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SYMPOSIA AND ORAL SESSIONS

Animal Health - Livestock and Poultry: Poultry and Swine II

685 Gene expression of alpha-toxin and *Clostridium perfringens* colonization in the development of necrotic enteritis disease in broiler chickens. W. Si¹, J. Gong¹, Y. Han*², H. Yu¹, H. Zhou¹, and S. Chen³, ¹Food Research Program, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada, ²Maple Leaf Foods Agresearch, Guelph, Ontario, Canada, ³Laboratory Service Division, University of Guelph, Guelph, Ontario, Canada.

Alpha-toxin produced by *Clostridium perfringens*(CP) is considered one of the major virulence factors for necrotic enteritis (NE). This study was conducted to study alpha-toxin production at the gene expression level and its relationship to CP proliferation in the development of NE lesions in broiler chickens. 600 d-old birds were reared in 12 pens (50 birds/pen) and were fed either a medicated diet (55ppm bacitracin) or a non-medicated diet. The birds were challenged with a type A CP inoculum at a concentration of 10⁷ CFU/ml on day 18. Two birds per pen were then humanely killed daily for 4 consecutive days. Ileal digesta was collected to analyze CP counts and alpha-toxin gene expression. NE lesions were scored on days 20, 21, and 22. The gene expression of alpha-toxin was quantified by two-step q-RT real-time PCR analysis. The birds fed the medicated control diet showed no NE lesions, undetectable alpha-toxin gene expression, and a mean CP counts of 0-13 CFU/g of digesta. In contrast, birds fed the non-medicated diet developed visible NE lesions 2 days after the infection (day 20). The average CP counts in the digesta of birds fed the non-medicated diet were 7 CFU/g before, and 1.3 x 10⁴-2.0 x 10⁷ CFU/g after the infection. Birds fed the non-medicated diet also had higher numbers of infected birds, NE scores, ileal CP counts, and alpha-toxin gene expression on day 20. Alpha-toxin gene expression reached a peak on day 20 and started to decline on day 21. All the infected birds fed the non-medicated diet demonstrated a highly positive correlation between the gene expression of alpha-toxin and cell proliferation of CP (R²=0.7606, P < 0.0001). It appears that there was a threshold of counts of the alpha-toxin producing CP for the development of the disease.

Key Words: *Clostridium Perfringens*, Necrotic Enteritis, Alpha-toxin

686 Comparison of the severity of Necrotic Enteritis caused by *Clostridium perfringens* in broiler chickens given either an attenuated or non-attenuated live coccidial vaccine. G. Mathis*¹ and C. Hofacre², ¹Southern Poultry Research, Inc., Athens, GA, ²University of Georgia, Athens.

Two studies were conducted to evaluate the degree of severity of Necrotic Enteritis in broiler chickens challenged or vaccinated with either an attenuated or non-attenuated live coccidial vaccine. The challenge study used battery cages. The treatments were no coccidia challenge, non-attenuated (NA) coccidia challenged, and attenuated (A) coccidia challenged. Both coccidial inoculums were mixtures of *E. acervulina*, *E. maxima*, and *E. tenella*. Birds were coccidia challenged on Day 14. All birds were dosed with *Clostridium perfringens* on Days 19, 20, and 21. The performance parameters measured were feed conversion and weight gain. The percent NE mortality for the A challenged birds was 2%, compared to 20% for the NA challenged birds. Significantly better performance and lower NE lesion scores were observed with the A challenged birds compared to the NA challenged birds. The vaccine study was conducted in a floor-pen facility. The treatments were non-vaccinated, non-challenged or challenged, coccidia vaccinated with attenuated or non-attenuated vaccine and challenged. Vaccination was performed on day of hatch, prior to placement. Challenged birds received *Clostridium perfringens*. Presence of litter oocysts in all vaccinated pens confirmed viability of both vaccines. The non-attenuated vaccinated birds had higher percent NE mortality and NE lesion scores and lower performance at Days 22 and 42 compared to attenuated vaccinated birds. The results demonstrate that the severity of Necrotic Enteritis was not as much in birds challenged with attenuated strains of coccidia than the non-attenuated coccidial strains.

Key Words: Necrotic Enteritis, Vaccine, Coccidia

687 Efficacy of CloSTAT™ direct-fed microbial for control of experimentally induced necrotic enteritis by *Clostridium perfringens* in broiler chickens. B. Boren*¹, G. F. Mathis², C. L. Hofacre³, and S. Moore¹, ¹Kemin AgriFoods North America, Des Moines, IA, ²Southern Poultry Research, Athens, GA, ³University of Georgia, Athens.

An experiment was conducted to determine if CloSTAT™ could affect the severity of experimentally induced Necrotic Enteritis (NE) and lessen related production losses. Chicks were assigned to 1 of 6 treatments each replicated 8 times. Birds were reared 8 chicks per battery brooder pen. Three doses of CloSTAT: 1 x 10¹⁰, 1 x 10⁹ and 1 x 10⁸ cfu/ton mash starter feed were tested in 384 male, Cobb x Cobb 500 broilers. The 3 control treatments were: an unmedicated, uninfected positive control; an unmedicated, infected negative control; and an infected, antibiotic control receiving 50g Bacitracin MD

(BMD)/ton feed. All birds were infected on day 14 with a mixed coccidial inoculum containing 25,000 oocysts of *E. acervulina*/bird and 5,000 oocysts of *E. maxima*/bird. On days 19, 20 and 21, all birds, with exception of positive controls, received a dose of 1×10^8 cfu of a *C. perfringens* strain proven to induce NE via cultured broth provided fresh daily. BW (0 to 22-day) of broilers fed CloSTAT at 1×10^{10} cfu/ton was higher vs. negative control ($P < .05$). By day 27, CloSTAT at all tested doses proved as efficacious ($P < .05$) in protecting against NE model as BMD and not significantly different (NSD) from uninfected positive controls. FCR of negative controls (0 to 27 days) was higher ($P < .05$) than those of all other treatment means and were NSD from one another. NE mortality of CloSTAT-treated chicks was lower ($P < .05$) for those receiving 1×10^9 cfu/ton (9.4%) than those fed 1×10^8 cfu/ton (20.3%); with both NSD from highest CloSTAT dose of 1×10^{10} cfu/ton (10.9%). Mortality due to NE of BMD was NSD from any CloSTAT treatment. Therefore, of the birds challenged with *C. perfringens*, those receiving CloSTAT lived as well as birds receiving BMD at 50g/ton. Based on the results of this study, CloSTAT proved effective in reducing NE mortality and related production losses.

Key Words: Clostridium, Chicken, Necrotic Enteritis

688 Immune interference of bacteriophage efficacy to treat colibacillosis in broiler chickens. W. E. Huff*, G. R. Huff, N. C. Rath, and A. M. Donoghue, *USDA/ARS Poultry Production and Product Safety Research Unit, University of Arkansas, Fayetteville, AR.*

Bacteriophage are viruses that kill bacteria, and may provide a natural and safe alternative to antibiotics. Colibacillosis is an important poultry disease caused by *Escherichia coli*. Previous work has indicated that bacteriophage could be used to both prevent and treat colibacillosis. However, bacteriophage may illicit an immune response in poultry, which could limit their effectiveness to treat bacterial diseases. The experimental design to investigate this possibility consisted of 5 treatments with 3 replicate floor pens of 20 birds per pen. The treatments were control, birds administered bacteriophage at 10 and 17 d of age, birds challenged with *E. coli* at 17 d of age, birds challenged with *E. coli* and administered bacteriophage at 17 d of age, and birds administered bacteriophage at 10 and 17 d of age and challenged with *E. coli* at 17 d of age. Five extra birds were placed in the pens of the control treatment and the treatment only administered bacteriophage. These extra birds were bled and euthanized at 17 d of age for viral neutralization assay development. All remaining birds were necropsied at 31 d of age. Bacteriophage were administered into the thigh muscle providing 6.7×10^8 pfu. The birds were challenged with an airsac inoculation of *E. coli* delivering 1×10^6 cfu per bird. Mortality was significantly reduced by bacteriophage from 55% in the *E. coli* treatment to 8% in *E. coli* challenged bacteriophage treated birds, and reduced, but not significantly, to 33% in the birds that were administered bacteriophage 7 d prior to the *E. coli* challenge, and consequently treated with bacteriophage. The neutralization assays suggest that the activity of bacteriophage was compromised by the bird's immune response. Although bacteriophage can be developed as an alternative to antibiotics for specific applications, bacteriophage efficacy to treat bacterial diseases may be affected by repeated intramuscular administration of bacteriophage due to immune interference.

Key Words: Bacteriophage, Colibacillosis, Poultry

689 Effect of lactic acid bacteria probiotic culture treatment timing on *Salmonella* in neonatal broilers. J. P. Higgins*, S. E. Higgins, V. Salvador, A. D. Wolfenden, G. Tellez, and B. M. Hargis, *University of Arkansas, Fayetteville.*

We evaluated the ability of a commercially available probiotic culture (FM-B11™ Ivesco LLC; LAB1) or a combination of 3 ATCC lactobacilli (LAB2) to reduce *Salmonella enterica* serovar Enteritidis (SE) in day-of-hatch broiler chicks. In Experiments 1 - 3, chicks were challenged with SE and then treated 1 h later with LAB1 or LAB2. Significantly less cecal SE was recovered from LAB1 treated chicks 24 h following treatment as compared to controls (48 - 76 % reduction) or LAB2 treated chicks (48 - 68 % reduction) in all three experiments ($P < 0.05$). LAB2 significantly reduced cecal SE recovery 24 h following treatment as compared to controls in Experiments 1 and 3 (24 - 29 % reduction) ($P < 0.05$), but not in Experiment 2. In Experiments 4 - 7, LAB1 was administered 24 h prior to SE challenge. LAB1 significantly reduced recovery of SE as compared to controls (60 - 78 % reduction) ($P < 0.05$) in Experiments 4 and 5, but not Experiments 6 and 7. In Experiments 8 - 10, chicks were first challenged with SE, then treated 24 h later with LAB1, and no reduction in cecal SE was observed. Together, these data demonstrate that the timing of probiotic treatment in relationship to SE challenge greatly affects the ability of LAB1 to reduce SE recovery. Furthermore, probiotic LAB1 significantly reduced cecal SE incidence as compared to LAB2 treatment, demonstrating that not all lactic acid bacteria are equally effective at reducing enteric pathogens in poultry.

Key Words: *Salmonella*, Probiotic, *Lactobacillus*

690 Evaluation of methods for detecting influenza viruses in wild aquatic birds. T. V. Dormitorio*, J. J. Giambone, K. Guo, and G. Hepp, *Auburn University, Auburn, AL.*

It is generally accepted that wild aquatic birds are the natural reservoirs of influenza viruses that infect other species including humans. There are a number of variables to consider when developing laboratory protocols for detecting avian influenza viruses (AIVs) in wild waterfowl. Egg embryo inoculation, hemagglutination, AC-ELISA and real-time RTPCR (RRT-PCR) were used to detect AIVs from cloacal swabs of wild ducks from Southeastern United States. No AIV was detected when cloacal samples (HA positives) were used directly for either RRT-PCR or AC-ELISA tests. AIV was detected 12 cycles earlier on allantoic fluid of egg inoculated on the 2nd passage. AC-ELISA detected influenza A only when the HA titer of the virus in the allantoic fluid sample was 256 or higher. Many factors such as inhibitors, RNA extraction, etc., could be responsible for the failure of the test to detect AIVs. The RRT-PCR appeared to be a more sensitive, cost-effective and rapid assay however, it still needed embryo inoculation, which is time-consuming and labor intensive.

Key Words: Real Time RT-PCR, Hemagglutination, ELISA

691 Evaluation of a novel recombinant salmonella vaccine vector for avian influenza. K. Cole*¹, S. L. Layton¹, M. M. Cox¹, Y.M. Kwon¹, L. R. Berghman², W. G. Bottje¹, and B. M. Hargis¹, ¹University of Arkansas, Fayetteville, ²Texas A&M University, College Station.

Avian influenza is a significant public health concern and serious economic threat to the commercial poultry industry worldwide. Previous research demonstrates that antibodies against M2e confer protection against influenza challenge. Using the Red recombinase system in combination with overlapping extension PCR, we recently developed several novel attenuated Δ SE)aroA Salmonella enteritidis strains (Δ SE) that express a protective M2e epitope alone or in combination with a potential immune-enhancing CD154 peptide sequence on the outer membrane protein lamB. These recombinant Salmonella strains (Δ SE, Δ SE-M2e, Δ SE-M2e-CD154, or Δ SE-M2eX4-CD154) were evaluated in chicken vaccination studies (challenge dose: 105-107 cfu/chick via oral gavage at day-of-hatch). Liver/spleen and ceca tonsils were aseptically removed at 7 d post-challenge for detection of Δ SE strain recovery, and blood samples were obtained for determination of M2e-specific IgG antibody response at 10 and 20 d post-challenge. Within challenge doses, no significant ($p > 0.05$) differences were observed in Δ SE strain recovery from cecal tonsils (colonization) at d 7. However, marked differences in organ invasion were observed ($p < 0.05$). Vaccinated chickens exhibited significantly increased M2e-specific IgG responses, which were further enhanced by simultaneous expression of CD154 ($p < 0.05$). Virus neutralization assays using chicken embryos gave neutralizing indices of 7.5, 8.3 and 8.3 for Δ aroASE-M2e, Δ aroASE-M2e-CD154, and Δ aroASE-M2eX4-CD154, respectively, indicating effective neutralization of AI by serum IgG of vaccinated chickens. Continuing experiments are focused on duration of response, effect of booster vaccination, and protection against influenza challenge. Our preliminary data suggest that these Salmonella-vectored vaccines expressing M2e in association with CD154 are effective against AI.

Key Words: Vaccine, Salmonella, Avian Influenza

692 Differential antibody response to AIV vaccination in chickens with different Mx genotypes. X. Y. Li, L. J. Qu, Z. H. Ning, G. Y. Xu, J. Y. Li, Z. C. Hou, and N. Yang*, *China Agricultural University, Beijing, China.*

The chicken Mx gene has been shown to be associated with the resistance to avian influenza virus (AIV). In this study, heterozygote (AG) parents with respect to the Mx gene S631N mutation were identified in a line of egg-type chickens and used to reproduce a segregation population. Chicks were divided into three groups based on the genotypes at the S631N site of the Mx protein: 58 chicks with AA, 56 with AG and 53 with GG genotypes, whereas A and G represent resistant and susceptible alleles to AIV, respectively. All experimental birds were vaccinated with 0.3 mL inactivated H5N2 AIV vaccine twice at 14 and 45 days of age. Blood samples were taken for each chicken before vaccination and at week 1, 2, 3, 4, 5, 7, 9 and 15 after the first vaccination, and the antibody titers to AIV measured using HI test. For the initial vaccination, the antibody levels were high with substantial maternal antibody, and there was no significant difference in antibody response among different genotypes. After the boosting vaccination, however, chickens with homozygous resistant allele (A) showed the lowest antibody response, whereas the heterozygous chickens (AG) presented the highest antibody level. The result implied that genetic background might interfere the effects of AIV vaccination.

Key Words: Avian Influenza, Mx Gene, Antibody

693 Impact of ergot infested sorghum on the reproductive performance of sows. G. M. AbdRahim*¹, R. C. Richardson², and A. Gueye³, ¹Alabama A&M University, Normal, ²Texas State University, San Marcos, ³Mt. Ida College, Newton, MA.

Over two parities, the impact of three levels of sorghum infected with ergot alkaloids on the reproductive performance of sows was evaluated. The three levels of ergot alkaloids fed to sows were 0 ppm, 12 ppm, and 24 ppm. Eighteen sows similar in weight, age and breed were used in the experiment. Sows were selected randomly and assigned to the three treatments and fed 2.3 kg of DM/d of a diet based on ground grain sorghum during the gestation and lactation periods. With the exception of the control treatment, the same sows were used during the first and second parities. The number of pigs farrowed, and weights of pigs within 24 hours after birth and at 28 days of age were recorded for each litter. Lactation feed intake, interval to estrus, and weight at d-56, were determined throughout each parity. In the first parity, ADG at d-28, and interval to estrus were affected by the level of ergot alkaloids in the diet. ADG at d-28 was greater ($P < 0.05$) for born pigs from sows fed the 24 ppm ergot alkaloids treatment than born pigs from sows fed the 12 ppm. In the same parity, the level of 12 ppm ergot alkaloids resulted in prolonged estrus interval ($P < 0.05$) over the 24 ppm treatment. In the second parity, variables that were affected by the level of ergot alkaloids were average weight of live pigs born and lactation feed intake. Average weight of live pigs born was greater ($P < 0.05$) in sows fed the 24 ppm and 12 ppm ergot alkaloids treatment than in sows fed the for 0 ppm treatment. The lactation feed intake was greater ($P < 0.05$) for sows fed the 0 ppm than for sows fed the 12 ppm. Statistical analysis of the combined results from parity one and parity two showed that the only variable affected by the treatments was ADG at d-28. ADG at d-28 was greater ($P < 0.05$) for born pigs from sows fed the 0 ppm and 24 ppm ergot alkaloids than for born pigs from sows fed the 12 ppm. Overall, variables that were affected by the infestation of grain sorghum by the ergot alkaloids were pigs gain to d-28, lactation feed intake, and interval to next estrus.

Key Words: Alkaloids Ergot, Sorghum, Sows

694 The effect of dam parity on circulating immunoglobulins (Ig) in neonatal swine. T. E. Burkey*, R. K. Johnson, P. S. Miller, D. E. Reese, and R. Moreno, *University of Nebraska, Lincoln.*

The belief that dam parity can affect progeny performance and survival has contributed to the establishment of parity-segregated management systems in the pork industry. To date, little experimental evidence exists that directly challenges the idea that dam parity affects progeny health status and performance. The objective of this experiment was to evaluate and compare concentrations of IgA and IgG in serum obtained from the progeny of first parity (P1) and third parity (P3) females in order to gain insight into parameters that may be affected by dam parity. Neonatal piglets ($n = 9$) were selected from the progeny of either P1 or P3 females ($n = 4$ each from the P1 or P3 population) for inclusion in the study. Whole blood was collected via jugular venipuncture from piglets at 1, 8, 15, 20 (weaning), 29, and 37 d following parturition and concentrations of IgA and IgG were determined via swine-specific enzyme-linked immunosorbent assays (ELISA). Serum samples were diluted 1:2000 for IgG determination and 1:100,000 for IgA determination. Individual BW were recorded at similar time points. A significant parity by time interaction existed for circulating IgA. Within time point (d), P3 progeny had greater ($P < 0.05$) concentrations of IgA than P1 progeny on d 1, 8, and 37.

A similar interaction was not observed for IgG. Averaged across all time points, P3 progeny had greater ($P < 0.009$) concentrations of circulating IgG than P1 progeny. Piglet BW did not differ between parities. These results suggest that circulating Ig concentrations in neonatal pigs may be affected by dam parity. It remains to be determined whether the increase in circulating Ig observed in P3 progeny occurred because P3 females had greater capacity to provide passive transfer of Ig during lactation. Additional work is needed to determine whether these effects afford the progeny of dams of increasing parity advantages in health and performance during subsequent growth phases.

Key Words: Dam Parity, Immunoglobulins, Swine

695 Impact of ochratoxin A and zearalenone on weaning piglets and counteracting. V. H. Starkl*¹ and M. Forat², ¹Biomim GmbH, Herzogenburg, Lower Austria, Austria, ²Instituto Internacional de Investigacion Animal, Queretaro, Mexico.

Mycotoxins are ubiquitously present, secondary metabolites of different fungal species. Ochratoxin A (OTA) is nephro- and hepatotoxic, carcinogenic and immunosuppressive at low concentrations. Zearalenone (ZON) is known to mimic estrogen and thus leading to hyperestrogenism. A trial was performed to evaluate the effects of 500ppb OTA and 250ppb ZON on health and performance of weaned piglets. The trial started at day 21 and ended on day 63. 96 piglets were randomly separated into 4 treatments. As both, OTA and ZON, are only partly adsorbable, Mycofix[®] Plus (MP) a product based on biotransformation was tested. The product consists of two microorganisms, *BBSH 797* and *Trichosporon mycotoxinivorans*, that produce specific enzymes to biotransform trichothecenes, ZON and OTA. The following four treatments were applied: T-1 Control diet; T-2 500ppb OTA + 250ppb ZON, no MP; T-3 500ppb OTA + 250ppb ZON, 500g MP/ton of feed; T-4 500ppb OTA + 250ppb ZON, 1000g MP/ton of feed. Non-purified toxins provided by Biomin[®] were used. ANOVA was used for statistical evaluation. Measured parameters included performance parameters (feed consumption, body weight, mortality) and histopathology of liver, kidneys and uterus. Daily weight gain over trial period and subsequently final bodyweight were significantly lower in T-2, whereas both treated groups T-3 and T-4 did not show any difference to the control group in performance parameters. No clinical signs were diagnosed in treatment T-1. In treatment T-2 swollen vulva (41,6%), swollen prepuce (50,0%), rectum prolapse (25,0%), vomiting (4,1%) diarrhea (4,1%) and frequent urination (50,0%) were diagnosed. Symptoms in MP-treated

groups T-3, T-4 were significantly reduced or completely overcome. Histopathology showed clear impact of ochratoxin A as kidney- and liver-toxin. Remarkable was the effect of 250ppb zearalenone on the epithelia of uterus of piglets of 63 days of age: Focal squamous cells and mitotic cells were observed in epithelia of uterus of piglets in T-2. As a conclusion can be stated that OTA and ZON strongly affected weaning piglets and that Mycofix[®] Plus was able to overcome the negative impact.

Key Words: Ochratoxin A, zearalenone, Piglet

696 Dietary supplementation with *acanthopanax senticosus* extracts beneficially modulates the gut microflora in weaned pigs.

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This study was conducted to investigate the effects of *acanthopanax senticosus* extracts (ASE) as a dietary additive on gut microflora in weaned pigs. Sixty pigs weaned at 21 d of age were randomly assigned to one of the three 3 treatments (20 pigs/treatment), representing supplementation with 0 or 0.1% ASE, or 0.02% colistin (an antibiotic) to a corn- and soybean meal-based diet. On d 0, 7, 14 and 28 after initiation of the supplementation, five 5 pigs from each treatment were euthanized/sacrificed to obtain the luminal contents of the ileum, jejunum, and cecum. The intestinal luminal samples were analyzed for the gut microflora using the polymerase chain reaction-denaturing gradient gel electrophoresis technique. The concentrations of lactobacillus and *E. coli* were determined using the in vitro culture methodology. The results indicated that the gut microflora of ASE-supplemented piglets was more diverse than the other 2two groups of piglets ($P < 0.05$). Particularly, the number of lactobacillus was higher ($P < 0.05$) and the number of *E. coli* was lower ($P < 0.05$) in ASE-supplemented pigs, compared with the other 2two groups of pigs. These findings suggest that dietary supplementation with ASE beneficially modulates the development of the gut microflora, suppresses the number of bacterial pathogens, and promotes a healthy intestinal environment in weaned pigs. We suggest that ASE is an effective alternative to a feed antibiotic for young pigs. (Supported by NSFC and CAS)

Key Words: *Acanthopanax senticosus* Extracts, Weaned Pigs, Gut Microflora

Nonruminant Nutrition: Poultry Nutrition - Ingredient and Mineral Nutrition

697 Investigation of antagonism and absorption of zinc and copper when different forms of minerals were fed to chicks. T. Ao*, J. L. Pierce, R. Power, A. J. Pescatore, K. A. Dawson, A. H. Cantor, M. J. Ford, and B. L. Shafer, *Alltech-University of Kentucky Nutrition Research Alliance, Lexington, KY.*

The aim of this study was to investigate the antagonism of Zn and Cu when organic or inorganic forms of these minerals were fed to chicks. A practical corn-soybean meal diet without Cu and Zn supplementation,

containing 31 mg Zn/kg diet and 5.4 mg Cu/kg diet, was used as a basal diet. Bioplex Zn[®] (a chelated Zn proteinate) and Bioplex Cu[®] (a chelated Cu proteinate) were used as the organic sources. Reagent grade sulfate salts provided the inorganic sources of Zn and Cu. Supplements provided 20 ppm Zn and 8 ppm Cu. Ten groups of six day-old male broilers were assigned to each of seven treatments. Tap water with no detectable Zn and Cu (<0.001 ppm) and feed were supplied on an *ad libitum* basis during the 3 wk trial. Treatments consisted of the following dietary supplementation: 1) none, 2)