

## SCAD I

**M79 Dietary immunomodulation of gut immunity to enhance disease resistance against mucosal pathogens.** H. S. Lillehoj and S. H. Lee\*, *ARS-USDA, Beltsville, MD.*

For many economically important diseases of poultry, there is an increasing interest to develop drug-free alternative control strategies against many infectious diseases due to increasing consumers' concerns about chemical residues in poultry meat. One promising new avenue to achieve this goal is the use of natural and herbal products, hyperimmune IgY antibodies, oligodeoxynucleotides (ODNs), or probiotics to enhance host defense against microbial infections. Recent studies from our laboratory provided clear evidence that dietary supplements which enhance innate immunity decreased immunopathology associated with infections with pathogenic enteric pathogens. In this report, molecular changes associated with enhanced innate immunity following dietary immunomodulation will be presented using microarray analysis. Alternative prevention and/or treatment measures such as non-chemical dietary supplements that effectively enhance productivity and activate non-specific immunity will help limit the use of antibiotics. However, there is a critical need for more fundamental research to understand poultry immune system and host-pathogen immunobiology in order to develop effective disease prevention strategy.

**Key Words:** passive immunity, immune enhancement, innate immunity, cell-mediated immunity, mucosal pathogens

**M80 Evaluation of clinical and sub-clinical *Eimeria* challenge methods in broilers.** S. Pohl\*<sup>SC1</sup>, J. Lee<sup>1</sup>, S. Anderson<sup>1</sup>, S. Fitz-Coy<sup>2</sup>, L. Oden<sup>1</sup>, A. Klein<sup>1</sup>, and D. Caldwell<sup>1</sup>, <sup>1</sup>Texas A&M University, College Station, <sup>2</sup>Intervet-Schering Plough Animal Health, Millsboro, DE.

The current study was conducted to determine the effectiveness of various *Eimeria* challenge methods in broilers. The main experimental objective was to compare clinical against sub-clinical or natural exposure-based challenge methods. Challenge methods expected to induce clinical infection were performed seven days post-placement and included oral gavage, feed delivery, and direct litter application of field strain *Eimeria*. Sub-clinical or natural exposure methods were performed on day of placement and included a low-level oral gavage and pre-seeded litter application of *Eimeria*. Broilers from clinical challenge groups were subjected to necropsy for gross and microscopic intestinal lesions 14 days and 21 days post-placement. Subclinical or natural exposure challenge groups were necropsied on days nine and 16 post-placement. Oocyst output was determined microscopically by counting shed oocysts per gram of feces collected in all experimental groups beginning six days post-challenge. Of all broilers necropsied in clinical challenge groups, broilers subjected to feed-based *Eimeria* challenge were associated with higher overall gross and microscopic intestinal lesions in all regions on day 14. Litter challenge broilers were associated with the highest overall gross and microscopic lesion scores on day 21. Of the sub-clinical challenge groups, pre-seeded litter challenged broilers were characterized by higher gross lesion scores in the mid-intestine and lower intestine, compared to low-level gavage broilers on days nine and 16. Microscopic lesion scores supported gross lesion scores in sub-clinical challenge groups. Oocyst output in all groups, on all sample days supported lesion scores. These data suggest that various challenge methods can be used under different research settings to more

accurately simulate natural routes of *Eimeria* exposure to broilers within commercial rearing environments.

**Key Words:** *Eimeria*, challenge, coccidiosis, lesion, broilers

**M81 Immunomodulation of the avian gastrointestinal tract with probiotics and coccidiosis vaccine.** M. B. Farnell\*<sup>1</sup>, J. T. Lee<sup>1</sup>, A. E. Klein<sup>1</sup>, K. D. Stringfellow<sup>1</sup>, M. Mohl<sup>2</sup>, R. Beltran<sup>2</sup>, G. Schatzmayr<sup>2</sup>, S. Fitz-Coy<sup>3</sup>, C. Broussard<sup>3</sup>, and D. J. Caldwell<sup>1</sup>, <sup>1</sup>Texas A & M University, Department of Poultry Science, College Station, <sup>2</sup>Biomin GmbH, Herzogenburg, Austria, <sup>3</sup>Schering-Plough Animal Health, Summit, NJ.

The oral administration of probiotics has been demonstrated to improve gut health, reduce incidence of pathogens, increase feed efficiency and stimulate immune function in chickens. The gastrointestinal tract is arguably the largest and most important immune organ; interacting with sizeable numbers of commensal and pathogenic organisms on a continual basis. The hypothesis of this study was that stimulation of the avian mucosa with probiotics (Biomin PoultryStar®, Biomin GmbH) would prime the immune system to better respond to coccidiosis vaccination (Coccivac®-B, Schering-Plough Animal Health). Day-of-hatch, straight-run broilers were placed onto equal parts fresh pine shavings and used litter for 49 days. Treatments consisted of a negative control, a probiotic only treatment, a vaccine only treatment and a combination probiotic vaccine treatment. Four pools of peripheral blood were collected from each group on days 15, 30, 40 and 49. Heterophil and mononuclear cell fractions were each assayed for oxidative burst using a fluorescent substrate and a synthetic agonist. Lymphocyte proliferation was determined using a formazan based colorimetric indicator and a plant derived mitogen. Heterophil oxidative burst was increased ( $P < .05$ ) in each of the treatment groups on days 40 and 49, when compared to the negative control. The probiotic and vaccine combination was the only group with improved ( $P < .05$ ) heterophil oxidative burst on day 15. Monocyte oxidative burst was increased ( $P < .05$ ) in each of the treatment groups on day 15, when compared to the negative control. Increases ( $P < .05$ ) in monocyte oxidative burst were also observed on day 30 with the probiotic group, days 40 and 49 with the vaccine group and day 49 with the combined treatment. Lymphocyte proliferation was greater ( $P < .05$ ) on day 15 with the combination group, day 40 with the vaccine group and day 49 with the probiotic and vaccine groups, when compared to the negative control. These data demonstrate the immune potentiating effects of probiotic bacteria and coccidiosis vaccine on the avian mucosa.

**Key Words:** probiotic, coccidiosis, mucosal immunity, vaccine, heterophil

**M82 In vitro activity of industrial plant by-products against *Eimeria tenella*.** K. Teichmann\*, S. Hummelberger, A. Köstelbauer, and G. Schatzmayr, *BIOMIN Research Center, Tulln, Austria.*

Coccidiosis is one of the most important diseases in poultry farming worldwide with high impact on production economy and animal health. Outbreaks in broiler flocks are mainly controlled by preventive use of

anticoagulant drugs. Frequent emergence of parasite resistances implies the need of effective and cost-efficient alternatives.

The EU-funded research project SAFEWASTES (<http://www.safe-wastes.info>) had the aim of investigating organic waste material and by-products from food-processing and the pharmaceutical industry. These materials are assumed to still contain various interesting and valuable compounds which could justify their use as feed additives.

In this study, their effects on different stages of *in vitro* development of *Eimeria tenella* were investigated by a set of bioassays: a sporozoite vitality assay (SVA) measured parasite vitality after incubation with test compounds, an invasion inhibition assay (IIA) detected effects on the invasion of animal host cells and a merozoite production assay (MPA) measured the production of first generation merozoites. MDBK (Madin Darby bovine kidney) cells were used as animal host cells for the parasites.

Of 16 tested samples, eight showed activity in the MPA, four of which also inhibited host cell invasion (IIA), while one of them also showed direct effects against free sporozoites (SVA). A heptane extract of willow bark (*Salix alba*) exerted >50 % parasite inhibition in each assay in concentrations ranging from 125 - 500 ppm. Monensin as a positive control exhibited reproducible anticoagulant activity in a range of 25 - 100 ppb. It is concluded that the assays provided valid information on the activity of plant extracts.

More testing is necessary with respect to elucidation of the active principle(s) of extracts. Furthermore, *in vivo* challenge trials will have to prove that the applied methods provided transferrable results.

**Key Words:** coccidiosis, *Eimeria*, SAFEWASTES, plants, *in vitro*

**M83 Quantitative real-time PCR assay for *Clostridium septicum* in poultry gangrenous dermatitis associated samples.** A. P. Neumann\*, S. M. Dunham, T. G. Rehberger, and G. R. Siragusa, *Agtech Products, Inc., Waukesha, WI.*

*Clostridium septicum* is a spore-forming anaerobe frequently implicated in cases of gangrenous dermatitis (GD) and other spontaneously occurring clostridial infections of poultry. Virulence of the organism is primarily attributable to the secretion of a lethal, pore-forming cytotoxin designated as alpha toxin. *C. septicum* can be readily cultured from diseased tissues but is difficult to isolate and is often overgrown by more predominant organisms on nonselective media typically used for its cultivation. Here we developed and validated a quantitative real-time PCR assay in order to more accurately evaluate the levels of *C. septicum* in healthy as well as GD poultry samples. The assay was specifically designed to target the *C. septicum* alpha toxin gene, *csa*, which is to our knowledge carried by all strains of *C. septicum* and has been shown to be essential for virulence. Genomic DNAs from 22 bacterial species other than *C. septicum*, including the closely related species of *C. chauvoei*, *C. carnis*, *C. tertium* and *C. perfringens*, all failed to produce a positive reaction using this assay. A diverse collection of twelve poultry *C. septicum* strains and the type strain, ATCC 12464, all produced a positive signal. The sensitivity of the assay was determined to be approximately 10fg of genomic *C. septicum* DNA. Standard curves were generated by spiking extracts from conventionally raised 6 week old broiler GI tracts, livers and breast muscles as well as poultry litter

with known quantities of *C. septicum*. Approximately 10<sup>3</sup>cfu/g of *C. septicum* was consistently detected in spiked GI tract, liver and litter samples. Surprisingly, a detection limit for spiked breast muscle samples could not be determined due to apparent background levels of resident *C. septicum* populations in the two muscle samples tested. This assay will serve as an important tool in the continuing research effort aimed at gaining a better understanding of the nature of *C. septicum* and how this organism contributes to the development of GD.

**Key Words:** gangrenous dermatitis, *Clostridium septicum*, quantitative PCR

**M84 Comparison of physiological responses of broilers during depopulation using water based foam, carbon dioxide gas and argon-carbon dioxide gas mixture.** K. J. Johnson\*<sup>SC</sup>, E. R. Benson, R. L. Alphin, M. K. Rankin, and G. W. Malone, *University of Delaware, Newark.*

This research was conducted to measure the differences in physiological responses of broiler chickens during three methods for emergency mass depopulation. The depopulation methods tested were carbon dioxide (CO<sub>2</sub>) gas, argon-carbon dioxide (70% to 30% mixture) gas and water based foam. Physiologic responses to each of the three methods were quantified using electrocardiogram (ECG), electroencephalogram (EEG), and motion sensing equipment. Each broiler was placed individually into a treatment chamber and exposed to a single depopulation method. Physiological responses and motion of the birds were monitored and recorded for fifteen minutes post-treatment application. The mean time to EEG silence for broilers treated with foam was 161 seconds. Mean EEG silence for CO<sub>2</sub> gas was 158 seconds and EEG silence for Ar-CO<sub>2</sub> was 224 seconds. Mean times of ECG suppression (cardiac relaxation) 142 seconds for foam, 158 seconds for CO<sub>2</sub> gas and 195 seconds for Ar-CO<sub>2</sub> treatments. The times for EEG silence and cardiac relaxation will be independently compared to motion cessation for each depopulation treatment.

**Key Words:** depopulation, foam, accelerometer, electrocardiogram (ECG), electroencephalogram (EEG)

**M85 Comparison of physiological responses of broiler chickens during water based and carbon dioxide foam depopulation.** M. K. Rankin\*<sup>SC</sup>, K. J. Johnson, E. R. Benson, R. L. Alphin, and G. W. Malone, *University of Delaware, Newark.*

The current control strategies for Avian Influenza (AI) and other highly contagious poultry diseases include surveillance, quarantine, depopulation, disposal, and disinfection. The purpose of this experiment was to determine the physiological effects of depopulating broiler chickens with water based foam compared to that of foam infused with carbon dioxide (CO<sub>2</sub>) gas. The effects of the treatment were monitored by the use of an electrocardiogram (ECG), electroencephalogram (EEG) and motion detection instrumentation (accelerometer). The results of this experiment show that when the foam infused with CO<sub>2</sub> is compared directly to the water based foam it was equally effective at depopulating the birds based on the motion cessation and the EEG silence data. EEG silence using the water based foam occurred within 240 seconds with one outlier and in 140 seconds with one outlier using the foam

infused with CO<sub>2</sub> gas. The motion cessation data showed that with the water based foam induced cessation within 240 seconds and within 180 seconds for the foam infused with CO<sub>2</sub> gas.

**Key Words:** depopulation, foam, electrocardiogram (ECG), electroencephalogram (EEG), carbon dioxide

**M86 Scald tank water and foam as sources of carcass contamination during early poultry processing.** K. Liljebjelke\*, K. D. Ingram, A. Hinton, Jr., and J. A. Cason, *USDA/ARS, Russell Research Center, Athens, GA.*

*Salmonella* remains a leading cause of bacterial foodborne illness in the United States, with poultry consumption associated with forty percent of outbreaks for which vehicles are identified. Identifying sources of *Salmonella* contamination and cross-contamination within poultry processing is imperative to developing effective control programs. We enumerated Enterobacteriaceae, coliforms, and *Escherichia coli*, and isolated *Salmonella* from scald tank water, scald tank foam, and defeathered carcasses

obtained from a commercial poultry processing plant during the second processing shift for nine consecutive weeks. Using Biolog GM metabolic fingerprinting, we identified Enterobacteriaceae from these samples to species. Sixty percent of isolates were identified as *E. coli*, with *Vibrio* sp., *Escherichia* sp., *Salmonella* sp., *Aeromonas* sp., *Enterobacter* sp., and *Raoultella* sp. making up the majority of other Enterobacteria identified. *Salmonella* serotypes were isolated from carcass rinsate, water, and foam samples. The total Enterobacteria count from carcass rinsates (n=102) was mean log 3.7 cfu/ml. Total Enterobacteria counts from scald tank water decreased significantly (P<0.05) from the first tank to the third (mean log cfu/ml: 3.4, 2.5, 1.7). Enterobacteria counts from foam samples did not decrease significantly from tank-to-tank (mean log cfu/ml: 2.6, 2.2, 2.1), but contained significantly (P<0.05) fewer cfu than equal volumes of water from the same tanks. Despite high temperatures in the scald tanks (mean 50–53 °C), Enterobacteria survive in the water and in lesser amounts in surface foam. The organic foam layer that builds up on the scald tanks during processing may serve as a source of bacterial contamination during the early steps of poultry processing when carcasses pass through the foam.

**Key Words:** *Salmonella*, *E. coli*, processing, scald water, broilers