1 High-throughput screening for natural host defense peptide-inducing compounds as novel alternatives to antibiotics. W. Lyu*, Z. Deng, R. Matts, and G. Zhang, Oklahoma State University, Stillwater, OK.

A rise in antimicrobial resistance demands novel alternatives to antimicrobials for disease control and prevention. As an important component of innate immunity, host defense peptides (HDPs) are capable of killing a broad spectrum of pathogens and modulating a range of host immune responses. Enhancing the synthesis of endogenous HDPs has emerged as a novel host-directed antimicrobial therapeutic strategy. To facilitate the identification of natural products with a strong capacity to induce HDP synthesis, a stable macrophage cell line expressing a luciferase reporter gene driven by a 2-Kb avian β-defensin 9 (AvBD9) gene promoter was constructed through lentiviral transduction and puromycin selection. A high throughput screening assay was subsequently developed using the stable reporter cell line to screen a library of 584 natural products. A total of 21 compounds with a minimum Z-score of 2.0 were identified. Secondary screening in chicken HTC macrophages and jejunal explants further validated most compounds with a potent HDP-inducing activity in a dose-dependent manner. A follow-up oral administration of a lead natural compound, wortmannin, confirmed its capacity to enhance the AvBD9 gene expression in the duodenum of chickens. Besides AvBD9, most other chicken HDP genes were also induced by wortmannin. Additionally, butyrate was also found to synergize with wortmannin and several other newly-identified compounds in AvBD9 induction in HTC cells. Therefore, these natural HDP-inducing products may have the potential to be developed individually or in combinations as novel antibiotic alternatives for disease control and prevention in poultry and possibly other animal species including humans.

Key Words: host defense peptides, antimicrobial peptides, high-throughput screening, wortmannin, antibiotic alternatives

2 Effect of dietary spray-dried plasma on immunocompetence in broiler chicks. C. Blue*1, Y. Jababu1, L. Young1, R. Ali2, M. Koci2, and Y. Fasina1,1 North Carolina A&T State University, Greensboro, NC, 2 North Carolina State University, Raleigh, NC.

Due to consumer pressure, there is a gradual reduction in the use of antibiotic growth promoters (AGPs) for broiler production. Spray-dried plasma (SDP) is a potential alternative to AGPs because they contain various functional proteins such as albumin, immunoglobulins, growth factors, and biologically active peptides that are capable of stimulating the immune system. An experiment was conducted to evaluate the effect of porcine SDP supplementation on immunocompetence in broiler chicks. Day-old (240) Ross 708 male chicks were obtained from a commercial hatchery, weighed, and randomly assigned to 6 dietary treatments. Treatment 1 (CX) consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP. Treatment 2 (MX) consisted of chicks given unmedicated corn-SBM basal into which Bacitracin methylene disalicylate (BMD) was added at 0.055g/kg diet. Treatments 3 (SP1), 4 (SP2), 5 (SP3), and 6 (SP4) consisted of chicks given unmedicated corn-SBM basal into SDP was added at 10, 20, 30, and 40 g/kg diet, respectively. Each treatment consisted of 4 replicate pens, with each pen housing 10 chicks. Chick growth performance (body weight and feed conversion ratio (FCR)) was monitored throughout the 28-d experiment. On d 14 and 21, blood was collected and subjected to differential leukocyte count (DLC) analysis and the estimation of serum IgG concentration by enzyme-linked immunosorbent assay. Lymphoproliferative response to kidney bean lectin (PHA-P) as an indicator of T-cell-induced delayed type hypersensitivity (DTH) was also evaluated from d 26 to d 27 of experiment. Data collected was subjected to one-way ANOVA, and means separation was done using Duncan’s multiple range test. Although differences were not observed in average body weight (P > 0.05), FCR of chicks in SP3 (1.25) and SP4 (1.24) was superior (P < 0.05) to CX (1.31), and similar to MX (1.29). Circulating IgG concentration was higher for SP1, SP2, and SP3 treatments (2955–2975 ng/mL; P < 0.05), compared with MX (2933 ng/mL). DTH response of MX treatment was higher (P < 0.05) was higher than that of CX, while those of SDP-supplemented treatments were in between. Specifically, SP1 and SP3 had similar DTH response to MX (P > 0.05). It was concluded that incorporation of SDP at a minimum of 30g/kg diet could enhance immunocompetence in broiler chicks.

Key Words: spray-dried plasma, immunocompetence, delayed type hypersensitivity, growth performance

3 In ovo application of epidermal growth factor in late embryonic stage on growth performance, gut development, and nutrient utilization in broiler chickens. E. Kim*, N. Akhtar, J. Li, and E. Kiarie, University of Guelph, Guelph, ON, Canada.

We previously reported that epidermal growth factor (EGF), a protein known for its mitogenic and antiapoptotic effects improved broiler growth performance and increased gene expression for digestive enzymes, nutrient transporters, cytokines, proliferating cell nuclear antigen, and tight junction proteins upon challenge with Eimeria. The aim of this study was to investigate the efficacy of in ovo application of EGF in late embryonic stage on growth performance, nutrient retention, and gastrointestinal (GIT) development. A total of 600 Ross 708 fertile eggs were allocated to 5 treatments (120 eggs/treatment). The treatments included: 1) intact (no punching and no injection), 2) punched and no injection, 3) control (injected with fermentation supernatant without EGF), 4) 80 µg of supernatant containing EGF/kg of egg, and 5) 160 µg of supernatant containing EGF/kg of egg. The eggs were incubated and candled for live embryos on d 19. The viable eggs were subsequently injected and transferred to the hatchet. Upon hatching, 90 chicks of each treatment were placed in cages (15 birds/cage) and the remaining chicks were necropsied for baseline GIT weights (gizzard, small intestine, and ceca) and jejunal histomorphology. Birds had free access to water and standard antibiotic-free corn-soybean diet containing TiO2 as indigestible marker for 21 d post-hatch. Feed intake and BW was monitored weekly and excreta samples were taken on d 3–5 and d 17–19 for AR of DM. On d 7, 14, and 21, 5 birds per cage were necropsied for GIT weights (gizzard, small intestine, and ceca) and jejunal histomorphology. All parameters were subjected to a one-way ANOVA with treatment as a fixed factor and contrast statements to look at the effect of injection, punching, and EGF. There was no effect of punching, injection, or EGF on (P > 0.05) on any response criteria. The final BW were 845.5, 855.9, 891.4, 902.9, and 898.0 g respectively and corresponding FCR were 1.34, 1.40, 1.49, 1.34, 1.34, respectively. The AR of DM for d 3–5 were 70.29%, 70.71%, 70.68%, 70.1%, 70.4% respectively and the corresponding values for d 17–19 were 74.19%, 74.9%, 74.39%, 74.96%, 74.16%. These results demonstrated that in ovo injection of EGF had negligible effects on growth performance, nutrient retention, and indices of gut development. The lack of EGF effect was surprising, as EGF binds to the EGF receptor to activate signal transduction pathways involved in

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regulating cellular survival, growth, proliferation, and differentiation. However, it is unclear whether chick embryos possess the receptors or if the injected EGF was degraded by proteases in the amniotic fluid before delivery into the developing gut.

**Key Words:** broiler, in ovo, epidermal growth factor, gut health and function

### 4 Identification of cells expressing avian beta defensin mRNA in the chicken yolk sac and small intestine

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During the embryonic and early posthatch periods, the chicken immune system has not fully matured, yet chicks are susceptible to infection from pathogens. The chicken yolk sac and small intestine play a similar role in nutrient absorption and serve as a physical barrier to invasion by potential pathogens present in the yolk or intestinal lumen, respectively. Avian β defensins (AvBDs) are host defense peptides that are part of the chicken innate immune system. The objectives of this study were to profile the expression of AvBD mRNA using quantitative PCR (qPCR) and to identify by in situ hybridization cells that express AvBD mRNA in the yolk sac and small intestine during the embryonic and early posthatch periods. qPCR data were analyzed by ANOVA and further separated by Tukey’s test with the significance level at 0.05. The transcriptional profile of AvBD mRNA in the yolk sac showed that AvBD1, 2, 7, and 10 were highly and differentially expressed from embryonic d 7 (e7) to e19. In situ hybridization and Giemsa staining revealed that AvBD1, 2, and 7 mRNA were mainly expressed in activated acidophilic granulocytes in the yolk sac, which were presumably chicken heterophils. AvBD10 mRNA was predominantly expressed in yolk sac endodermal epithelial cells. The expression profile of AvBD10 mRNA showed an increase from e7 to e11 and then a decrease to low levels at e15 and e19, when analyzed by both the in situ hybridization and qPCR assays. In the small intestine, AvBD10 mRNA was expressed by intestinal epithelial cells along the intestinal villus at e19 and day of hatch. However, by d 2, only a few cells above the intestinal crypts expressed AvBD10 mRNA. In summary, AvBD10 was expressed in the epithelial cells of the yolk sac and intestinal villi, while AvBD1, 2, and 7 were expressed in acidophilic granulocytes, suggesting that these AvBD play an important role in chicken innate immunity.

**Key Words:** avian β defensins (AvBD), yolk sac, small intestine

### 5 Immunoassay and immunoaffinity purification of chicken high mobility group box 1 (chHMGB1), a potential new inflammation marker

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High mobility group box 1 (HMGB1) was originally described as a highly conserved, 215 amino acid chromosomal protein that served as a DNA chaperone. However, more recently HMGB1 was revealed to also play an important role as a potent pro-inflammatory immune modulator. When released into the circulation by necrotic cells, HMGB1 becomes as a prototypical danger-associated molecular pattern (DAMP), although it can also be secreted by cells of the monocyte lineage under physiological conditions. As such, HMGB1 may serve as a biomarker for inflammation in various poultry diseases, such as coccidiosis and necrotic enteritis. Therefore, mouse monoclonal antibodies were generated against the amino-terminus (1–16) and the cytokine-inducing region (90–111) of chHMGB1. In addition, polyclonal rabbit sera were developed against chHMGB1 (156–177), the RAGE-binding region of chHMGB1. Different sandwich ELISA formats based on all possible antibody capture and detection antibody pairs were tested with a nuclear chromatin extract as the source of chHMGB1. The configuration featuring rabbit anti-chHMGB1 (156–177) as the capture antibody and either mouse anti-chHMGB1 (1–16) or mouse anti-chHMGB1 (90–111) produced by far the best signal to noise ratio. Both mAbs were successfully used for the immunoaffinity purification of chHMGB1 from a nuclear chromatin extract using mAb-functionalized magnetic beads, and the resulting chHMGB1 preparation will be used to standardize the above sandwich ELISA. Immunoblotting analysis of both affinity-purified chHMGB1 preparations using rabbit anti-chHMGB1 (156–177) for immunodetection revealed identical bands with an MW of approximately 65 kDa. These results suggest that chHMGB1 dimerized upon purification, similar to what has been described for mammalian HMGB1. Future research will focus on validating this ELISA for the measurement of chHMGB1 in chicken serum samples under physiological and pathological conditions.

**Key Words:** chicken, HMGB1, sandwich ELISA, monoclonal antibody, polyclonal antibody

### 6 Immunofluorescent characterization of professional antigen-presenting cells in chicken Peyer’s patches using monoclonal antibodies against CD205 and CD40

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In the chicken, Peyer’s patches (PP) represent a crucial gut-associated lymphoid tissue (GALT) responsible for antigen sampling and activation of T-cells and B-cells. This involves antigen presentation in the context of major histocompatibility complex class II (MHC-II) by professional antigen-presenting cells (APC). This study aims at elucidating the microanatomical organization of the APCs in the PP to better understand their role in initiating the response to orally administered vaccines. PP can be most readily identified in young birds (3–12 weeks of age) as an ovoid white patch about 1-cm in length on the antimesenteric side of the mucosa in the distal ileum between the ceca and cecal tonsils. The hallmarks that make the PP different from the adjacent intestinal tissue include thickened villi, heavy lymphocyte filtration and isolated follicles deeply embedded in the muscularis mucosa/submucosa of the intestine. For this study, PP and adjacent tissue were rolled up as a Swiss roll, snap frozen in liquid nitrogen (vapor phase), cryosectioned at 5μm and 10μm and fixed by cold acetone and 80% methanol before immunofluorescent visualization of CD205 (DCs), CD40 and/or MHC-II (APCs), IgM (B-cells) and CD3 (T-cells). In the center of the PP follicle, CD40 and surface IgM were abundantly expressed, while expression of MHC-II and CD205 was relatively scarce. CD3+ cells were predominantly distributed in the peripheral zone of PP follicle and localized intraepithelially and in lamina propria of the adjacent villi. MHC-II+ APCs were packed subepithelially throughout lamina propria and some were penetrating the follicle-associated epithelium (FAE) toward the lumen. CD205+ DCs appeared as single cells near the crypts and were occasionally found inside the follicles. CD40+ APCs were clustered both inside and outside the follicles. These results show that, much like in mammalian PP, naïve B-cells are the major cell type occupying the follicles of chicken PP, while T-cells can be found in the interfollicular areas.

**Key Words:** chicken Peyer’s patches, dendritic cells, antigen-presenting cells, CD205, CD40