110 Effect of fructooligosaccharide supplementation on leukocyte composition, immunoglobulin G level and ileal cytokine gene expression under Salmonella Enteritidis lipopolysaccharide challenge in broiler chickens. Y. Shang1, M. Alizadeh1, J. H. Kim2, and W. K. Kim1, 1Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada, 2Poultry Science Division, National Institute of Animal Science, Rural Development Administration, Chungnam, Republic of Korea, 1Department of Poultry Science, University of Georgia, Athens, GA.

The effect of fructooligosaccharide (FOS) supplementation on leukocyte count, immunoglobulin (Ig) G level and ileal cytokine gene expression in respond to Salmonella Enteritidis lipopolysaccharides (LPS) challenge was investigated in broiler chickens (n = 180). The study was based on a 3 × 2 factorial arrangement which included (1) 3 dietary treatments from d 1 to 21: positive control (PC), a wheat-corn-soybean meal based diet contained antibiotics (virginiamycin and monensin); negative control (NC), as PC without antibiotics; and NC+FOS, as NC supplemented with 0.5% of FOS; and (2) 2 immunological challenges on d 21: intraperitoneal injection with 2 mg/kg of sterile saline or Salmonella Enteritidis LPS. Four hours after the immunological challenge, blood samples were collected (6 replicates/treatment) for leukocyte differentiation and IgG determination. Segments of ileum were excised to evaluate cytokine gene expression using quantitative real-time polymerase chain reaction (qRT-PCR). The LPS challenge demonstrated significant difference on relative heterophils (H) and lymphocytes (L) concentration, H:L ratio, interleukin (IL)-2, IL-18 and interferon (IFN)-γ expression (P < 0.001), as well as serum IgG levels, IL-1β, IL-6, IL-10, and toll-like receptor (TLR)-4 expression (P < 0.05). The NC+FOS group had reduced heterophil but increased monocye count when compared with NC (P < 0.05). Diet × challenge interaction was observed in IgG measurements (P < 0.001). Natural and specific IgG levels were elevated in NC+FOS group under the LPS challenge (P < 0.01). Supplemental FOS has also upregulated ileal IL-1β, IL-2, IL-10, IL-18, TLR-4 and IFN-γ expression in saline groups when compared with PC and NC (P < 0.05). In conclusion, Salmonella Enteritidis LPS challenge established significant difference toward the immune responses of broiler chickens. FOS supplementation upregulated ileal cytokine gene expression, altered leukocytes composition and serum IgG levels in respond to LPS, and it may play a positive role in improving the immunity of broiler chickens. 

Key Words: fructooligosaccharide, Salmonella Enteritidis LPS, broiler chicken

111 Chicken anti-CD40 Mab upregulates CCR10 gene expression in chicken B-cells: A potential gateway bridging the systemic and mucosal immune systems. W. K. Chou∗, C. Vuong, and L. R. Berghman, Texas A&M University, College Station, TX.

Secretory IgA (sIgA) is the first line of defense against pathogenic microorganisms entering a host’s mucosal surface, the major portal of pathogen entry. Current vaccines for chicken mucosal defense are either not effective or are considered time and labor consuming. Recently, we reported a new platform, in which an anti-CD40 monoclonal antibody (Mab) complexed with biotinylated synthetic peptides was able to induce a rapid antigen-specific mucosal sIgA immune response as well as a circulatory IgG titer by single administration via various routes, including mucosal and subcutaneous administration. Systemic immunization was not expected to have an effect on specific mucosal immunity due to distinct homing receptors on peripheral resting B-cells and mucosal B-cells. However, several recent studies in mammals have demonstrated that polyclonal anti-CD40 induced the mucosal homing receptor C-C chemokine receptor type 10 (CCR10) by upregulating both mRNA and protein levels in peripheral resting B-cells. This suggests that B-cells can be activated in the circulation and then migrate to mucosal effector sites in mammals. In this study, the effect of Mab CD40-antigen complex and LPS stimulated HD11 produced proinflammatory cytokines on gene expression of CCR10 in the chicken B-cell line DT40 was examined by real-time PCR. DT40 cells were treated for 8 h with (1) cytokines, (2) 5 µg/mL Mab CD40-antigen complex, or (3) cytokines plus 5 µg/mL Mab CD40-antigen complex. The results show that Mab CD40-antigen complex alone was able to induce significant (P < 0.001) CCR10 gene expression in DT40 cells compared with cytokines-only treated cells. Moreover, the combination of Mab CD40-antigen complex and cytokines boosted the expression of CCR10 on DT40 cells in a synergistic manner, up to 5.5-fold (P < 0.05) compared with nontreated cells. These results suggest that Mab CD40-antigen complex initiates the expression of CCR10 in chicken B-cells in vitro. If the same phenomenon also appears in vivo, then our data suggest a gateway between systemic immunization and mucosal immunity in birds, similar to what has recently been shown in mammals.

Key Words: CD40, CCR10, B-cell, mucosal, systemic immunization

112 Maternal immunization with NetB-based vaccines protects broiler chickens from necrotic enteritis. A. L. Keyburn1,4, R. D. Portela1, M. E. Ford1, T. Bannam2,3, X. Yan2,3, J. I. Rood2,3, and R. J. Moore∗1,2, 1Australian Animal Health Laboratory, CSIRO Animal, Food & Health Sciences, Geelong, Victoria, Australia, 2Department of Microbiology, Monash University, Clayton, Victoria, Australia, 3ARC Centre of Excellence in Structural and Functional Microbial Genetics, Monash University, Clayton, Victoria, Australia, 4Poultry Cooperative Research Centre, Armidale, New South Wales, Australia, 5Health Sciences Institute, Federal University of Bahia, Salvador, Bahia, Brazil.

Clostridium perfringens-associated necrotic enteritis in broilers remains a major global economic and welfare issue in the poultry industry. The disease is primarily controlled through the use of antibacterial feed additives. However, the industry is moving away from these practices. This situation has increased the need to develop other methods to control necrotic enteritis and vaccination is an alternative approach that could be deployed to manage this disease in the future. Previously we have shown that birds vaccinated with inactivated C. perfringens Type A culture supernatant (toxoid) combined with recombinant NetB (rNetB) were significantly protected from homologous and heterologous challenge. In the present study, the effect of maternal immunization against the disease was examined. Broiler breeder hens were injected subcutaneously with rNetB alone, C. perfringens Type A toxoid alone, and toxoid combined with additional rNetB. Vaccination resulted in a strong serum immunoglobulin Y response to NetB in parent hens immunized with rNetB formulations, and specific antibodies were transferred to their progeny. Subclinical necrotic enteritis in broilers was induced and the occurrence of specific enteric lesions was evaluated. Birds from hens immunized with rNetB combined with toxoid and challenged at either 14 or 21 d, had significantly lower levels of disease compared with birds from adjutant only vaccinated chickens. In addition, birds from hens immunized with rNetB alone were significantly protected when
challenged at 14 d only. From these results, maternal immunization with a NetB enhanced toxoid vaccine appears to be a promising method for the control of necrotic enteritis in young broiler chickens.

**Key Words:** necrotic enteritis, *Clostridium perfringens*, vaccine, NetB, toxoid

### 113 Immune potentiating role of protein, probiotic, and synbiotic supplementation in molted White Leghorn hens.

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The current study was planned to investigate effect of high protein, probiotic, and synbiotic supplementation on immunity of culled molted White Leghorn hens. About 70 week old cage-housed hens were zinc molted. The molted hens were randomly divided in 4 treatment groups: control group (CONT; CP16% in basal diet); high protein group (HP; 18% CP in basal diet); probiotic-supplemented group [PRO; probiotic (Protexin) at 85 mg/L in drinking water]; and synbiotic group [SYN; synbiotic (Pefectin) at 85 mg/L in drinking water and fed 16% CP in basal diet]. Ten birds from each group were killed at 5% peak (SP), full peak (FP), and end of production (EP), and blood samples were taken. Serum was collected from blood samples for estimating geometric mean antibody titer (GMT) against Newcastle disease virus (NDV) and egg drop syndrome (EDS) virus. Abdominal exudates (AEC) were used to isolate macrophages, which were cultivated in vitro to observe their engulfment response and their nitric oxide production assay against sheep RBC as antigen. 2,4-Dinitrochlorobenzene (DNCB) was inoculated on skin of 5 birds of each group at 3 specified production stages to evaluate the delayed type hypersensitivity response by determining skin thickness. The overall humoral antibody GMT against NDV was higher (*P* < 0.05) in the HP till FP as compared with the other groups. The GMT value against EDS was higher (*P* > 0.05) in the HP group compared with the CONT group. A significant increase (*P* ≤ 0.01) in macrophage engulfment response and macrophage nitric oxide production response was seen in the HP and PRO groups compared with the CONT. The DNCB inoculated skin thickness response remained higher (*P* > 0.01) in the HP till FP as compared with the other groups. In conclusion, feeding a diet rich in protein or supplementation with a probiotic or a synbiotic enhanced humoral as well as cell mediated immunity of molted hens.

### 114 v-src tumor growth differs among recombinant congenic strains identical at the major histocompatibility complex.


Rous sarcoma virus (RSV) or v-src DNA from the RSV oncogene induces chicken tumors whose fate is determined by major histocompatibility complex (*Mhc*) genes. In addition, genes outside the *Mhc* influence tumor growth. USDA inbred lines 61 and 72, which possess the B2B2 *Mhc* genotype, differ in RSV or v-src tumor growth, as well as multiple immune characteristics. These 2 lines constitute the prime example of non-*Mhc* gene effects on tumors. Recombinant congenic strains (RCS) were developed to identify genes other than the *Mhc* that affect immune responses. Line 72 males were crossed to Line 61 females followed by 2 backcrosses to Line 61. Subsequent brother-sister mating generated strains designated A through X, each having a different 87.5% Line 61 and 12.5% Line 72. Two replicates including 393 chicks from Line 61, Line 72 and 16 RCS were injected with 40 μg of v-src DNA at 6 wk of age. Tumor size was measured for 7 consecutive weeks. Birds that were terminated or died during the study were assigned subsequent tumor sizes of 100. A tumor profile index (TPI), ranging from 1 (complete regression by 28 d post-injection) to 5 (a terminal tumor) was assigned to each bird based on individual tumor scores. Tumor size and TPI data were evaluated by ANOVA followed by separation of significant (*P* < 0.05) means using Bonferroni’s method. Line 72 had the greatest tumor growth and highest TPI (4.89 ± 0.1), which was significantly (*P* < 0.05) greater than all other strains. The next grouping of significant differences (*P* < 0.05) for tumor size and TPI included strains C (3.67 ± 0.1), L (3.63 ± 0.2), A (3.46 ± 0.2), and Line 61 (3.06 ± 0.1). Finally, strains R (2.91 ± 0.1), W (2.81 ± 0.2), and J (2.72 ± 0.3) had the smallest tumor growth. USDA inbred lines 61 and 72 developed to identify genes other than the *Mhc* gene effects on tumors. Recombinant congenic strains (RCS) were developed to identify genes other than the *Mhc* that affect immune responses. Line 72 males were crossed to Line 61 females followed by 2 backcrosses to Line 61. Subsequent brother-sister mating generated strains designated A through X, each having a different 87.5% Line 61 and 12.5% Line 72. Two replicates including 393 chicks from Line 61, Line 72 and 16 RCS were injected with 40 μg of v-src DNA at 6 wk of age. Tumor size was measured for 7 consecutive weeks. Birds that were terminated or died during the study were assigned subsequent tumor sizes of 100. A tumor profile index (TPI), ranging from 1 (complete regression by 28 d post-injection) to 5 (a terminal tumor) was assigned to each bird based on individual tumor scores. Tumor size and TPI data were evaluated by ANOVA followed by separation of significant (*P* < 0.05) means using Bonferroni’s method. Line 72 had the greatest tumor growth and highest TPI (4.89 ± 0.1), which was significantly (*P* < 0.05) greater than all other strains. The next grouping of significant differences (*P* < 0.05) for tumor size and TPI included strains C (3.67 ± 0.1), L (3.63 ± 0.2), A (3.46 ± 0.2), and Line 61 (3.06 ± 0.1). Finally, strains R (2.91 ± 0.1), W (2.81 ± 0.2), and J (2.72 ± 0.3) had the smallest tumor growth and the lowest TPI, were significantly (*P* < 0.05) less than strains C, L, A, and Line 61. Genetic comparisons of Line 72, Line 61, and RCS having significant TPI differences will help to identify non-*Mhc* genes that affect v-src DNA tumor growth.

**Key Words:** oncogene, Rous sarcoma virus, tumor, regression