 21 d old. During 38–48 d of age challenge trial was run using velogenic NDV. Supplementation of 1.0g MOS or 80 mg Vit E significantly decreased growth than the control group and the other groups, but differences in feed conversion ratio was not significant. The 1.0 g MOS or 80 mg Vit E significantly increased liver and gizzard percentage. Chickens given 0.5g MOS significantly increased thymus percentage compared with only groups on 1.0 g MOS or 40 mg of Vit E. Total serum proteins and globulin were significantly greater of broilers on 0.5 g MOS or 40 mg of Vit E. The broilers on 80 mg Vit E had significantly lower serum urea than those on 0.5 g of MOS, however, 0.5 g MOS increased plasma creatinine than only those on 1.0g of MOS. Broilers on the control diet and those on the 1.0 g MOS had significantly greater alanine amino transferase/ aspartate amino transferase ratio than the other groups except that on 80 mg Vit E. The antibody titters against ND increased numerically in the group on 80 and 40 mg Vit E compared with the control diet and the other groups. The antibody titer against the AI was increased significantly in the broilers on MOS at 0.5 g/kg diet compared with the other groups. Different MOS and Vit E doses significantly increased villi height and width in the intestine as compared with the control diet. In conclusion, 0.5 g MOS or 40 mg of Vit E may be considered growth promoting agents; MOS had positive effect on gut morphology, leading to better performance and immunity to AI while 40 mg of Vit E improved immune response to NDV.

**Key Words:** broilers, MOS, vitamin E, immunity, gut health

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**87 Effect of diet fat source on broiler immune system.** A. R. Seidavi,* M. R. Poorghasemi, and A. A. A. Qotbi, Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Guilan, Iran.

An experiment was conduct to investigation on effect of diet fat source on broiler immune system based on completely randomized design using 5 treatments and each treatment was included 4 replicate by means of 2 hindered broiler chickens Ross 308. Experimental treatments included (1) standard diet containing 4% tallow as animal fat; (2) standard diet containing 4% canola oil as plant fat, (3) standard diet containing 4% sunflower oil as plant fat, (4) standard diet containing 2% tallow as animal fat + 2% + canola oil as plant fat, and (5) standard diet containing 2% tallow as animal fat + 2% + sunflower oil as plant fat. All immune traits were recorded. The obtained results showed that inclusion and changing of fat source of diet from vegetable to animal fats, and vice versa had no significant effect on response of immune system against SRBC injection (first or second injection), Newcastle vaccination, Gambro vaccination, infectious bronchitis vaccination; or spleen weight, spleen relative weight, bursa fabricius weight, bursa fabricius relative weight, or thymus weight ($P > 0.05$). However, fat source of diet had significant effect on thymus relative weight ($P < 0.05$).

**Key Words:** immune system, chick, fat, oil, thymus