

during subsequent incubation in two experiments. Treatment groups had four replicate incubation trays of 150 eggs each. As expected, hatchability of fertile eggs declined with length of egg storage in both experiments ($P < 0.01$). This was due to increases in both percentage early and late dead embryos in both experiments. Hatchability of fertile eggs was increased by SEU storage and turning during storage in Experiment 1, and by turning 96 times daily during incubation in Experiment 2. There was a consistent turning during storage \times storage position interaction for hatchability of fertile eggs that demonstrated that turning during storage benefited only eggs stored LEU. Other interactions suggested that turning during storage and storage in the SEU position worked best with long-stored eggs. Furthermore, the data suggested that storage in the SEU position or turning during storage were equally effective in ameliorating the adverse effects of storage for 14 days.

Key Words: Turning, Egg storage, Egg position

34 Egg weight, fertility, and hatchability of broiler breeders as influenced by time of oviposition and breeder flock age. A. H. Zakaria², P. W. Plumstead^{*1}, H. Romero-Sanchez¹, N. Lekrisompong¹, J. Osborne³, and J. Brake¹, ¹North Carolina State University, Raleigh, ²Damascus University, Damascus, Syria, ³North Carolina State University, Raleigh.

Time of oviposition has been reported to affect the fertility of eggs produced by caged and artificially-inseminated broiler breeders but this was not found with naturally-mated broiler breeders housed in conventional, partial slat houses. To clarify the situation, two experiments were conducted to investigate egg weight, fertility, and fertile hatchability in relation to time of oviposition of young (34 wk) and old (59 wk) broiler breeder flocks. In Experiment 1, eggs were collected from young and old flocks for two days at hourly intervals from 0700 hr to 1900 hr. Most eggs for the young flock were laid in a three-hour window from 0800 to 1000 hr while the old flock had a broader, less defined peak of egg production from 0900 to 1400 hr. Egg weight of early laid eggs was significantly greater than that of late laid eggs at both flock ages. In Experiment 2, eggs were further categorized as early laid first-in-sequence (C1) eggs (0700-0800 hr), mid-sequence (Cs) eggs (0900-1200 hr), and late laid terminal-in-sequence (Ct) eggs (1300-1700). These eggs were incubated to determine fertility and fertile hatchability relative to oviposition time and flock age. Fertility declined with flock age but was not affected by time of oviposition nor were there differences in fertile hatchability relative to time of oviposition (sequence position) or flock age. These results showed that while the distribution of ovi-

position time changed with flock age, the important production variables of fertility and fertile hatchability were not affected by the egg sequence position in either young or old naturally-mated broiler breeders.

Key Words: Broiler breeders, Egg sequence, Oviposition time

35 Effect of full feeding broiler breeder pullets until 1 or 3 wk of age on frame size, fatness and fleshing at 4, 8, 12, and 16 wk of age. R. Renema^{*1}, A. Pishnamazi¹, F. Robinson¹, and M. Zuidhof², ¹University of Alberta, Edmonton, AB, Canada, ²Alberta Agriculture, Food and Rural Development, Edmonton, AB, Canada.

This study examined the effects of smoothing the transition to feed restriction by feed restricting from 1 wk of age. This may improve pullet welfare by reducing the degree of change in feed allocation. A total of 720 Ross 308 pullets were placed at day of hatch (8 pens). Chicks were provided ad libitum access to feed for 1 (1WK) or 3 wk (3WK) of age, after which a 5:2 feed restriction program was initiated. BW was recorded twice/wk to allow the growth profiles to be gradually converged (target of 8-10 wk of age). Individual BW was recorded at 2 wk intervals for calculation of BW uniformity. At 4, 8, 12, and 16 wk, external carcass and fleshing scores were recorded for all birds, and 14 birds/pens were dissected for assessment of muscle mass, fatness, and reproductive development.

By 3 wk of age, the daily gain of the 3WK pullets was double that of the 1WK pullets. Whereas the sudden drop in allocation to 3WK birds improved feed efficiency, these birds had more fleshing at 4 wk of age. The 3WK birds weighed 30% more, had a larger shank and keel length, and carried a higher proportion of breast muscle (12.6% compared to 11.5%) than the 1WK birds. By 8 wk of age, the 3WK birds still weighed 100 g more than 1WK birds ($P = 0.006$) and carried a higher proportion of breast muscle. The BW profiles met at 10 wk of age, resulting in carcass measures being similar at 12 wk of age, although the 3WK birds still had a wider chest ($P = 0.048$). The BW uniformity of the 1WK birds was better than that of the 3WK birds from 14 wk of age (CV of 13.0% compared to 16.7%). At 16 wk of age, the 3WK birds were slightly fatter than the 1WK birds ($P = 0.046$), while the frame size of 1WK birds was increasing more quickly than in 3 wk birds ($P < 0.0001$). Changing the starting age of feed restriction altered growth and conformation traits to 8 wk of age, after which the conformation of birds became similar.

Key Words: Broiler breeder, Feed restriction, Fleshing

Immunology: Immunology A

36 Expression of reovirus sigma C protein in Arabidopsis thaliana. H. Wu^{*1}, Y. Williams¹, K. Gunn¹, R. Locy², N. Singh², and J. Giambrone², ¹Alabama State University, Montgomery, ²Auburn University, Auburn, Alabama.

The sigma C protein is the major host-protective immunogen of avian reovirus (ARV). Transgenic lines of Arabidopsis thaliana, expressing recombinant sigma C, were developed. The S1 gene, which encodes the sigma C protein, from the S1133 reovirus strain was isolated, amplified by RT-PCR, and introduced into a plant expression vector. This vector, (pE1857), has a strong promoter for plant expression. A construct, with the Bar gene cassette for bialaphos selection in plants (rpE-S1), was introduced into Agrobacterium tumefaciens by electroporation. Agrobacterium, containing the rpE-S1 construct, was used to transform A. thaliana, and transgenic plants were selected using bialaphos. The sigma C transgene was confirmed by PCR analysis and its expression confirmed by a real-time quantitative RT-PCR. Western blot analysis, using chicken anti-ARV sera, confirmed the expression of Sigma C protein in the plants. Six transgenic lines, with high expression of sigma C protein, were selected with real-time quantitative PCR. The immunogenic and immunoprotective potential of the plant-derived recombinant sigma C protein will be tested in future oral feeding and live ARV challenge experiments in chickens

37 Characterizing Rous sarcoma growth for major histocompatibility (B) complex haplotype B61. R.L. Taylor, Jr. ^{*1}, W. E. Briles², and J. E. Fulton³, ¹University of New Hampshire, Durham, ²Northern Illinois University, DeKalb, ³Hy-Line International, Dallas Center, IA.

The major histocompatibility (B) complex has a crucial role in Rous sarcoma virus (RSV) tumor outcome. The immune response to RSV tumors was tested in chickens segregating for haplotype B61 which occurs in a commercial line. A single B8B61 sire mated to B2B5 dams (50% Line 6.15-5) produced B2B61, B5B61, B2B8, and B5B8 progeny. Genotypes bearing B61 were studied further. The first mating consisted of one B2B61 male bred to five B2B61 dams. A second mating used the same procedure for B5B61 parents. Six-week-old chicks from each mating were challenged with 20 pock forming units (pfu) of subgroup A RSV. Tumors were scored for size six times over a ten week period post-inoculation. Tumor size scores were used to assign each bird a tumor profile index (TPI). The TPI criteria were 1 = complete regression by 28 days, or earlier; 2 = complete regression by 42 or 56 days; 3 = complete regression by 70 days, or a decreasing slope, or complete regression by 56 days followed by recurrence; 4 = general upward trend, or plateau or slight regression after 56 days; and 5 = terminal tumor prior to 70 days. Mean tumor sizes and rank transformed TPI values were evaluated using a repeated measures design and ANOVA, respectively. Hatch, sex, dam, and B genotype were the main effects.

Significant means were separated with Fisher's Protected LSD at $P < 0.05$. *B2B61* and *B2B2* genotypes had significantly lower TPI than did *B61B61* chickens. Among *B5* and *B61* segregants, heterozygous *B5B61* chickens had TPI significantly lower than either *B61B61* or *B5B5* chickens. Haplotype *B61* is a relative progressor to RSV subgroup A tumors. However, *B61* combined with the progressive *B5* haplotype produces complementation between alleles (heterosis), resulting in lower tumor scores.

Key Words: *B* complex, Oncogene, Tumor

38 Controlled replication of chicken anemia virus: Implications for commercial breeders. M. Miller and K. Schat*, *Cornell University, Ithaca, NY*.

Chicken anemia virus (CAV) can cause subclinical infection with severe immunosuppression and an absence of pathogen-specific cytotoxic T lymphocytes when infection occurs after 3 weeks of age. We had previously shown that CAV may remain present in gonadal tissues of SPF birds independently of their antibody status suggesting that CAV can become latent. Using two different approaches, we investigated the potential mechanisms that may regulate transcriptional control of CAV. We demonstrated that transcription from the CAV promoter can be upregulated by some hormone receptors, including the estrogen receptor in the presence of estrogen. In addition, repressive factors can downregulate transcriptional expression from the CAV promoter. One of these factors is the orphan nuclear receptor COUP-TF. In addition, we identified a regulatory element at the transcription start point of CAV, which may be important for the control of expression. This E box-like element binds δ EF1, a protein, which has been linked to transcriptional control during embryonal development and in some organs including lymphocytes. Based on these results, it is likely that viral reactivation, and thus replication, depends on the balance between activators, e. g., estrogen-activated hormone receptors, and repressors, e. g., COUP-TF and δ EF1. These findings are highly relevant for the understanding of the pathogenesis of CAV infection in SPF and commercial flocks. CAV infection remains highly problematic for the SPF industry and seroconversion has been reported in these flocks. We suggest that these outbreaks are the consequence of reactivation of latent virus. Similarly, virus may be transmitted from commercial, serologically positive breeders to their offspring when the balance between repressors and activators is altered. This may lead to the presence of CAV in the offspring. In addition, it offers an explanation for the high doses of virus needed to establish infection after challenge with CAV in commercial flocks. Under the conditions of intensive virus challenge the repressors, especially δ EF1, may not be able to control transcription.

Key Words: Chicken anemia virus, Transcription, Hormone receptors

39 Gut humoral immune response and resistance to salmonella challenge of progeny from breeders vaccinated with killed antigen. A. Rolon¹, J. S. Bailey*², P. S. Holt³, C. Hofacre¹, and J. L. Wilson¹, *¹The University of Georgia, Athens, ²USDA Poultry Microbiological Safety Research Unit, Athens, GA, ³USDA Russell Research Center, Athens, GA.*

Salmonella vaccination programs using killed bacterins in breeders and live auxotrophic-strain vaccines early in the life of their progeny have gained popularity in today's poultry industry. In this study we evaluated the gut humoral immune response to a live auxotroph vaccine used on hatchlings with and without maternal antibody, and related this response to challenge with a blend of two antibiotic-resistant *Salmonella* marker strains. Forty week-old ISA Brown breeders from a *Salmonella*-free flock were vaccinated twice at a three week interval with commercially-prepared autogenous trivalent bacterin, serogroups B, C and D1, or a serovar Enteritidis bacterin. Half of the progeny were given a live auxotroph mutant vaccine (LiveST), by coarse spray on arrival to the brooding premises. On days 3, 13 and 34 of age, gut lavage samples were taken, and ELISAs for IgA and IgG measured. On the same days, another group of birds was challenged with a blend of antibiotic-resistant serovars Enteritidis and Typhimurium strains. Cecal and composite heart-liver-spleen samples obtained 7 days post-challenge were cultured and colonies enumerated. Maternal IgG observed up to 13 days of age had no effect on subsequent LiveST-stimulated Ig

production. No protective effect of maternal Ig was demonstrable, except when combined with LiveST given to the progeny. Killed vaccines to the breeders combined with a live vaccine to the progeny resulted in reduced invasiveness after challenge, as shown by a reduction in Liver-Heart-Spleen *Salmonella* counts. One dose of LiveST enhanced gut IgG up to 34 days when measured on STLPS, but only to 13 days when measured on SELPS, with titers decreasing with time. Increased IgA was observed only at 13 days. The LiveST vaccine decreased cecal and liver-heart-spleen composite counts at 3 and 13 but not 34 day challenges, indicating that a second dose might be necessary for prolonged protection. Results lead us to hypothesize that protection might be a combined effect of stimulus of cell-mediated gut immunity, and a competitive exclusion effect of the LiveST.

Key Words: *Salmonella*, Vaccines, Immunity

40 Expression of innate immune functions in developing broiler gut associated lymphoid tissue in the immediate pre and posthatch period. E. Bar-Shira* and A. Friedman, *The Hebrew University of Jerusalem, Rehovot, Israel*.

In chicks, gut associated lymphoid tissue (GALT) serves as a major site for generation and induction of immune responses. Previous studies from our laboratory showed that the development of GALT is concomitant with the structural and functional development of intestinal tissue, and that its full immune maturation is obtained by the end of the second week posthatch, as demonstrated by cytokine gene expression and antibody production. As adaptive immunity appears to mature towards the second week of life, the question of immune protection during the first week of life was raised. We hypothesized that in addition to protection provided by maternal antibodies, innate immune functions of the enteric immune system provide immediate solution to enteric challenges. To test the protective potential of the innate enteric immune system immediately posthatch, we studied expression of functional genes representing different activities of innate immune cells. Expression of IL1 β , IL8 and K203 was basal at hatch and increased rapidly thereafter so that at day 2 posthatch levels were similar to those observed at day 7. The rapid increase in expression of these genes is concomitant with the exposure to environmental antigens and demonstrates the capability of the enteric immune system to respond rapidly to inflammatory stimuli of external origin. However the expression of Gallinacin 1 and 2 (β -defensins secreted by heterophils) decreased after hatch and began to increase again only towards the end of the second week of life. To verify high β -defensin expression at hatch, we studied their expression in prehatched chicks. We found that expression of both β -defensins increased towards hatch thus suggesting a unique mechanism by which the intestine might be preconditioned for the posthatch encounter with external antigens. In conclusion two types of protective mechanisms were found to be conferred by innate immune cells during the first week of life: protection of the intestine prior to hatch reinforced by an immediate response to inflammatory signals encountered posthatch.

Key Words: Innate, Immune response, Broiler chicks

41 Temporal expression of immunoglobulin transporter genes in broiler gut epithelial barriers during the immediate pre- and post-hatch period. I. Bromberger* and A. Friedman, *The Hebrew University of Jerusalem, Rehovot, Israel*.

Two types of receptors mediating Ig transfer across epithelial barriers have been previously described in mammals. The polymeric immunoglobulin receptor (pIgR) participates in transporting polymeric IgA (pIgA) and IgM, while the MHC class I-related Fc receptor (FcRn) participates in IgG transport. Receptor mediated transport of antibodies through epithelial tissue has yet to be described in poultry. Recent studies have demonstrated receptors for Ig transport in chicks: The *Gallus gallus* polymeric Ig receptor (GG-pIgR) that binds pIgA, and the chicken Yolk Sac IgY Receptor (FcRY) that binds IgY. As contents of the yolk sac are absorbed from the gut as well as from the yolk sac membrane, we hypothesized that the FcRY gene is expressed in epithelial cells of the intestinal lining. In contrast to IgY, most IgA found in hatchlings is not of maternal origin. Thus while the FcRY gene is expected to be fully expressed at hatch, pIgR gene expression is expected to be minimal at hatch and to increase with age. To investigate these hypotheses we determined FcRY and pIgR gene expression in

intestine, liver and cloacal bursa tissue slices sampled from pre and post hatched chicks. As expected, we found constant levels of FcRY gene expression in the intestine and liver during the immediate pre and post hatch period. Interestingly, we also detected significant FcRY gene expression in bursal tissue. The expression of pIgR gene was low in the tested tissues during late embryonic development, but increased significantly during the first few days post hatch. The increase was found to correlate with increasing IgA levels in bile, intestinal washings and bursal secretions. These findings are the first to demonstrate expression of Ig transporters in the chicken gut. Furthermore, they indicate an excretory function for the cloacal bursa that has been previously described to function as an active site of antibody production. The differential temporal expression of both transporters indicate that epithelial barriers in the gut are coordinated with the development of the immune system in terms of antibody availability.

Key Words: GALT, Ig receptors, Transport

42 Optimization of an invasion assay for *Eimeria tenella* sporozoites. D. Abi-Ghanem*, K. Ameiss, D. J. Caldwell, and L. R. Berghman, *Texas A&M University, College Station*.

Coccidiosis, an economically important and problematic poultry disease, is caused by *Eimeria* parasites. These invade and multiply in the avian intestinal tract, causing tissue damage that results in blood loss, dehydration, malabsorption, and increased susceptibility to other diseases. Many of the events involved in the invasion of intestinal cells by coccidial parasites have not been completely elucidated. Characterization of the invasion processes and visualization of the dynamic host-parasite interactions have proved to be daunting tasks. Cells used for invasion studies have so far included primary avian kidney cells and the Marbin-Darby bovine kidney cell line. We are in the process of optimizing a new, more realistic *in vitro* invasion assay that uses primary chicken enterocytes, the natural target cells of *Eimeria*. Enterocytes were isolated from a mid-intestine section of a 4-week old broiler chicken. Chicken fibronectin was used to anchor the cells to the bottom of a cell culture flask. *Eimeria tenella* oocysts (8×10^6) were broken with glass beads, and sporozoites were excysted by incubation of the sporocysts in excysting solution at 41 C for 1 hour. Sporozoites were purified on a Percoll gradient and resuspended in DMEM containing chicken serum. A total of 1×10^7 sporozoites/ml were labeled with PKH67, a green fluorescent cell linker dye incorporated into lipid regions of the cell membrane. Stained sporozoites were shown to remain viable for several hours and fluorescence is claimed to be stable for 2 weeks at 4 C. Further optimization of various assay parameters is underway. Labeled sporozoites will be added to cultured chicken enterocytes for 1, 3, or 5 hours. At each time point, extracellular sporozoites will be washed off, cells will be observed and fluorescence will be used to monitor invasiveness. In addition to reflecting a more real-life situation, the invasion assay we propose will do away with the need for immunohistochemical detection, and hence is expected to be much faster than the currently used assays.

Key Words: Coccidiosis, Chicken enterocytes, Invasion assay

43 Prolactin receptor gene expression in chicken immune tissues during embryogenesis and post-hatch period. Z. Kang* and G. Bedecarrats, *University of Guelph, Guelph, ON, Canada*.

Prolactin (PRL) is a neuroendocrine pituitary hormone with multiple homeostatic roles among vertebrates. In mammals, PRL is important to maintain immuno-competence and regulate lymphocyte function. In avian species, PRL receptor (PRLR) is present in many different tissues, and PRL has mainly been studied for its involvement during the expression of incubation behavior. Although PRL can also modulate the avian immune system, its mechanisms of action still remain to be clarified. The objective of our study was to determine if PRLR gene is expressed in chicken immune tissues during embryonic development and in post-hatch chicks. Furthermore, PRLR mRNA tissue distribution was analyzed by in situ hybridization. Spleen and bursa of Fabricius were collected from chicken embryos at day 8, 10, 12, 14, 16, 18, 19, 20 and 21, and from chicks at 1, 7 and 14 days of age. Thymus could not be isolated in embryos before day 12 but were collected for all subsequent development stages. Messenger RNA was extracted, and PRLR gene expression was detected by RT-

PCR. Tissue samples were also collected to generate frozen sections used for in situ hybridization studies. It was found that PRLR is expressed in all immune tissues examined from embryonic day 8 to post-hatch day 14. Morphometric analyses of lymphoid tissues by in situ hybridization confirmed the presence of PRLR mRNA in splenic pulp, around forming follicles in the bursa, and around lobules in the thymus. The observed pattern was consistent with a specific PRLR gene expression in lymphocytes and lymphocyte precursors. In conclusion, our results indicate that in the chicken, PRLR is expressed early during the development of lymphoid cells and tissues, and PRL may act on the maturation and differentiation of lymphocytes.

Key Words: Prolactin, Immune tissues, Chicken

44 Somatostatin receptor subtype 2 is expressed in the chicken thymus and in a chicken T-cell line. X. Zhang* and L. Berghman, *Texas A&M University, College Station*.

Somatostatin is a neuro-peptide produced in various organs, including the hypothalamus and the pancreas, with multiple and complex biological roles including immune-regulatory functions, both in the primary and peripheral lymphoid organs. We have previously reported that somatostatin is expressed locally in some neuro-endocrine cells in chicken thymus. To examine whether the somatostatin receptor is also expressed in thymus, total RNA from chicken thymus was extracted and RT-PCR was performed, based on the sequence of chicken somatostatin receptor subtype 2 (gi:50757970). The results clearly showed that somatostatin receptor 2 is indeed expressed in chicken thymus, suggesting that somatostatin may exert its immune regulatory function in a paracrine way. To further study whether chicken T lymphocytes, which differentiate and mature in the thymus, produce any somatostatin receptors, a virally transformed chicken T-cell line (ConA-C1-VICK) was investigated. RT-PCR results indicated that mRNA transcripts of somatostatin receptor subtype 2 do exist in mature chicken T-cells. This study, combined with our previous data, supports the notion that locally expressed somatostatin has physiological effects on thymocyte proliferation and maturation, through a paracrine mechanism. A molecular level screening of putative biological effects of somatostatin on chicken T-cells in vitro is now underway. Genes of interest include those involved in cytokine induction, apoptosis, as well as transcription factors.

Key Words: T cell, Somatostatin, Thymus

45 Is variation among broilers in their pulmonary hypertensive responsiveness to lipopolysaccharide (LPS) attributable to innate variation in nitric oxide (NO) production by mononuclear cells? O. T. Bowen*, R. F. Wideman, and G. F. Erf, *University of Arkansas, Fayetteville*.

Variability among broilers in their pulmonary hypertensive (PH) responsiveness to LPS apparently reflects innate variation in the types or proportions of vasodilators and vasoconstrictors released by leukocytes. Accordingly, experiments were designed to determine whether an inverse relationship exists between the PH responsiveness to LPS in vivo and the quantities of NO (a potent pulmonary vasodilator) produced by mononuclear cells in vitro. Blood samples were collected from male broilers that then were anesthetized and injected i. v. with LPS to assess the magnitude of their PH response. Within 40 min after the peak PH response to LPS began to subside, the nitric oxide synthase (NOS) inhibitor L-NAME was injected, revealing a strong modulating influence of NO. Mononuclear cells separated from each blood sample were cultured at two million cells/well, stimulated with LPS, and the 24 h in vitro production of NO was measured by nitrite assay. The in vitro production of NO was partially inhibited by L-NAME and fully inhibited by aminoguanidine, a specific inhibitor of inducible NOS (iNOS). The leukocytes also were evaluated by flow cytometry after pre-treatment with 4-amino-5-methylamino-2,7-difluorofluorescein diacetate (DAF-FM), a compound that enters cells and fluoresces in the presence of NO. Flow cytometry demonstrated that approximately 10% of LPS-stimulated mononuclear cells produce NO (presumably monocytes). There was no correlation between the peak PH response to LPS in vivo and the quantity of NO produced by LPS-stimulated cultured mononuclear cells in vitro. These observations suggest that most of the modulatory NO generated in vivo during the PH response to LPS likely is produced by NOS in the vascular endothelium rather than by iNOS in mononuclear cells.

Key Words: Mononuclear leukocytes, Nitric oxide production, Pulmonary hypertensive response to LPS

46 Effect of broiler strain and sex on macrophage inflammatory responses in cell culture. M. Torres* and E. Koutsos, *California Polytechnic State University, San Luis Obispo.*

HD11 cells are an important avian macrophage-like cell line, which are often used to examine macrophage responses in cell culture. These experiments examined HD11 inflammatory immune responses when cultured with plasma from males and females of different broiler strains. First, we determined that confluent HD11 cells incubated with ≥ 111 pg LPS/ml media had increased nitric oxide (NO) production at 24 and 48 h post-LPS ($P < 0.01$ for each). Next, we examined inflammatory responses of confluent HD11 cells incubated with 5% plasma from 15 d old chicks (3 strains- fast feathering-Ross x Ross (RR), Ross x Cobb (RC), Cobb x Cobb (CC); all males). At 24 h post-LPS, NO was increased in all strains ($P < 0.01$), however HD11 cells plated with plasma from CC males produced more NO ($P < 0.05$) than HD11 cells plated with plasma from RC and RR males. Additionally, when females vs. males were compared (RC, CC only) all sexes and strains responded to LPS ($P < 0.01$). A sex x strain interaction ($P = 0.01$) demonstrates that within the CC strain, males produced more NO than females ($P < 0.05$), but within the RC strain there was no difference between sexes ($P > 0.05$). At 48 h post-LPS, NO production/cell was greater in RR compared to CC and RC (males only; $P < 0.05$), due to a significant decrease in cell numbers for RR plasma only ($P < 0.05$). HD11 cells plated with female plasma maintained greater cell numbers than did those plated with male plasma (RC and CC only; $P < 0.05$). A trend for an effect of sex was also evident at 48 h post-LPS; cells plated with male plasma had increased NO/cell as compared to cells plated with female plasma ($P = 0.052$). These data demonstrate that there are differences in HD11 inflammatory responses when cultured with plasma from broilers of different strains and sex. Further studies are needed to examine whether these sex and strain differences affect macrophage inflammatory immune response in vivo as well.

Key Words: Nitric oxide, HD11, Macrophage

47 When do natural antibodies become unnatural? Observations from WUR selected lines. P. Cotter*, A. Lammers, and H. Parmentier, *Wageningen University, Wageningen, The Netherlands.*

Natural antibodies (Nab) are those that arise spontaneously in non-immunized animals, are mostly of the IgM class, and occur in low titers. Chickens possess a repertoire reactive with erythrocytes from several mammalian species. Antibodies to the alpha-Gal epitope (anti-Gal) are expressed on rabbit and pig cells, as well as by viruses and bacteria. They are unusual Nab's because they are found in high titer and likely composed of IgG as well as IgM. Anti-Gal titers were temporarily affected by selection for acquired SRBC response in WUR experimental lines H, C, and L (Cotter, et al. *Poultry Sci.* 84: 220-225, 2005). This suggests that Nab's and acquired antibody are regulated by a common pathway.

The source of anti-Gal in chickens is unknown but gut microbes expressing the alpha-Gal epitope may provide a developmental stimulus. Support for this origin is derived from carbohydrate inhibition studies. The disaccharides, galactose and melibiose, lowered rabbit cell anti-Gal agglutination in lines H, C and L. Day 0 (pre SRBC immunization) plasma agglutination scores were reduced approximately 30% by galactose and 35% by melibiose. Day 5 galactose inhibition was 5 and 10% in Lines H and C but 37% in L. Post-SRBC immunization (day 5) agglutination was inhibited by melibiose in each line approximately 73%. These observations suggest that changes in anti-Gal specificity and avidity result from SRBC immunization.

Cellobiose enhanced anti-Gal titers in line L and it seemed to affect the ability of L plasma to agglutinate SRBC as well. The quality of agglutination of SRBC's that do not express the alpha-Gal epitope, in Line L is currently quite low as a result of downward selection. Including cellobiose in the diluent had an agglutination enhancing effect that was non-linear and appeared to be maximal at 0.25%.

Collectively these observations support the idea of physiologic linkage between innate and acquired antibody and further document the utility of the WUR experimental lines in the investigation of avian immunity.

Key Words: Natural antibody, Anti-Gal, Carbohydrate inhibition

48 Similarities between chicken and turkey leukocyte surface markers. M. Koci*¹, R. Ali¹, and G. Huang², ¹*North Carolina State University, Raleigh,* ²*Southern Biotechnology Associates Inc, Birmingham, AL.*

The promotion and maintenance of poultry health is essential to their overall performance. Understanding how various cells of the host immune system coordinate and respond to disease is central to our ability to design effective therapies and vaccine strategies. In recent years, we have seen a dramatic increase in reagents specific for the chicken immune system due to the rapidly expanding worldwide consumption of chicken and the completion of the chicken genome project. While our understanding of the chicken immune system has increased, our understanding of other poultry species has not kept pace. The current study examined the extent to which monoclonal antibodies (mAbs) specific for chicken surface markers would cross-react with their homologues in turkeys. We examined the ability of 16 chicken specific mAbs to bind peripheral blood leukocytes (PBLs) from two different commercial turkey lines. The cross-reactivity of the mAbs was first examined by flow cytometry. The percentage of PBLs and the intensity of staining of the two turkey lines was compared to that of chickens. Flow cytometry was used as an initial screen for the specific binding of chicken mAbs to turkey PBLs. From this study, 9 mAbs were identified that appear to cross-react with turkey cells, and an additional 3 were identified as potentially cross-reactive. To confirm these results, immunoprecipitation assays were performed. Cell surface proteins from both chickens and the two turkey lines were labeled with biotin, precipitated using the above mAbs, and detected by western blot analysis using HRP labeled avidin. The number and size of proteins precipitated from chicken cells was compared with those identified from turkey cells as additional evidence that these mAbs specifically recognized the same antigen on both chicken and turkey cells. The results from these experiments expand the reagent base for immunological studies involving turkeys. In addition, these results suggest differences in conservation of leukocyte markers between chickens and turkeys and amongst different lines of turkeys. Further studies are required to understand the implications of these potential polymorphisms.

Key Words: Immunology, Leukocyte marker, Turkeys

49 Cell-mediated immunity in chickens: Time-course study on lymphocyte infiltration profiles during the wattle response in Ag-sensitized chickens. I. R. Ramachandran and G. F. Erf*, *University of Arkansas, Fayetteville.*

T cell-dependent cell-mediated immune (CMI) responses have been shown to exist in chickens. As in mammals, the skin in chickens can be used to test CMI responses by injecting the recall antigen into the wattle, wing web or toe web and measuring the resulting swelling. In this study, a temporal approach was used to examine qualitative and quantitative aspects of lymphocyte infiltration during a delayed wattle swelling response (DWSR). Hens were sensitized with *Mycobacterium butyricum* (20 mg in 1 mL, i. m.). Three weeks post-antigen injection, the left wattle was injected with 4 mg M. butyricum in 0.1 mL and the right wattle with 0.1 mL of vehicle (PBS). Wattle swelling measurements were conducted and wattles collected from euthanized hens at 4 h-, 24 h-, 48 h- or 72 h-post-wattle injection. To identify subsets of lymphocytes in wattle tissues, frozen sections were immunohistochemically stained using an indirect enzyme staining method. Detection antibodies included monoclonal mouse anti-chicken CD4, CD8, TCR1, TCR2, TCR3, and Bu-1. Percent positive staining area was determined using Image-Pro software. In antigen-injected wattles, significant DWSR was observed at each time point, whereas minimal swelling was observed in PBS-injected wattles. In DWSR+ wattles, lymphocyte infiltration was observed by 24 h. Lymphocytes were primarily located in perivascular areas, forming loose and nodular aggregates. Within a lymphoid aggregate, T cells predominated. B cells tended to be located in the periphery of the aggregates. At 24 h, CD4+ cells predominated in the lymphoid infiltrate and stayed at the same level for the remaining time points. Infiltration of CD8+ cells increased from 24 to 72 h, surpassing the levels of CD4+ cells by 72 h. The quantitative and qualitative changes in lymphocyte populations infiltrating the wattle during a DWSR are consistent with antigen-specific CMI responses in mammals.

Key Words: Cell-mediated immunity, Lymphocytes, Chicken