323P Marginal deficiency of trace minerals causes footpad dermatitis in broiler chicks. F. Alemi*, R. Kurzbard, and K. C. Klasing, University of California, Davis, CA.

The food value of the chicken feet is often impaired by discoloration, hyperkeratosis, lesions and ulcerations foot pads. This pathology is known as footpad dermatitis (FPD) and is an animal welfare concern. FPD usually results from an initial injury (scratch or chemical burn) to the skin that becomes infected and chronically inflamed. The inflammatory response results in hyperkeratosis, discoloration, and granulomatous lesions. The etiology of FPD is complex and multifactorial. Nutrition, litter type, and litter moisture often interact to cause FPD. Litter quality is affected by many factors including particle size, moisture content, fecal content and levels of irritants such as ammonia. Inflammatory diseases increase the requirement for copper, zinc and manganese (CMZ). Therefore, an experiment using broilers was conducted to examine the effect of a marginal deficiency of CMZ on FPD. Four broods of chicks were raised successively in batteries with raised wire floors and litter was allowed to accumulate until it slightly covered the wire floor. The moisture content of the used litter was brought to 25%. Two diets were fed to day old broiler chicks: CMZ supplied as hydroxyl minerals at: copper, 6 ppm; zinc, 40 ppm; manganese, 40 ppm (CMZ-OH) or a control corn-soy meal diet with no added CMZ (CONT). There were 32 pens per dietary treatment with 4 birds per pen. On d 19, the foot pads of all birds were scored macroscopically and the epidermis was evaluated histologically. Birds fed basal diet had severe FPD. CMZ-OH decreased the severity of FPD as scored macroscopically \((P < 0.04)\) or histologically \((P < 0.02)\). The correlation between visual scoring FPD and histological scoring was significant \((P < 0.02; R^2 = 0.88)\). CMZ-OH improved growth rate and performance \((P < 0.01)\). Much of the effect on performance occurred during the first 11 d but this effect was maintained, though not increased, during the remainder of the experiment. Most of the effect on performance was likely due to a marginal zinc deficiency because it affects feed intake and growth more than copper or manganese.

Key Words: zinc, copper, chicken, performance, footpad


The objectives of the study were to investigate effects of aflatoxin on growth performance and immune indices for Cherry Valley ducks, and evaluate the efficacy of different kinds of binders in eliminating aflatoxin-induced adverse effects. A total of 360 1-d-old Cherry Valley male ducks were randomly divided into 4 dietary treatments with 6 replications per treatment. The 4 diets formulated included a basal diet (negative control), a contaminated diet with 100.19 mg/kg aflatoxin (positive control) and 2 contaminated diets supplemented with 2 g/kg binders of hydrated sodium calcium alumino silicate (produced by R&L Chemical Inner Mongolia Co. Ltd.) or yeast cell wall (produced by Guang Dong Jiangmen Center For Biotechnology Development Co. Ltd.; NCH, NCY). Of those, the contaminated diet was formulated by replacing common cottonseed meal with 8% naturally moldy one on the equal nutrient level basis. Ducks had access to feed and water ad libitum throughout the whole study period, starter (0–14d) and finisher (15–35d). Compared with the negative control, moldy cottonseed meal significantly decreased ADG and ADFI \((P < 0.05)\) and increased F/G in whole experiment period \((P < 0.05)\). Moldy cottonseed meal significantly increased the relative weight of liver, thymus, spleen and bursa of fabricius \((P < 0.05)\), but decreased the serum albumin and ALP at 14d \((P < 0.05)\), and increased ALT at both 14d and 35d and AST at 14d as well \((P < 0.05)\). NCH group had significant lower relative weight of the liver and immunity organs at both 14d and 35d \((P < 0.05)\) than NCY group. Ducks in NCY group had lower serum albumin at 14d \((P < 0.05)\), but higher ALT at both 14 and 35 d \((P < 0.05)\), AST at 14 d than that in NCK group. It suggested that feeding diet with the natural moldy cottonseed meal could result in a deleterious effect on Cherry Valley ducks, and addition of hydrated sodium calcium alumino silicate could more effectively ameliorate the negative effect of the moldy cottonseed meal than the yeast cell wall.

Key Words: duck, aflatoxin, binder, growth performance, immune indices

325P Effects of dietary phosphorous supplementation on laying performance, egg quality and immunological parameters of laying hens challenged with Escherichia coli lipopolysaccharide. W. Nie, Y. Guo*, and Z. Wang, China Agricultural University, Beijing, China.

The effects of dietary phosphorous supplementation on laying performance, egg quality and immunological parameters in laying hens under acute conditions of lipopolysaccharide (LPS) challenge were investigated. Three hundred laying hens at 28 wk were randomly divided into 2 dietary treatments with 10 replicates of 15 birds. The wheat-soybean based diets contained either 1.2 g or 4.0 g nonphytate phosphorous per kg of diet. At 32 wk of age, half of the birds from each dietary treatment were injected intramuscularly with 1.5 mg/kg of BW of either LPS or saline once a day for continuous 5 d. Three hours after last injection, blood was collected and the hens were euthanized to obtain cecal tonsils. Compared with saline-injected hens, LPS-injected hens had higher rectal temperature at 3 h post challenge and lower feed intake and egg production \((P < 0.05)\). Eggshell thickness, strength, albumin height and Haugh unit were significantly increased in LPS-injected hens compared with saline-injected hens \((P < 0.05)\). Furthermore, laying hens challenged with LPS had higher relative weight of liver and spleen, lower villus height/crypt depth ratio than those received saline \((P < 0.05)\). Serum calcium, phosphorus, total protein, albumin and superoxide dismutase (SOD) activities significantly decreased in the LPS-injected hens compared with the control \((P < 0.05)\). LPS upregulated expression of IL-1β, IL-6 and IL-10 in cecum, and serum concentration of methane dicarboxylic aldehyde (MDA), IL-1β and IL-6 \((P < 0.05)\), whereas dietary nonphytate phosphorous supplementation at 4.0 g/kg significantly increased \((P < 0.05)\) vili height/crypt depth ratio, decreased \((P < 0.05)\) serum MDA and IFN-γ concentration compared with the 1.2 g/kg nonphytate phosphorus group. Dietary non-phytate phosphorus addition at 4.0 g/kg could decrease immunological stress in laying hens injected with LPS.

Key Words: laying hen, nonphytate phosphorus, egg production, egg quality, immune stress
326P Effects of dietary nonphosphate phosphorus level on intestinal immune response of laying hens upon oral Salmonella Typhimurium challenge. S. Bai1, Y. Huang2, Y. Luo1, L. Wang1, X. Ding1, J. Wang1, Q. Zeng1, and K. Zhang.1, 1Institute of Animal Nutrition, Feed Engineering Research Centre of Sichuan Province, Sichuan Agricultural University, Ya’an, Sichuan, China, 2College of Veterinary Medicine, Sichuan Agricultural University, Ya’an, Sichuan, China.

The experiment was conducted to investigate the effects of dietary nonphosphate phosphorus (NPP) level on intestinal immune response of hens upon oral Salmonella challenge. A total of 72 hens (50-wk-old; free of Salmonella) were allotted to 3 dietary treatments with 24 chicks each. The hens were fed the diets containing 0.12%, 0.22%, or 0.42% NPP, and 3.48% calcium for 6 weeks, and then 12 birds per group were challenged or mock-challenged with Salmonella Typhimurium (1 mL, 5 × 10⁷ cfu). The ileum and cecal tonsils were collected at 2 and 7 d post-infection (dpi). The 0.12% NPP diet increased (P < 0.05) Salmonella counts in ileum of Salmonella challenged hens at 2 and 7 dpi when compared with 0.22% or 0.42% NPP diets. Salmonella challenge increased (P < 0.05) ileal CD4⁺ and CD8⁺ T-cells ratios at 2 and 7 dpi, and ileal CD8⁺ T-cells ratio increased (P < 0.05) with dietary NPP level increasing in Salmonella challenged or non-challenged hens. Salmonella challenge increased (P < 0.05) the mRNA expression of toll-like receptor (TLR4, TLR5, interleukin (IL)-1β, IL-6, IL-8, and thymic-helper (Th1) cytokines [interferon γ (IFN-γ), IL-12 and IL-18] in the cecal tonsil of hens. The 0.12% NPP diet decreased (P < 0.05) TLR4, IL-6, IL-8, IFN-γ, IL-12, and IL-18 mRNA level in the cecal tonsils of Salmonella challenged hens, while decreased (P < 0.05) only IL-6, IFN-γ, and IL-18 mRNA level in the cecal tonsils of non-challenged hens when compared with 0.22% or 0.42% NPP diet. There was no significant difference in the above Th1 cytokines mRNA expression in the cecal tonsil of Salmonella challenged or non-challenged hens between 0.22% and 0.42% NPP groups. These results suggested that 0.12% NPP diet had negative effect on and 0.42% NPP diet did not influence ileal Salmonella invasion and Th1 immune response in the cecal tonsils of hens upon oral infection with Salmonella Typhimurium when compared with 0.22% NPP diet.

Key Words: innate immune response, chicken, intestinal cell, Clostridium perfringens

327P Inflammatory responses to Clostridium perfringens type A in primary intestinal cells of chicken embryos. S. S. Guo*, C. W. Li, D. Liu, and Y. M. Guo, China Agricultural University, Beijing, China.

The causative agent of necrotic enteritis is the gram-positive bacterium Clostridium perfringens. Its main cell wall component is peptidoglycan (PGN), which could be recognized by receptors on intestinal epithelial cells, Toll-like receptor (TLR) 2 and nucleotide-binding oligomerization domain (NOD). Consequently, nuclear factor kappa B (NF-xB) pathway is activated and innate immune responses are initiated. A study was conducted to investigate the molecular mechanism of immune responses in primary chicken intestinal cells stimulated with C. perfringens type A. The cells were prepared from 17-d-old chicken embryos and stimulated with C. perfringens at multiplicity of infection of 100:1 for 1, 3, 6, and 9 h (n = 3). Mock stimulation and positive control of commercially available PGN at 50 μg/mL were set as control groups (n = 3). The mRNA expression of inflammatory response genes and interleukin secretion were measured. A significance level of 0.05 was used. Both bacterial and PGN stimulations induced greater IL-8 and IL-6 mRNA expression than that in control at 1 h and 3 h post infection (p.i.). At 6 h and 9 h p.i., C. perfringens but not PGN treatment increased IL-8 and IL-6 mRNA expression compared with control. The mRNA expression of iNOS in bacterial group was highest among treatments at various time points p.i.. At 6 h p.i., bacterial and PGN stimulations increased IL-8 secretion in supernatant of cell culture compared with control. No significant difference in TLR2 expression was observed among treatments. At 1 h p.i., bacterial infection upregulated mRNA expression of NOD1 compared with control and PGN treatment. The level of NOD1 expression in bacterial group was higher than that in control and lower than that in PGN treatment at 6 h p.i.. The PGN stimulation decreased NF-xB p65 mRNA expression compared with control at 1 h p.i.. Bacterial infection boosted NF-xB p65 mRNA expression in contrast to control and PGN treatment at 6 h and 9 h p.i.. In conclusion, C. perfringens infection caused intense innate immune responses in primary chicken intestinal cells, which might be partly mediated by NOD1 recognition and NF-xB pathway.

Key Words: innate immune response, chicken, intestinal cell, Clostridium perfringens

328P The development of poultry-specific, high-throughput, kinase peptide arrays. R. J. Arsenault*, L. B. Trost, and M. H. Kogut1, 1United States Department of Agriculture, College Station, TX, 2University of Saskatchewan, Saskatoon, SK, Canada.

While use of the high-throughput technology to study phosphorylation-mediated signal transduction and the field of kinomics in general has seen a rapid expansion, these tools are often not available outside the study of human and mouse. For example, 2 of the most commonly used tools to study phosphorylation events are phospho-specific antibodies and peptide arrays. The majority of phospho-specific antibodies are generated for human and mouse targets, and the design of peptide arrays relies on information from phosphorylation databases which contain predominantly human and mouse data. Our group has developed a method using well curated species data to find orthologous phosphorylation target sites in a species of interest to design a species-specific tool for kinomic study. Two first-generation chicken-specific peptide arrays were designed, one focused on immunological functions and the other on metabolic functions. Subsequently a second generation, combined immuno-metabolic peptide array was designed incorporating signaling peptides involved in both physiological functions. Two of these second generation arrays were designed, one chicken-specific and one turkey-specific. Of the 177,000 phosphorylation events used to query the poultry proteome approximately 43,000 were predicted in chicken. Only 10% of these 43,000 peptide sequences were identical between the database and the chicken proteome. This limited degree of conservation highlights the need to design chicken-specific proteomic tools for the study of this species, as it is quite distinct from the more common laboratory research species. In addition, turkey and chicken display significant proteome level differences, further emphasizing the need of species-specific designs. This effort has resulted in the production of a high-throughput, species-specific, kinomic tool for physiological research in poultry species.

Key Words: kinome, immunology, metabolism, phosphorylation, signaling

329P Salmonella enterica serovar Enteritidis modulate intestinal cell signaling responses that activate T regulatory cell functions and mediates persistent infections in chickens. M. H. Kogut*, R. Arsenault1, C. L. Swaggerty1, R. Shanmugasundaram2, and R. Selvaraj2, 1SPARC, USDA-ARS, College Station, TX, 2Ohio Agricultural Research Center, Ohio State University, Wooster, OH.
**Salmonella enterica** induce an early pro-inflammatory response in chickens, but the response is short-lived, asymptomatic of clinical disease, and results in a persistent colonization of the cecum. The underlying mechanisms that control persistent colonization of chickens by *Salmonella* are unknown. We hypothesize that a tolerogenic response is induced by alterations of host signaling pathways that mediate the influx and functional activation of T regulatory (Treg) cells. Using chicken-specific kinomic immune arrays, cell isolations, and T cell suppression biosays of infected cecal tissue, we evaluated the development of immunological tolerance in chickens infected with *Salmonella enterica* serovar Enteritidis in a persistent infection model. The induction of a tolerogenic response in the cecum infected with *S. Enteritidis* began around 4 d post-infection. The response was induced by a series of phosphorylation-mediated changes in the ceca characterized by alterations in T cell signaling (dephosphorylation of phospholipase C-γ1 [PLCG1]) and mTOR signaling pathways (increased phosphorylation of AMP-activated protein kinase [AMPK]) and blockage of IFN-γ protection through the disruption of the JAK-STAT signaling pathway (dephosphorylation of JAK2, JAK3, and STAT4). Further, the response is characterized by a reduction in pro-inflammatory cytokine mRNA expression (P < 0.05) and an increase in anti-inflammatory cytokine mRNA expression (P < 0.05). Last, we found an expansion of the Treg population and subsequent immunosuppressive functions at the site of the *Salmonella* infection. These studies define a mechanism by which *Salmonella* infection influences the host responsiveness resulting in the establishment of a persistent colonization of the avian cecum. The identified tissue protein kinases also represent potential targets for future antimicrobial compounds for decreasing *Salmonella* loads from the intestines of food animals.

**Key Words:** *Salmonella*, kinome analysis, regulatory T cells, signaling pathways

### 330P Differential profile of local inflammatory response after challenge with Brazilian field isolates of avian infectious bronchitis virus


Avian infectious bronchitis virus (IBV) causes a worldwide economically important disease in poultry. IBV replicates primarily at the tracheal mucosa, though virus pathology at local sites of IBV replication remains poorly elucidated. The present experiment aimed to evaluate the gene expression of inflammatory mediators, and compare viral load and scores of lesions, in chickens challenged with 2 Brazilian IBV field isolates (F3736 and F3715) previously identified as variants by S1 analysis. Thirteen-day-old SPF chickens were housed in 3 isolators (G1, G2 and G3) with positive pressure. At 39 d of age, 3 chickens in G1 were mock infected with diluted media, while 5 chickens from G2 and G3 were infected with 10^3EID_50/bird of F3736 and F3715 strains, respectively. At 5 d post-infection, birds were necropsied and tracheal samples collected from each group; a portion was processed for histopathology and the remaining part submitted to RNA extraction. RNA was processed by RT-qPCR using SYBR Green I for relative quantification analysis of cytokines IL6, IL1β and T-bet (Th1 lineage transcription factor), and for absolute quantification of IBV S1 gene. Comparisons of the mean relative changes in gene expression were performed using the Mann Whitney test, probability level for significance was set as P < 0.05. Our results showed that in both groups (F3736/G2 and F3715/G3) there was a significant increase of histopathology scores and viral load, compared with negative control group (G1), though no significant differences were observed between the challenged groups. IL6 and IL1β mRNA, pro-inflammatory cytokines precursors, were significantly upregulated only in the F3715 challenged group. TBET mRNA was upregulated in both challenged groups, with highest significant increase for F3715 group. Although similar profiles of tracheal viral load and scores of lesions were observed for both challenged groups, we found an exacerbated inflammatory response for F3715 group, indicating relevant differences in the pathology of the distinct IBV genotypes studied here.

**Key Words:** avian infectious bronchitis virus, IBV, RT-qPCR, inflammatory response, Brazilian isolates.

### 331P Efficacy of commercial H120 strain vaccine against avian infectious bronchitis virus isolates from Brazil


Avian infectious bronchitis is a widespread economically important poultry disease difficult to control. One possibility is that available vaccines are not able to protect fully against some serotypes or genotypes variants, once the causative infectious bronchitis virus (IBV) readily undergoes mutations during mixed infections, resulting in the emergence of new variants. In Brazil, only live-attenuated vaccines of the Massachusetts serotype are licensed, and full protection against IBV variants remains scarcely elucidated. This study aimed to determine efficacy of commercial H120 strain vaccine against viruses classified in genomic groups distinct from the vaccine strain. Among several IBV isolates recovered from breeders or broilers with respiratory signs and/or decrease in egg production, 4 viruses clustered as a group phylogenetically distinct form of Mass serotype were selected (F3137, F3738, F2771 and F3561). SPF birds were divided into 9 groups with 13 birds each and maintained in isolators with positive pressure; G1) non-vaccinated and non-challenged; G2 to G5 were only challenged with selected isolates; G6 to G9 were vaccinated and challenged 4 weeks after. Five days post-challenge, all birds were euthanized and tracheas removed for evaluation of ciliary movement. The scores used were: 0) all cilia vigorously beating; 1) all cilia slowly beating; 2) some cilia very slowly beating; 3) no cilia beating. All birds with median ciliary scores ≤1 were classified as protected and scores ≥2 unprotected. The live vaccine is suitable for use if at least 90% of the challenged and vaccinated birds show no evidence of IBV in their trachea, while 90% or more of the control birds should have evidence of the presence of the virus. Efficacy of challenge was confirmed for all challenged controls groups (G2 to G5), as 100% of birds presented ciliary activity score 3. On the other hand, all vaccinated groups were classified as protected, with median ciliary scores ≤1. Finally, according to the criteria here applied, H120 vaccine was able to induce cross-protection against 4 avian infectious bronchitis virus isolated from Brazil.

**Key Words:** avian infectious bronchitis, Brazilian isolates, Massachusetts vaccine

### 332P Supplemental organic zinc in broiler breeder diet enhances immunity in progeny

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To investigate the effects of high maternal zinc nutrition status on immunity in offspring chicks, 1,200 Ross 308 broiler breeders (45-wk-old) were allocated randomly into 5 groups fed basal diets (containing 26.80–33.52 mg/kg zinc) supplemented with 0, 50, and 300 mg/kg zinc in either organic (methionine hydroxy analog chelated zinc, MHA-Zinc)
or inorganic (zinc sulfate) form. After 6 wk, fertilized eggs were collected and incubated. Newly hatched chicks were divided into 2 groups fed diets containing low level (50 mg/kg) or normal level (100 mg/kg) of inorganic zinc, respectively. Maternal zinc deficiency significantly reduced maternal antibody (IgY and IgA) contents in yolk and serum of chicks, and resulted in adverse immune response to Newcastle Disease virus (NDV) and infectious bursal disease virus (IBD) in offspring at 14-d-old \((P < 0.05)\). Adequate or excess zinc supplementation in hens increased maternal non-specific (IgY and IgA) and specific (anti-NDV and anti-IBD) antibody production. Offspring from hens received extra 300 mg/kg organic zinc in breeder basal diets had a more beneficial effect on progeny immunity compared with that from hens supplemented with extra 50 or 300 mg/kg inorganic zinc \((P < 0.05)\). Offspring chicks supplemented by normal zinc had improved immunity \((P < 0.05)\). Collectively, supplemental MHA-zinc 300 mg per kg of broiler breeder diet is shown to have significant long-term beneficial effects on humoral immunity regardless of zinc states of offspring.

**Key Words:** organic zinc, maternal antibody, IgY, broiler breeder