

Animal Health: Monogastric health

W40 Changes in liver and white adipose tissue metabolism induced by postnatal nutritional restriction in piglets with intra-uterine growth restriction. Liang Hu*, Xie Peng, Fali Wu, Chuan Yan, Qin Xu, Yan Liu, De Wu, Shengyu Xu, Yan Lin, Zhengfeng Fang, and Lianqiang Che, *Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan, China.*

It is well known that intrauterine growth restricted (IUGR) neonates lead to escalation in the incidence of metabolic syndrome due to post-natal early catch up growth. We hypothesized that postnatal nutritional restriction may improve metabolic status of liver and white adipose tissue (WAT) in IUGR neonates. Piglets with a birth weight near the mean litter birth weight (SD 0.5) were defined as normal-birth weight (NBW), whereas those with at least 1.5 SD lower birth weight were defined as IUGR. Twelve pairs of NBW and IUGR piglets at 7 d of age were randomly assigned to adequate nutrient intake (ANI) or restricted NI (RNI) for a period of 21 d, which produced 4 experimental groups as NBW-ANI, IUGR-ANI, NBW-RNI and IUGR-RNI ($n = 6$ per group). The NBW-ANI and IUGR-ANI piglets had free access to formula milk, the NBW-RNI piglets were provided the same amount of formula as the IUGR-ANI piglets, and the IUGR-RNI piglets were provided approximately 70% of the formula intake of the IUGR-ANI piglets. At d 28, blood, liver and WAT samples were collected at necropsy and analyzed for hormone, metabolites and gene expressions. Data were analyzed by SPSS software using the MIXED procedure. The results indicated that IUGR significantly increased leptin concentration ($P < 0.01$). Compared with NBW piglets, the mRNA abundance of glucose-6-phosphatase and acetyl-CoA carboxylases were significantly decreased ($P < 0.05$) while sterol regulatory element-binding protein-1c (*SREBP1c*) was markedly increased ($P < 0.01$) in the liver of IUGR piglets; moreover, the mRNA abundances of *CD36*, hormone-sensitive lipase, adipose triglyceride lipase and carnitine palmitoyltransferase-1A in WAT were significantly decreased by IUGR ($P < 0.05$). Irrespective of BW, the hepatic mRNA abundances of *SREBP1c*, glucose transporter 4, phosphoenolpyruvate carboxykinase, lipoprotein lipase and peroxisome proliferator activated receptor α were significantly increased by RNI ($P < 0.05$). In summary, postnatal nutritional restriction changed blood metabolites and hormone concentrations by the metabolic related genes in piglets with IUGR.

Key Words: nutritional intake, metabolism, white adipose tissue

W41 MicroRNA expression profile of the mouse lung infected with a virulent avian H5N2 virus. M. K. Shim*¹, E. J. Choi², S. H. Hong¹, Y. K. Choi², and H. B. Kim¹, ¹Dankook University, Cheonan, Chungnam, Republic of Korea, ²Chungbuk National University, Cheongju, Chungbuk, Republic of Korea.

The aim of this study was to investigate the differences of the microRNA (miRNA) expression profiles in mouse lungs infected with wild type low pathogenic H5N2 avian influenza A (w81) or mouse-adapted virulent H5N2 avian influenza A (ma81) virus. Different sensitivities of influenza A virus strains to the hosts cause variations in miRNA expression. Therefore, some of miRNAs can be used as potential prognostic targets during the avian influenza A virus infections in mammalian hosts. Five-week-old C57BL/6 female mice were inoculated with 30 μ l of 10^4 TCID₅₀ of w81 or ma81. Lung tissues from 3 mice per group were harvested at 1 and 3 dpi. A small RNA library was constructed from the total RNAs of lung samples and sequenced using the Solexa platform. Sequence reads were normalized to determine the number of transcripts

per million, and fold changes of miRNAs were evaluated. P -value was calculated using following formula as previously described. $p(x|y) = (N2/N1) (x+y)! / x!y!(1 + N2/N1)(x+y+1)$ where x , y , $N1$, and $N2$ represent number of miRNAs surveyed, number of homologous miRNAs in controls, total number of clean reads in controls, and total number of clean reads in treatments, respectively. Gene ontology analysis was conducted by miRanda and DAVID. The w81 virus induced a higher number of differentially expressed miRNAs compared with the ma81 virus. It is interesting to see that only 9 miRNAs (miR-100-5p, miR-130a-5p, miR-146b-3p, miR-147-3p, miR-151-5p, miR-155-3p, miR-223-3p, miR-301a-3p, and miR-495-3p) were significantly upregulated in both lungs infected either with the w81 or ma81 strain at both time point ($P < 0.05$). Especially, expression levels of 4 miRNAs were higher in the lungs of mice infected with the ma81 virus than those infected with the w81 virus ($P < 0.05$). These 4 miRNAs have been implicated in immune responses (miR-223-3p and miR-147-3p), viral infection (miR-155-3p), and cell migration (miR-151-5p). Our results suggest that the mammalian adaptation of avian influenza A virus results in a different miRNA expression pattern in lungs of virus-infected mice compared with its parental strain. Thus these might be used as potential prognostic targets during the avian influenza A virus infections in mammalian hosts.

Key Words: influenza A virus, microRNA, virulence

W42 Relationship between *Salmonella* translocation patterns and immune responses in orally inoculated pigs. Paul R. Broadway*¹, Jeffery A. Carroll¹, Nicole C. Burdick Sanchez¹, E. V. Gart², L. K. Bryan², R. M. Gold², C. Yang², and Sara D. Lawhon², ¹Livestock Issues Research Unit, USDA-ARS, Lubbock, TX, ²Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.

Salmonella is a pathogen of interest with broad implications ranging from animal health to food safety. Translocation patterns of *Salmonella* from the gastrointestinal tract to peripheral tissues have not been fully elucidated. Also, the mechanisms by which immunological responses influence translocation and fecal shedding are not fully understood. The objective of this study was to determine the translocation patterns of orally inoculated *Salmonella* in response to changes in immune biomarkers. Male pigs ($n = 12$; 6.1 ± 2 kg) were orally inoculated with 4.7×10^9 cfu of *Salmonella* Typhimurium. Whole blood samples were collected and analyzed for serum cortisol concentrations and complete blood counts. Fecal samples were collected daily from -1 to 3 d relative to the bacterial challenge on d 0. At 72 h post-inoculation, animals were humanely euthanized and tissues were collected to determine the presence of the inoculated *Salmonella* [mesenteric lymph node (LN), subiliac LN, liver, spleen, kidney, and gallbladder]. There was a tendency ($P = 0.06$) for fecal shedding quantity to be similar to mesenteric LN *Salmonella* concentrations at 72 h. There was no interaction ($P > 0.05$) between the presence of *Salmonella* in LN and any of the immune parameters measured. Subiliac LN *Salmonella* concentrations were highly correlated ($P = 0.0001$; $r = 0.99$) with liver *Salmonella* concentrations. Fecal shedding at d 3 was negatively correlated with kidney and gallbladder *Salmonella* concentrations ($P = 0.04$). Cecum concentrations of *Salmonella* were negatively correlated with white blood cell ($P = 0.02$ $r = -0.72$) and neutrophil ($P = 0.0003$) counts. These data suggest that orally inoculated *Salmonella* may translocate to tissue not only within the gastrointestinal tract but to organs and peripheral tissues such as musculoskeletal LN. Additionally, negative

correlations between immune biomarkers and *Salmonella* migration suggest that translocation of *Salmonella* may be inhibited following the peak immunological response. Further information is needed to fully elucidate the mechanisms by which pathogens interact with their host and how an immune response alters migration patterns.

Key Words: *Salmonella*, swine, immune

W43 Isolation and characterization of *Clostridium tertium* in poultry feces. S. H. Hong*, S. A. Seok, M. K. Shim, and H. B. Kim, Dankook University, Cheonan, Chungnam, Republic of Korea.

The aim of this study was to isolate and characterize *Clostridium tertium* from chicken feces. *C. tertium* is found in soil as well as gastrointestinal tracts of humans and other animals. *C. tertium* is an anaerobic, motile, gram-positive, rod-shaped bacterium that forms highly resistant endospores without generating an exotoxin. Although it is considered an uncommon pathogen and its pathogenicity is often unclear, this bacterium has been implicated in bacteremia, pneumonia, enterocolitis, septic arthritis and abscess in humans and animals. Even though there is no direct evidence that *C. tertium* can be transmitted from animals to humans, it is of importance to see the prevalence and distribution of *C. tertium* in animal feces. Chicken feces samples were collected from the research farm at Dankook University, South Korea. Ten chickens per cage were housed together and 10 chicken droppings per cage (a total of 10 cages) were collected using sterile disposable culture loops starting when chickens were 1 week of age then at one-week interval until they were 4 weeks of age. After ethanol treatment (50% ethanol for 1 h), dropping samples were streaked onto TCCFA agar plates with 5% sheep blood, and incubated in anaerobic jars at 37°C for 72 h. The isolates were identified based on morphological criteria. Then identification was confirmed by the Gram staining and sequencing of the V1-V3 of 16S rRNA gene. After 72 h incubation in an anaerobic jar, a pure culture of colonies was recovered. A Gram stained smear of colonies showed weak-staining, gram-positive rods. These rod-shaped bacteria were detected from all chicken feces. The cloning and subsequent sequencing of the 16S rRNA genes from 2 representative isolates identified them as *C. tertium* with a probability of 99%. To our knowledge, this is the first isolation of *C. tertium* from chicken droppings. In depth studies on pathogenesis of *C. tertium* will be needed to better understand potential roles of *C. tertium* in poultry health.

Key Words: *Clostridium tertium*, chicken, feces

W44 Antimicrobial resistance strategy and effect on the veterinary feed directive regulation. David B. Edwards*, Dragan Momcilovic, Sharon A. Benz, and Jo W. Gulley, Division of Animal Feeds, Center for Veterinary Medicine, Rockville, MD.

On December 12, 2013, the Food and Drug Administration (FDA) proposed to change its animal drug regulations to improve the efficiency for stakeholders using veterinary feed directive (VFD) drugs while continuing to protect human and animal health. The VFD drug class was created in 1996 by the Animal Drug Availability Act, and they are intended for use in or on animal feed under a veterinarian's order and professional supervision. The FDA established the VFD program in 2000. Changes to the VFD regulation are important as FDA implements the judicious use principles for medically important antimicrobial new animal drugs approved for use in food-producing animals, based on the framework set forth in Guidance for Industry (GFI) #209 issued April 13, 2012 and the process and timeline in GFI #213 issued December 13, 2013. Antimicrobials must continue to be available to combat disease in

animals, including treatment, control, and prevention, while preserving availability of effective drugs. The primary concern is the continued effectiveness of "medically important" drugs. One important change in GFI #213 is to include veterinary involvement in the use of these antimicrobials by changing the drug marketing status of antimicrobial drugs from over the counter to prescription or VFD drugs to be used for therapeutic uses only. This means a VFD order from a licensed veterinarian will be needed to obtain medicated feeds containing VFD drugs. This order must include information on the number and type of animals to be treated. This keeps veterinarians included in the decision-making process to use medically important antimicrobials. FDA has secured the cooperation of drug manufacturers to change the drug marketing status and remove the production uses from these drug approvals. To improve the VFD process, FDA proposed several key changes in the proposed rule such as removing the federally defined veterinarian-client-patient-relationship requirement, eliminating the automatic classification of VFD drugs as Category II, and modifying recordkeeping requirements. FDA received about 2000 comments on the proposed rule and is reviewing those comments to publish a final rule.

Key Words: antimicrobial, veterinary feed directive, regulation

W45 Estimating glucose requirements of an activated immune system in growing pigs. Sara K. Stoakes*, Erin A. Nolan, Mohanad Abuajamieh, Maria V. Sanz Fernandez, and Lance H. Baumgard, Iowa State University, Ames, IA.

Activated immune cells are obligate glucose utilizers and a large lipopolysaccharide (LPS) IV dose causes severe hypoglycemia. Therefore, study objectives were to use the quantity of glucose needed to maintain euglycemia following an endotoxin challenge as a proxy of immune cell glucose requirement. Fifteen fasted crossbred gilts (30 ± 2 kg) were jugular catheterized bilaterally and assigned one of 2 IV bolus treatments: control (CON; 10 mL sterile saline; n = 7) or LPS-infused + euglycemic clamp (LPS-Eu; *E. coli* 055:B5; 5 µg/kg BW; 50% dextrose infusion to maintain euglycemia; n = 8). Following infusion, blood glucose was determined every 10 min and dextrose infusion rates were adjusted in LPS-Eu pigs to maintain euglycemia for 8 h. Rectal temperature was increased in LPS-Eu pigs relative to control (39.8 vs 38.8°C, $P < 0.01$). After 3 h, blood glucose content gradually declined for CON pigs while LPS-Eu glucose levels remained unchanged ($P = 0.01$). Plasma insulin, BUN, BHBA, and L-lactate were increased in LPS-Eu pigs compared with CON (69, 57, 21, and 60%, respectively; $P < 0.05$). By 8 h, plasma LPS binding protein was increased 24% in LPS-Eu pigs relative to controls ($P < 0.01$). Plasma NEFA increased with time in CON pigs, but remained unchanged in the LPS-Eu pigs ($P < 0.01$). White blood cells, lymphocytes, monocytes, eosinophils, and basophils were decreased in LPS-Eu pigs relative to CON ($P < 0.01$). Additionally, the neutrophil-to-lymphocyte ratio was increased in LPS-Eu pigs relative to CON (72%, $P < 0.01$). During the 8 h, 232 ± 16 g of infused glucose was required to maintain euglycemia. If the amount of glucose required to maintain euglycemia can be used as a proxy, then the glucose requirements of an activated immune system are approximately 29 g/h.

Key Words: lipopolysaccharide, immune challenge, glucose homeostasis

W46 The influence of sodium alendronate on performance and bone densitometry of broilers at 42 days of age. Thays Cristina Oliveira Quadros*¹, Sarah Sgavioli¹, Giuliana Milan de Andrade Rocha Garcia¹, Liliana Longo Borges¹, Elaine Talita Santos¹, Diana

M. Correa Castiblanco¹, Albaraa Hisham Sarsour², Lizandra Amoro-oso¹, Joao Batista Matos Junior¹, Joao Paulo M. Chiquini¹, Otto Mack Junqueira¹, and Silvana Martinez Baraldi Artoni¹, ¹*Paulista State University Julio de Mesquita Filho, Jaboticabal, Sao Paulo, Brazil*, ²*North Carolina State University, Raleigh, NC*.

Sodium alendronate (SA) is a drug that shows effects on bone mass, which may contribute to better bone development of animals, allowing for more efficient production performance. This study was conducted to evaluate the influence of SA on performance and bone mineral density of Cobb broilers at 42 d of age. Twelve hundred broilers used in this study were derived from 2 treatments that included injection or no vitamin D injection during incubation. After the eggs hatched, the broilers received water containing different levels of sodium alendronate (0, 2, 4, 6 or 12 mg/mL). The water solution with SA was supplied through esophageal inoculation in broilers chicken once a week for 3 weeks. One milliliter of solution was given to each bird on 7, 14 and 35 d of age, to ensure that all animals ingested the treatment. Experimental design was completely randomized in a 2 × 5 factorial for a total of 10 treatments (injected or not × sodium alendronate level). Broiler performance was evaluated by calculating feed intake, body weight gain and feed conversion. Bone density of the tibiotarsus was analyzed at 42 d using DEXA equipment. Data were analyzed using the JMP program for ANOVA. For the performance parameters of the broilers there was no significant effect ($P > 0.05$), as well as for the densitometry results, though one can observe an increased bone density value (0.242 g/cm²) in broilers derived from eggs injected with vitamin D and subsequently treated with 4mg of SA level. Treatments with SA can increase total skeletal mass, but the data suggest that despite the non-significant results of SA in this experiment, a numerical increase in bone density was observed, hence an improvement in bone development. It was concluded there was no influence on broiler performance ($P = 0.798$) and for bone mineral density the level of 4 mg of SA was numerically ($P = 0.246$) better when compared with other treatments, which encourages us to continue our research to establish a significant level of SA. SA may enable us to increase bone mineral density, and possibly contribute to weight gain of broilers.

Key Words: bone development, chicken, densitometry

W47 Potential of a new probiotic strain, *Bifidobacterium longum* ssp. *infantis* CECT 7210, to improve health status of weaning piglets orally inoculated with *Salmonella* Typhimurium or ETEC K88. E. Barba-Vidal¹, L. Castillejos¹, C. Sol^{*1}, M. Rivero², JA Moreno², and SM Martín-Orúe¹, ¹*Animal Nutrition and Welfare Service, Animal and Food Science Department, Universitat Autònoma de Barcelona, Bellaterra, Spain*, ²*Laboratorios Ordesa S.L., Parc Científic de Barcelona, Barcelona, Spain*.

A new probiotic strain of *Bifidobacterium longum* subsp *infantis* CECT 7210 (Laboratorios Ordesa, S.L.) was evaluated in 2 oral challenges against *Salmonella* Typhimurium or enterotoxigenic *E. coli* (ETEC) K88. Seventy-two piglets, 21 and 24 (±2) days old were used in each trial. Animals were distributed in 24 pens and 4 groups in a 2 × 2 design: with and without probiotic and oral challenge. The animals were fed a plain diet and the probiotic was administered orally on a daily basis (10⁹ cfu/d). After a 1-week adaptation, a double oral inoculation was done with *Salmonella* Typhimurium (2 × 10⁹ and 6 × 10⁹ cfu/day) (trial 1) or ETEC K88 (5 × 10⁹ and 6 × 10¹⁰ cfu/day) (trial 2). Intake, live weight, fecal excretion of the pathogen, fecal consistency and rectal temperature were registered. Results were analyzed with a GLM and adjusted by Tukey. Microbiological frequencies were analyzed by Fisher. On d 4 and 8 post inoculation (PI), blood samples were obtained from one animal

from each pen to assess inflammatory response (TNF- α and Pig-Map), being euthanized afterward for intestinal content and ileal scrapings sampling for microbiological and fermentation products analysis. Performance parameters were affected by the *Salmonella* challenge with reductions in feed intake and gains (ADG 118 g challenge animals vs. 209 g, $P < 0.001$), but not by *E. coli* (ADG 114g challenge animals vs. 120 g, $P = 0.801$). No significant differences were found related to the probiotic. The challenge also got worse fecal consistency ($P < 0.001$) and increased plasma TNF- α levels ($P < 0.05$). Although the probiotic did not ameliorate these clinical signs it was able to reduce fecal excretion of *Salmonella* (especially at d 3 PI; $P = 0.043$) and diminished the percentage of animals with countable (>10⁵cfu/g) coliforms in ileal scrapings at d 4 PI (58 vs. 91%; $P = 0.010$). A consistent interaction was seen in both trials for colonic short chain fatty acids (SCFA), were the probiotic increased their concentration in the control animals but not in the challenged ones. Results indicate administration of the probiotic can reduce intestinal colonization by *Salmonella* and ETEC K88 in mild enteric processes.

Key Words: probiotic, *Salmonella*, ETEC K88

W48 A comparison for IgG absorption between Minpig and Landrace piglets. Shiquan Cui^{*1,2}, Yuan Xu¹, Xuankai Huang¹, Xibiao Wang¹, and Yuzhi Li², ¹*Northeast Agricultural University, Harbin, Heilongjiang, China*, ²*West Central Research and Outreach Center, Morris, MN*.

The levels of IgG in colostrum is responsible for the natural passive immunity of piglets, so effectively obtaining colostrum is important to survival and growth of newborn piglets. A study was conducted to evaluate the ability of newborn Minpig piglets to obtain IgG from colostrum. Four pairs of Minpig and Landrace sows (3–5 parity), with each pair farrowing their first piglet within an hour, were used. The 6 first born piglets were removed from their dam immediately after birth to prevent them from suckling colostrum. Then 3 out of 6 piglets in each litter were cross-fostered to a sow of another breed, resulting in 4 treatment groups: MM (Minpig piglets nursed by Minpig sows), ML (Landrace nursed by Minpig), LM (Minpig nursed by Landrace), and LL (Landrace nursed by Landrace), with 12 piglets in each group. Blood samples were collected from all piglets at 12 h, 24 h, 36 h, 48 h, 72 h, and 7 d after birth to determine concentrations of IgG using ELISA. Serum IgG concentrations for MM, ML, LM, and LL groups were 58.95 ± 3.33, 53.81 ± 3.64, 54.03 ± 3.11, and 48.89 ± 2.60 mg/ml, respectively, at 24h after birth. Compared with Landrace piglets, Minpig piglets had higher IgG concentrations ($P < 0.05$) regardless of being nursed by Minpig or Landrace sows. To further verify the efficiency of Minpig piglets to absorb IgG, a piglet from each 5 litters of Minpig and Landrace were tube-fed bovine IgG (1000mg/kg·BW) at 0 h, 6 h, 12 h, 24 h, 36 h, 48 h, and 72 h after birth, respectively. Blood samples were collected from the piglets at 6h after eating BIgG, and the absorption efficiency of BIgG was calculated for each piglet based on: $\{[\text{serum BIgG concentration} \times \text{BW} \times 0.10 \times (1 - \text{hematocrit})] / \text{BIgG consumed}\} \times 100\%$. Results indicate that Minpig piglets had higher absorption efficiency of exogenous BIgG than Landrace piglets (19.82 ± 1.55 vs. 17.07 ± 1.18 at 24 h, $P < 0.05$; 17.25 ± 1.63 vs. 14.43 ± 1.43 at 36 h, $P < 0.05$). These results suggest that Minpig piglets had a stronger capability to obtain passive immunity through milk and feed compare with Landrace piglets during the suckling period.

Key Words: Minpig, piglet, IgG