

Animal Health: Immunity, Probiotics and Health Status

263 An experiment in transmission of *Mycoplasma bovis* in sand bedding to naive dairy calves. D. J. Wilson*¹, A. Justice-Allen¹, T. J. Baldwin¹, R. T. Skirpstunas¹, K. B. Cavender¹, and G. Goodell², ¹Utah State University, Logan, ²The Dairy Authority, Greeley, CO.

The study evaluated possible transmission of *Mycoplasma bovis* from sand bedding to naive dairy calves. Screening of a closed herd showed 99% probability that the herd was free of mycoplasma in calves. Neonatal calves (n = 12) from the herd were blocked by weight and height and randomly assigned as controls (n = 6) bedded with quarry sand, or exposed (n = 6) bedded with *M. bovis*-positive bedding sand (confirmed by PCR) from another farm. Calves were housed at Utah State University in calf hutches, fed commercial milk replacer and calf starter, with strict biosecurity and separation between groups. Exposed group sand cultured positive for *Mycoplasma* spp. during weeks 1, 5, 6, 7, 11 and negative for the rest of the 15 week study; control group sand was always mycoplasma-negative. Exposed group calves were bedded on mycoplasma bedding for 138 total calf-days. All 94 sera tested for antibody against *M. bovis* were negative. All 16 tracheal swabs and all 67 nasal and ear swabs collected from all calves were mycoplasma culture-negative. Two calves died and 3 were euthanized before the end of the study; the remaining 7 calves were euthanized after 15 weeks. All calves were necropsied and full diagnostic testing was performed. No exposed or control calves had any gross lesions of mycoplasma infection. All post-mortem culture (n = 60) and PCR (n = 48) tests on trachea (cultured only), deep lung, peri-bronchial lung, retropharyngeal lymph node, and carpal and tarsal joint fluid from all 12 calves were negative for *Mycoplasma* spp. The PCR could differentiate *M. bovis* if positive. Using test sensitivity and sequential probability, the probability of each calf being detected positive at least once if they had become infected with mycoplasma following 4 weeks of exposure was calculated. For the 9 calves that survived beyond 25 days of age, probabilities of detection were between 96.5% and 99.3%. There was no evidence that *Mycoplasma bovis*-positive bedding sand was a source of infection to naive dairy calves. Further studies in lactating cows are still indicated because of the possibility of infection through teat ends.

Key Words: mastitis, *Mycoplasma*, bedding

264 Effect of supplementing fatty acids to prepartum Holstein cows on transfer of passive immunity to calves. M. Garcia*, L. F. Greco, M. G. Favoreto, R. S. Marsola, L. T. Martins, D. Wang, W. W. Thatcher, J. E. P. Santos, and C. R. Staples, *University of Florida, Gainesville.*

The aim of this study was to evaluate supplementing linoleic acid (LA) to cows during the last 2 mo of pregnancy on transfer of passive immunity to calves. Cows (n = 89) were fed diets formulated to supply minimum amounts of LA and supplemented without fat, with saturated fatty acids (SFA; Energy Booster 100, MSC) at 1.75% of dietary DM, or with Ca salts of unsaturated fatty acids enriched in LA (UFA; Megalac-R, Church and Dwight, Co.) at 2% of dietary DM. Within 2 h of birth, calves were given 4 L of colostrum from their own dam or from a dam fed the same dietary treatment as the calf's dam using an esophageal feeder. Acquisition of passive immunity was assessed by measuring concentration of IgG in colostrum and in serum, as well as total protein concentrations in serum at 0 and 24 h of life. Apparent efficiency of absorption was calculated considering serum as 9.9% of BW. Body weight at birth did not differ among treatments (39.6 kg), however calves born from multiparous cows were heavier ($P < 0.05$)

than those born from primiparous cows (42.6 vs. 36.5 kg). Concentration of colostral IgG was greater from multiparous cows fed fat than from those not fed fat prepartum (116 vs. 96 g/L) whereas the opposite was true for primiparous cows (83 vs. 101 g/L). As a result intake of IgG by calves born from multiparous cows fed fat prepartum was greater than that by calves born to cows not fed fat (459 vs. 383 g). Therefore serum concentrations of IgG tended to be greater ($P = 0.10$) at 24 h in calves born from multiparous cows fed fat compared with those not fed fat (26.7 vs. 21.1 g/L). Concentrations of serum protein did not differ among treatments (3.8 and 5.3 g/100 mL at 0 and 24 h, respectively). Calves born from cows fed fat prepartum tended to be more efficient at absorption of IgG (26.8 vs. 23.3%). Feeding supplemental fat prepartum to multiparous cows resulted in greater IgG concentration in colostrum and better efficiency of IgG absorption.

Key Words: calves, passive immunity, linoleic acid

265 Effect of a yeast autolysate combined with probiotics on performance and gut health of broilers. A. Ganner*¹, S. Masching², N. Reisinger¹, G. Schatzmayr¹, and T. Applegate³, ¹BIOMIN Research Center, Tulln, Austria, ²BIOMIN Holding GmbH, Herzogenburg, Austria, ³Purdue University, West Lafayette, IN.

The present study was conducted to evaluate the efficacy of a product, consisting of yeast autolysate, lactobacilli, *Enterococcus* sp., *Pedio-coccus* sp. and bifidobacteria, on performance and jejunal structure of broilers. In a 35 d study, 300 1-d-old broilers were distributed to 2 experimental groups with 8 replicates: control group A, group B (1 kg yeast autolysate per ton feed combined with probiotic mixture of 10^8 CFU/kg feed). Directly after housing the chicks were supplied with the experimental diets. Feed and water were provided for *ad libitum* intake, feeding was done manually several times a day. On d 35 birds were killed and the distal jejunum taken from 8 chickens per group (1/pen). Paraffin sections were stained with periodic acid Schiff (PAS) and hematoxylin. The length of the villi and the depth of the crypt were determined with an ocular micrometer via light microscopy. The goblets cells were counted on 6 villi/ bird as well under the microscope and an average was taken. Similarly, 12 villi/ bird were measured for villus length and crypt depth. In the course of the feeding trial a positive influence could be observed by the product consisting of autolysate and probiotics. Weight on d 14 and daily weight gain (DWG) 1–14 were improved ($P = 0.0001$), as well DWG 15–35 ($P = 0.002$). Weight on d 35 (1433g) and DWG 1–35 (39.9g) were slightly improved in comparison to the control (1381g weight d 35, 38.4g DWG; $P = 0.08$). Mortality was reduced in the trial group (2.7%) in comparison to the control (5.3%). The goblet cell number was slightly increased by the trial group with 126 cells/villus (control 98cells/villus, $P = 0.1$). Villus height and crypt depth were not affected. Our results indicate that the product consisting of autolysate and probiotics is able to improve gut health and to enhance bird performance.

Key Words: yeast autolysate, probiotics, gut health, broiler performance

266 Effect of NuPro supplementation on intestinal *Clostridium perfringens* levels in broiler chickens. R. Thanissery*¹, J. L. McReynolds², D. E. Conner¹, K. S. Macklin¹, P. A. Curtis¹, and Y. O. Fasina¹, ¹Auburn University, Auburn, AL, ²SPARC-USDA-ARS, College Station, TX.

Clostridium perfringens (CP) is the causative bacteria for necrotic enteritis (NE) in poultry. Yeast extract contain immunomodulatory nucleotides, and may therefore serve as a non-antibiotic feed additive for reducing intestinal CP in broilers. In a 42-d floor pen trial, the efficacy of NuPro (a yeast extract) in reducing intestinal CP levels in broiler chickens was evaluated. Chicks (n = 600) obtained from a commercial hatchery were randomly assigned to 6 treatments. Treatment 1 (CX) consisted of chicks fed corn-soybean meal (SBM) diet without bacitracin methylene disalicylate (BMD) or NuPro added. Treatment 2 (MX) consisted of chicks fed corn-SBM basal into which BMD was added at 0.055g/kg. Treatment 3 (LN) consisted of chicks fed corn-SBM basal into which NuPro was added at 2% level throughout experiment. Treatments 4 (PCX), 5 (PMX), and 6 (PLN) consisted of chicks fed diets similar to those given to CX, MX, and LN treatments, respectively, and were additionally challenged with 3.5 mL of CP inoculum (10^8 CFU/mL) on d 14, 15, and 16 of experiment. Post-challenge (PC) assessment of intestinal CP levels was done on d 1, 7, and 21 PC. Growth performance (body weight (BW) and body weight gain (BWG)) was also assessed at 3 and 6 weeks. Results showed that by d 21 PC, the NuPro-containing diet significantly reduced intestinal CP levels ($P < 0.05$) in PLN treatment by $1.50 \log_{10}$ CFU/g compared with NuPro-free PCX treatment. Also, the efficacy of BMD antibiotic and NuPro in reducing intestinal CP in PMX and PLN, respectively, was similar throughout the experiment. BW and BWG were similar ($P > 0.05$) among CP-challenged chicks (PCX, PMX, and PLN treatments), indicating that the NuPro-induced reduction of CP in PLN treatment occurred without any adverse effect on bird performance. In conclusion, dietary supplementation of NuPro at 2% level of diet effectively reduced intestinal CP during broiler production cycle.

Key Words: *Clostridium perfringens*, NuPro, broiler chickens

267 A modified in vitro larvae migration inhibition assay using rumen fluid to evaluate *H. contortus* viability. T. R. Whitney*¹, D. R. Klein², A. E. Lee¹, C. B. Scott², and T. M. Craig³, ¹Texas AgriLife Research, San Angelo, ²Angelo State Univ., San Angelo, TX, ³Texas A&M Univ., College Station.

The objectives of this study were to evaluate how forage material added to an in vitro system affects rumen fluid parameters, which may unintentionally affect larvae viability (LV), and define effective concentrations of common additives, i.e., polyethylene glycol (PEG), quebracho tannins (QT), and ivermectin used in this modified in vitro larvae migration inhibition (LMI) assay. Rumen fluid was collected and pooled from goats (n = 3), mixed with buffer solution and a treatment (1 jar per treatment), and placed into an anaerobic incubator for 20 h. Ensheathed *H. contortus* larvae (<3 mo old) were then anaerobically incubated with treatment rumen and buffer fluid (repeated in 2 to 3 runs; 3 cups/treatment) for either 2, 4, or 16 h depending on the Trial; pH, ammonia N, and VFA were evaluated just before and after larvae were incubated. Larvae were then transferred into a well (n = 4 to 6 wells per treatment cup) containing treatment rumen fluid, within a Multi-Screen 96-well plate and incubated over night; larvae that passed through the 20- μ m screen were considered viable. Data were analyzed using the MIXED procedure with run as the repeated measure and cup (rumen parameters) or well (LV) as the subject. Adding dry or fresh juniper material (representative of 40% of diet DM intake) reduced ($P < 0.05$) pH, ammonia N, and isobutyric, butyric, and isovaleric acids, and increased ($P < 0.001$) acetic, propionic, and total VFA. The PEG concentrations of 0, 0.4, 0.8, 1.4, and 2% w/v quadratically reduced ($P = 0.07$) LV. The QT concentrations of 0, 0.15, 0.6, and 1.2% w/v quadratically reduced ($P < 0.001$) LV; 89.4, 65.5, 22.8, and 9.2%, respectively.

Ivermectin concentrations of 0, 0.05, 0.10, 0.50, 1, 1.5, 2, 3, 6 and 15 μ g/mL quadratically reduced ($P < 0.001$) LV; 90.2, 82.6, 73.6, 66.3, 51.9, 56.5, 43.5, 41.9, 29.3, and 19.9%, respectively. Effects of altering in vitro rumen parameters and the use of PEG on LV needs to be further investigated. Concentrations of QT and ivermectin sufficiently decreased LV; thus, can be used in trials evaluating effects of in vitro rumen treatments on *H. contortus* viability.

Key Words: juniper, secondary compounds, internal parasites

268 Effect of feeding nitarosone medicated ration on the acquisition and development of nematode parasites in the chicken. F. D. Clark*¹, C. A. Tucker¹, J. Reynolds¹, T. A. Yazwinski¹, S. Clark², V. Smith², and K. Dobson², ¹University of Arkansas, Fayetteville, ²Alpharma, Inc, Bridgewater, NJ.

This study was conducted to investigate the anthelmintic efficacies of nitarosone when fed at recommended dietary levels (Histostat at 0.01875%) to artificially infected chickens. Birds were obtained at 1 d of age (experiment d 1), and kept parasite free until artificially infected. Four experimental groups of chickens were established, 2 pens of 24 birds per pen in each group: TRT 1, non-medicated and uninfected; TRT 2, non-medicated and artificially infected on d 14, 21 and 28; TRT 3, medicated from d 42 to d 56 and artificially infected on d 14, 21 and 28; and TRT 4, medicated from d 7 to 56 and artificially infected on d 14, 21 and 28. For each day of scheduled artificial infection, each bird in a pen designated for infection was gavaged with a 1 mL suspension containing 500 *Ascaridia galli*, 500 *Heterakis gallinarum* and 1000 *Capillaria obsignata* eggs that contained infective larvae. Every 7 d from d 35 to 63, 5 fresh droppings were obtained from each pen, combined by pen and homogenized, and processed 3 times by homogenate for egg per gram counts (EPG). Additionally, randomly obtained birds from each pen were necropsied for nematode recoveries and counts on d 42 (3/pen; 6/exp grp) and 63 (12/pen; 24/exp grp). No ascarid eggs were found in any droppings during the study. *Capillaria* eggs were found in droppings from birds of treatment groups 2, 3 and 4 from d 35 to 63, with no quantitative effect of treatment on the EPG counts. *Heterakis* eggs initially appeared in droppings from birds of treatment groups 3 and 4 on d 42, but by d 63, a negative treatment effect was demonstrated for the respective EPG counts ($P < 0.05$). Worm counts performed on birds posted on d 42 and d 63 indicated a lack of treatment effect on total (larval plus adult) worm burdens as *Capillaria*, *Heterakis* or *Ascaridia*. However, for d 63 observations, negative treatment effects were noted for adult *Ascaridia galli* numbers, adult female sizes and the number of fully developed eggs per adult female ($P < 0.05$).

Key Words: nitarosone, chickens, nematode parasitisms

269 Effect of a *Lactobacillus* probiotic and nitrate in feed on *Salmonella* colonization in broiler chicks. A. D. Wolfenden*, N. R. Pumford, M. J. Morgan, S. L. Layton, C. Kremer, G. Tellez, and B. M. Hargis, University of Arkansas, Fayetteville.

In our previous studies, we have found a consistent reduction of *Salmonella enteritidis* (SE) using a lactic acid probiotic culture (B11) in neonatal chicks. In vitro studies suggest that nitrate (NO_3) may potentiate this effect. An in vitro crop assay model was used to evaluate the effect of NO_3 in combination with B11 against SE. B11 with NO_3 at 1000ppm reduced SE by $6.54 \log_{10}$ as compared with non-treated control during a 24h incubation at 40C. Two in vivo studies were then initiated to determine if addition of NO_3 in the feed could further reduce SE colonization in the crop and cecal tonsils of broiler chicks. Briefly, 180 d-of-hatch chicks were randomly assigned to 6 groups: control, B11,

or B11 with NO₃ at 1, 10, 100 or 1000 ppm. All groups were challenged by oral gavage with approximately 10⁴ cfu of SE (n = 30/group). One hour later, all groups except control were treated by oral gavage with approximately 10⁷ cfu of B11. Twenty-four hours and 72 h after treatment, chicks were humanely killed (n = 15/group), and crop and cecal tonsils were cultured for SE. In exp. 1, at 72h there was a significant ($P < 0.05$) decrease in the percent of SE positive crops from the B11+NO₃100ppm (57%) and B11+NO₃10ppm (67%) as compared with the control (93%). A significant reduction in the percent positive cecal tonsils in all groups was observed at 24h, however at 72h, only the B11 (47%) and B11+NO₃100ppm (14%) were different from the control (93%). Exp. 2 was similar to exp. 1 with the following exceptions: chicks assigned to 3 groups (n = 40/group): control, B11, B11+NO₃100ppm. In exp. 2, significant reductions of SE positive crops in both treated groups compared with control were noted: control: 24h 100%, 72h 100%; B11 24h 10%, 72h 65%; B11+NO₃100ppm 24h 60%, 72h 10%. Similar results were noted in the cecal tonsil: control 24h 95%, 72h 95%; B11 24h 10%, 72h 35%; B11+NO₃100ppm 24h 15%, 72h 25%. These experiments indicate that the addition of NO₃ potentiated the effects of the lactic-acid bacteria probiotic in vitro and in vivo. It is postulated that this effect is through increased production of nitric oxide by the beneficial bacteria.

Key Words: *Salmonella*, nitrate, probiotic

270 Effect of food additives on intestinal microflora in caeca of broilers challenged with *Eimeria* species analyzed using 16S rDNA pyrosequencing. A. Nalian*¹, M. Manoharan¹, J. Bray¹, S. Dowd², and A. Martynova-Van Kley¹, ¹Stephen F. Austin State University, Nacogdoches, TX, ²Research and Testing Laboratory, Lubbock, TX

The effect of food additives (cocciostat and natustat) on composition of cecal microflora of *Eimeria* challenged broilers was studied using 454 pyrosequencing of 16S rDNA. We collected a total of 36 cecal samples from pre- and post-challenged birds from 4 treatment groups: natustat, cocciostat, combination of both and control (no treatment). More than 170,000 sequences were obtained from pyrosequencing (~5000 sequences of an average 450 bp length per sample). The taxonomical lineage of the sequences was determined using RDP classifier and BLAST programs. Absolute majority of the sequences belonged to kingdom *Bacteria* and only 4 sequences belonged to kingdom *Archaea*. More than 94% of all the sequences belonged to phylum *Firmicutes* and 5% belonged to phylum *Proteobacteria*. Redundancy analysis of the relative percent abundance data from pre-challenged birds with Monte Carlo permutation test showed that there was no significant difference in microflora composition between different treatments. Whereas in post-challenged birds, we observed significant differences in microflora composition between treatment groups ($P = 0.01$) and also challenged and control groups ($P = 0.03$). In pre-challenged birds, the dominant taxa were *Lachnospiraceae* and *Faecalibacterium*. Generalized additive model showed that cocciostat ($P = 0.001$) and combination of cocciostat with natustat ($P = 0.002$) significantly lowered the number of bacterial taxa in ceca of chickens. In addition, in post-challenged birds, irrespective of treatment groups, the coccidia challenge lowered the relative percent abundance of *Faecalibacterium* taxa ($P = 0.001$). Our results show that pyrosequencing could be used to monitor the changes in microflora due to different treatments. We expect that pyrosequencing will be widely used in poultry for studying microbial communities and their interactions with the host and the environment.

Key Words: *Eimeria*, natustat, pyrosequencing

271 Genetic line and dietary immunomodulator effects on expression of CXCLi2 in chicken heterophils responding to *Salmonella enteritidis*. S. B. Redmond*, P. Chuammitri, D. Palic, C. B. Andreasen, and S. J. Lamont, Iowa State University, Ames.

The performance of leukocytes in the response to pathogens is influenced by genetic background and diet. Dietary immune modulation can alter the mechanisms by which leukocytes, such as chicken heterophils, respond to bacteria. This experiment evaluated the effects of genetic line and dietary immune modulation over time on chicken heterophil expression of interleukin-8 (CXCLi2), an important chemotactic chemokine which recruits leukocytes to the site of an infection. Chickens (n = 64) from highly inbred Leghorn and Fayoumi lines received basal or immune modulating diets enhanced with 0.1% β -glucans, 0.1% ascorbic acid, or 0.01% corticosterone from 8 to 11 weeks of age. Heterophils were isolated from blood samples pooled within line and diet (n = 4 birds/pool) on d 1, 3, 7, and 21 after the start of diet treatments, and then exposed to *Salmonella enteritidis* (SE) bacteria in vitro for 3 h. Heterophil isolates were assayed for CXCLi2 expression mRNA by quantitative RT-PCR. Cycle threshold values were analyzed by ANOVA for the fixed effects of genetic line, diet, and day post treatment, with hatch and plate as random effects. Collection day post treatment was not significant. Leghorn line heterophils expressed significantly higher levels of CXCLi2 than those from the Fayoumi line ($P < 0.01$), suggesting that the Leghorn response to SE relies more heavily on chemotactic signaling. Heterophils from birds fed the corticosterone diet expressed less CXCLi2 than those of birds fed the basal diet ($P < 0.05$), reflecting corticosterone's ability to inhibit immune response. This result suggests that stress-induced corticosterone increases can inhibit the ability of heterophils to produce chemotactic signals. There was no significant difference in CXCLi2 expression by heterophils of birds fed the β -glucans or ascorbic acid enhanced diets, indicating that these immune stimulants likely alter mechanisms other than chemotaxis to enhance immune response.

Key Words: dietary immune modulation, heterophils

272 Nitric oxide synthesis by chicken macrophages results in coordinated changes in the mRNA abundance of multiple arginine transporters. M. Moulds* and B. D. Humphrey, California Polytechnic State University, San Luis Obispo.

Nitric oxide (NO) is synthesized by macrophages when arginine (ARG) is cleaved by inducible nitric oxide synthase (iNOS). In mammals, ARG uptake for NO synthesis is controlled by CAT-2B, but in Aves the requisite system(s) are unknown. Therefore, ARG transporter mRNA abundance was quantified during a NO response from a chicken macrophage (HD-11) cell line and peripheral blood monocytes (PBM). PBM were isolated from Hyline W36 white leghorn hens (n = 3). PBM were cultured in RPMI complete media at 10⁵/well (n = 3) while HD-11 cells were cultured in IMDM complete media at 4 × 10⁵ HD-11 cells/well (n = 6). To induce NO production, cells were cultured with 0 (control) or 1 μ g/ml *E. coli* lipopolysaccharide (LPS) for 48 h. NO was measured indirectly by means of media nitrite concentration. Total RNA was isolated from cultured macrophages and was reverse transcribed for measurement of iNOS and ARG transporter mRNA abundance by quantitative real-time PCR. LPS increased HD-11 nitrite concentration by 7,600% and PBM by 8.7% ($P < 0.05$). LPS also increased HD-11 and PBM iNOS mRNA abundance 8.5-fold and 2.6-fold, respectively ($P < 0.05$). CAT-2B mRNA was undetectable in both HD-11 and PBM cell types. In HD-11 cells, LPS induced CAT-1 and CAT-3 mRNA abundance from undetectable levels, and also increased CAT-2A mRNA abundance 1.6-fold ($P < 0.05$). The exporter y⁺LAT1 mRNA abundance decreased by 71% in HD-11 cells ($P < 0.05$), but no change occurred in

γ^+ LAT2 mRNA abundance ($P > 0.05$). In PBM, LPS increased CAT-1 and CAT-3 mRNA abundance 11-fold and 13-fold, respectively ($P < 0.05$). In LPS treated PBM, mRNA abundance of γ^+ LAT2 decreased 67% ($P < 0.05$), but γ^+ LAT1 mRNA abundance did not change ($P > 0.05$). NO production increased ARG transporter mRNA in HD-11 cells (CAT-1, CAT-2A, CAT-3) and PBM (CAT-1, CAT-3) and decreased ARG exporter mRNA in HD-11 cells (γ^+ LAT1) and PBM (γ^+ LAT2). These data indicate that multiple ARG systems may be involved in ARG uptake for NO production in avian macrophages.

Key Words: arginine, macrophage, nitric oxide

273 Dietary cinnamaldehyde enhances intestinal protective immunity against *Eimeria acervulina*, *E. maxima* and *E. tenella* in broiler chickens. S.-H. Lee*¹, H. Lillehoj¹, S.-I. Jang¹, K.-W. Lee¹, M.-S. Park¹, and D. Bravo², ¹*Animal and Natural Resources Institute, Agricultural Research Service-U.S. Department of Agriculture, Beltsville, MD*, ²*Pancosma S.A., Grand Saconnex, Geneva, Switzerland*.

The protective effect of dietary treatment of cinnamaldehyde on challenge infection with *E. acervulina* (EA), *E. maxima* (EM), or *E. tenella* (ET) (20,000 oocysts/bird) was evaluated in broilers. Three days after hatch, broiler chickens were continuously fed with a standard diet (20/group) or standard diet supplemented with cinnamaldehyde (20/group) for 4 weeks and challenge infection was given at 2 weeks of age. Body weight gains, antibody titers, and cytokine gene expression were measured following oral challenge infection with EA, EM or ET. When body weight gains were measured at 5 and 9 d post infection (dpi), cinnamaldehyde-fed birds showed 10~30% increases over ($P < 0.05$) the untreated birds following challenge infection with EA, EM or ET. Cinnamaldehyde-fed chickens produced 2 folds higher IgY antibody titers ($P < 0.05$) against coccidia at 9 dpi compared with the control group. Finally, the levels of intestinal lymphocyte cytokine transcripts of IL-1 β , IL-6, IL-15, and IFN- γ were 2~5 folds higher in the cinnamaldehyde-fed chickens compared with the controls. This study

provides the first immunological evidence that dietary cinnamaldehyde significantly enhances host innate immunity against coccidiosis.

Key Words: Cinnamaldehyde, immunity, broiler

274 Immune system stimulation and sulfur amino acid intake alter the pathways of glutathione metabolism at transcriptional level in pigs. A. Rakhshandeh*¹, A. Holliss², N. A. Karrow¹, and C. F. M. de Lange¹, ¹*University of Guelph, Department of Animal and Poultry Science*, ²*University of Guelph, Advance Analysis Centre, Guelph, Ontario, Canada*.

The synthesis rate of GSH increases during immune system stimulation (ISS) and is highly dependent on availability of sulfur amino acids (SAA). The expression of key regulatory genes was determined to evaluate the impact of ISS and SAA intake on pathways of GSH metabolism. Restricted-fed barrows (BW 21.5 kg) were allotted to one of 2 levels of SAA intake (1.1 and 3.2, g/d) and injected with either saline (n = 8) or increasing amounts of *Escherichia coli* lipopolysaccharide (n = 16) every 48 h for 7 d. Pigs were then killed to collect liver tissue for total RNA extraction. Liver and an internal standard (KANr) RNA were then reverse transcribed, and expression of selected genes was simultaneously determined by multiplex PCR amplification of cDNA from liver, the housekeeping gene (β -2-microglobulin) and the internal standard in the presence of their corresponding fluorescent labeled primers. The interactive effect (ISS \times SAA) resulted in lower and higher expression, respectively, of GSH synthetase (GSH-S) and GSH reductase (GSR) at the low level of SAA intake in ISS animals ($P < 0.04$). Increased SAA intake decreased expression of GSH-S and GSR but increased expression of GSH peroxidase 3 (GPX3; $P < 0.03$). Expression of glutamate-cysteine ligase modifier (GCLM) was decreased by ISS ($P < 0.01$). However, ISS increased expression of glutamate-cysteine ligase (GCS), GPX1, GPX3 and GSR ($P < 0.4$). No treatment effect on expression of γ -glutamyl hydrolase (GGH) or GPX4 was observed. This study demonstrates that ISS and SAA intake alter GSH metabolism pathways at the transcriptional level in liver of pigs.

Key Words: sulfur amino acids, immune system stimulation, gene expression, multiplex PCR, glutathione