

# **ABSTRACTS**

## **Animal Welfare**

### **Evaluating humane methods to euthanize broilers**

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When production animals are sick, injured or immobile, they need to be euthanized promptly, which can be challenging. Manual cervical dislocation (CD) is a common method for euthanizing broilers. Mechanical approaches are an alternative especially for larger poultry, for instance with the Koechner Euthanizing Device (KED). The objective was to compare CD to KED and assess the onset of brain death in broilers. Broilers (n=200; 36, 42, 43 days old) were euthanized by CD or KED during 3 experimental days. After day 1, we added a KED treatment including head extension at ~90° (KED+). The loss of nictitating membrane reflex, gasping reflex and musculoskeletal movements indicated brain death. Additionally, blood loss and the skull-to-atlas gap distance were recorded. The induced nictitating reflex ended after 10 sec for CD, 62 for KED and 60 for KED+ (P <0.001). Gasping ended after 20 sec for CD, 66 for KED, and 62 for KED+ (P<0.001). Musculoskeletal movements ended after 89 sec for CD, 106 for KED and 109 for KED+ (P=0.0004). CD birds had no external blood loss, compared to 80% of KED and 88% of KED+ birds (P<0.0001). CD (1.8cm) and KED+ birds (1.7cm) had a larger skull-to-atlas gap than KED birds (1.1cm; P=0.0006)

Although similar skull-to-atlas gaps were achieved for KED+ and CD, CD resulted in quicker brain death compared to both KED or KED+. Therefore, CD would be recommended for 36-43 day-old broilers. Ongoing work includes developing and evaluating euthanasia methods for broiler breeders, turkeys, and possibly ducks and geese.

# **Environment**

## **LEGAL DEFENSES AND IMPACT ON AGRICULTURAL OPERATIONS**

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Legal Defenses and Impact on Agricultural Operations 2018 has been a year of development in right-to farm laws and potentially limiting how these defenses protect farms in the United States. A right-to-farm law is a nuisance defense law that has been enacted in all 50 states. If a farm meets the requirements in the law, then the farm gains a defense to nuisance claims. Right-to-farm laws were developed in the 1980s by state governments as a way to limit some of the impacts of urbanization in many rural areas. As residents moved in next door to farming operations, many of these new neighbors had limited experience with agriculture and were not expecting the impact living next to a working farm could have on their lives. Right-to-farm laws provide a defense to those qualifying farms who face nuisance suits. Today right-to farm laws have been in the news in the United States more than normal. Producers have seen recent court decisions that potentially limit these laws in some states, decisions that protect the changing nature of American agriculture, and finally state legislatures getting involved to expand these protections. In Alaska, North Carolina, and Iowa, court decisions potentially limit the types of agricultural operations that the right-to-farm law defense would apply too. At the same time, the North Carolina General Assembly acted to change the reach of the court decision in that state. In Pennsylvania and Georgia, courts have interpreted the respective state right-to-farm defenses to protect a changing face of American farms. This poster would fall under the Roles of Government. The poster would highlight recent court decisions and how these wide-ranging of decisions could impact agriculture on a state level in the United States and strategies that states have taken to move these laws to protect the changing face of agriculture.

## **Using Vegetative Buffers to Improve Neighbor Relations –Fan Noise**

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Vegetative buffers, composed of grasses, shrubs and/or trees, are a BMP (best management practice) used to help improve neighbor relations between poultry growers and the general public. Properly placed buffers can improve the “curb appeal” of the farm and can help reduce complaints and screen farms from view. Additionally, properly placed buffers can reduce dust caused by fans, which is of increasing importance as more homes are being built in agricultural areas. Another

benefit of buffers is that they may be able to help reduce noise from exhaust fans. In order to determine how effective buffers are at reducing fan noise, a preliminary study was undertaken. Two farms with established buffers by the fans where used. Fan noise was measured at varying distances from the fans with and without a buffer. Results showed a >20% reduction in horizontal sound energy, demonstrating that when properly designed and maintained, vegetative buffers can help reduce fan noise.

### **The Delaware Waterfowl Tracker**

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The state of Delaware finds itself in the middle of the Atlantic Flyway, posing a problem for stakeholders in the poultry industry (Karesh et al., 2012; Newell et al., 2010). Avian influenza occurs in migratory waterfowl populations and serve as primary vectors of the disease. When migratory waterfowl pass through Delaware via the Atlantic Flyway, Delawarean poultry are at risk of exposure to the virus (Olsen et al., 2006). Supplying stakeholders with locations of waterfowl can help better their surveillance and biosecurity efforts. The Delaware Waterfowl Tracker is an interactive web application that provides maps of the distribution and density of overwintering migratory waterfowl in Delaware and the surrounding region. Radar data are used to map waterfowl distribution by sampling them as they take off for evening feeding flights. Dover (KDOX) Weather Surveillance Radar data was pre-screened for contaminations such as precipitation, clutter, or anomalous propagation within 100 km of the radar using the data archives of the National Oceanic and Atmospheric National Climatic Data Center (NOAA-NCDC). Past data was used to train statistical models in R to predict waterfowl distributions based on environmental and geographic variables (e.g. temperature, cumulative days below freezing, proximity to refuge lands, hunting season) to produce maps of waterfowl distributions in near-real time that are posted in the web app. The app has been developed utilizing the same protocols used to develop the California Waterfowl Tracker, a similar web application developed to produce maps of waterfowl in California.

### **Anaerobic Digestion of Poultry Manure**

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This project is monitoring biogas production, nutrient extraction, and sustainability of a poultry litter-only anaerobic digestion (AD) system built by Planet Found Energy Development, (PFED). Specifically, we are determining energy production potential, nutrient transformations, and environmental impact of using poultry litter as a renewable energy source. A life cycle analysis is tracking the cradle-to-grave effects of AD in reducing greenhouse gas emissions and eutrophication potential and improving human health and ecosystem biota from this innovative technology. We are also testing our patented technology to remove and capture ammonia from the poultry litter AD

effluent. Removing nutrients from the AD effluent results in easier transportation of the extracted nutrients and allows for reuse of the AD effluent. This research will help us understand the effectiveness of ammonia stripping and capture from poultry litter AD effluent and the effects of this technology on transporting poultry litter nutrients and reuse of the poultry litter effluent in the AD process. The effects of nanoparticle (NP) addition to poultry litter digestion is also being tested to determine the most effective concentration of NP additives for increasing biogas production and reducing hydraulic retention time. The effects are being tested both in laboratory digesters and through post-digestion hydroponic lettuce growth to assess the risk of using the bio-slurry for growing crops based on NP degradation during AD and subsequent crop uptake. The results have shown that NP addition improves digestion performance (energy production). Nanoparticle uptake was seen in the lettuce biomass, and therefore, any NP additions should be removed prior to field application

### **Fluidized Bed Combustion of Poultry Manure**

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Poultry litter processing in a full-scale, fluidized bed combustion system built by BHSL is being monitored. This renewable energy technology aims to reduce nutrient runoff, create electricity, provide direct thermal energy for heating poultry houses to reduce propane use, and create an ash to be used as a soil amendment. The poultry litter influent, ash output, bird weight, bird feed and water use, energy production (thermal and electrical), energy use, and air quality were monitored to determine the overall sustainability of this technology. Continuous energy production and biomass use data for the combustion system were quantified, and poultry litter and ash samples were collected monthly for lab analyses over a 16-month period. Data obtained for the six flocks showed that 45.1% of the poultry litter input was combustible, with an average moisture content of 39.2% and an average heating value of 2386 cal/g. The average C:N ratio was 7.8:1. Total energy production was 871.1 MWh, with 858.6 MWh in the form of heat and 12.5 MWh in the form of electricity. Efficiency of converting the poultry litter to energy was 55.3%, with an average conversion rate of 1,534 kWh/ton of biomass. The process converted 567 tons of poultry litter into 35.8 tons of ash. A life cycle analysis is tracking the cradle-to-grave effects of combusting the poultry litter in reducing greenhouse gas emissions and eutrophication potential and improving human health and ecosystem biota from this innovative technology.

# **Food Safety and Processing**

## **The Effect of the Life Cycle Phase on the Proteomic and Transcriptomic Profiles of Salmonella Typhimurium in Brain Heart Infusion and Ground Chicken Extract**

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Salmonella Typhimurium can survive long-term starvation and harsh conditions; however, adequate information is not available on the molecular mechanisms underlying its survival and persistence in poultry environments. In this study, gene and protein expression profiles of *S. Typhimurium* in Brain Heart Infusion (BHI) and Ground Chicken Extract (GCE) were investigated to identify potential intervention targets. Protein expression of Salmonella in early stationary phase (ESP, 24 h) to early death phase (EDP; 720 h) and in ESP and EDP log phase cells (after reinoculation) in BHI and GCE were compared. Additionally, gene expression based on RNA-Seq data on ESP and EDP log phase cells after reinoculation in BHI and GCE was investigated. The expression of 581 genes (98 up-regulated, 483 down-regulated) from cells in EDP (7.5h in BHI) and 398 genes (138 up-regulated, 260 down-regulated) in EDP (7.5h in GCE) was measured. Despite limited overlap between RNA-Seq and proteomics data; together the results provided information for understanding the adaptations of Salmonella in GCE. In the EDP, expression of heat shock response, DNA protection, and catalase-peroxidase genes may have played a vital role in protecting it from starvation and harsh conditions. In GCE, acid shock response and the tripartite efflux system genes were up-regulated possibly aiding in its survivability. Thus, RNA-Seq and proteomics data can be used in combination to provide a comprehensive analysis of Salmonella survival under different conditions. The information may be useful for identification of genes that can be employed as potential targets for interventions to control this bacterium.

## **Prevalence of Shiga Toxin-Producing Escherichia coli within Nontraditional Irrigation Water Sources on the Delmarva Peninsula**

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The presence of microbial pathogens in irrigation water has increasingly become a public health concern; as a source of contamination for fruits and vegetables. Six sites comprising of tidal, non-tidal, and reclaimed water sources were periodically sampled and analyzed for the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) from October 2016 to November 2017. A sampling apparatus containing a modified Moore swab was used to collect the samples. The swabs were enriched with Universal Pre-enrichment Broth. This enriched broth was added to R&F *E. coli* Enrichment broth, incubated, and then streaked on both R&F Non-O157 STEC Chromogenic

plating medium and CHROMagar O157. A total of 1046 presumptive STEC isolates were tested for the “Big 7” serogroups and four virulence factors using an 11-plex traditional PCR. The non-tidal sites had 40 positive samples (40/162), the tidal site had 11 positive samples (11/54), and the reclaimed water sites had 6 positive samples (6/66). Overall, the majority of the positive samples were positive for the virulence *eae* (44), followed by *stx2* (7). No samples were positive for *E. coli* O157:H7. The 76 *eae* isolates were further tested for enteropathogenic *E. coli* (EPEC) and *Escherichia albertii*. All were determined to be atypical EPEC. From the water samples, 2.5% (7/282) contained an STEC isolate, while 15.6% (44/282) contained an atypical EPEC isolate. These results indicate a low prevalence of STEC in nontraditional irrigation water sources on Delmarva. The outcomes of our study will be used to develop and implement next-generation on-farm water treatment technologies.

### **Influence of Poultry Based-Biological Soil Amendments in Pathogen Persistence and Transfer Dynamics in a Pre-harvest Environment**

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Poultry-based biological soil amendments of animal origin (BSAAO) provide nutrients to soil but may contribute to contamination; their use has been questioned following implementation of the FDA-Produce Safety Rule. BSAAO type and soil parameters influence pathogen survival in soils and transfer to raw agricultural commodities. This study aimed to evaluate poultry-based BSAAOs for *Escherichia coli* survival dynamics in soils and transfer to cucumbers along with soil extrinsic and intrinsic-factors. Twenty-plots (3m<sup>2</sup>) were amended with: poultry litter (PL), heat-treated poultry litter pellets (HTPP), composted PL (CPL), or unamended inorganic fertilizer (UN). Each plot was spray-inoculated with *E. coli* TVS355 (1L, 6logCFU/ml). Cucumbers (Supremo) were planted 5 days post-inoculation (dpi). Composite soil samples (n=234) were collected every 10 days up to 120dpi, with cucumbers upon maturation (n=160), to determine *E. coli* populations, *Salmonella* spp. presence/absence, extrinsic (temperature, moisture) and intrinsic (conductivity, soluble-carbon) factors. Results showed significant ( $P < 0.05$ ) decrease in *E. coli* populations (4.7 to  $< 0.4$ logMPN/g), soil conductivity (1.5 to 0.1mmhos/cm), and soluble-carbon (1984.4 to 103.2mg/kg) between 0dpi and 120dpi across amendments. At 60dpi, PL and HTPP plots had higher *E. coli* populations (2.4-5.3logCFU/g) along with soluble-carbon (171.3-175.6mg/kg) compared to CPL and UN ( $< 0.4$ logMPN/g) with lower soluble-carbon (76.8-95.3mg/kg), suggesting soluble-carbon could be critical for bacterial survival in soil. Although UN-plots had lower bacterial populations than PL and HTPP, supported significantly ( $P < 0.05$ ) greater *E. coli* transfer to cucumbers (1.7logMPN/g) at 60dpi. *Salmonella* spp. was confirmed in HTPP-treated plots. BSAAO type affected the duration of *E. coli* survival in amended soils, with lowest populations in CPL-plots; however, longer survival did not correlate with greater transfer to cucumbers.

## **Mitigating Effects of Early Heat Conditioning against Heat Stress in Growth, Oxidative stress, and Breast Meat Quality of Broilers**

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Heat stress is a major cause of the loss of broiler productivity and meat quality and its intensity has drastically increased due to climate changes. Early heat conditioning (EHC) is a technique taking advantage of chick's perinatal epigenetic temperature adaptation ability to enhance heat resistance of broilers. This study was aimed to determine effects of EHC against chronic (C-HS) and acute heat stress (A-HS) during the finishing period on growth performance, oxidative stress, and breast meat quality of broilers. male broiler chicks (180) were randomly allotted to 5 groups and raised for 42 days; Two EHC groups were exposed, at Day 3, to EHC at 36 °C for 12h, then subjected to C-HS (cyclic temperature control at 34/27 °C for the last one week) and A-HS (at 36 °C for 2h at Day 41), respectively. Two noEHC groups were subjected to both heat challenges as negative controls, respectively, and one group was Control without EHC and HS. Growth performance, plasma oxidative stress markers, and quality parameters of breast meat were determined. Growth performance was adversely affected by both HS, but EHC alleviated their effects. EHC and/or HS did not change plasma oxidative stress markers and meat quality parameters. Protein oxidation and antioxidant capacity in meat were degraded by both HS but more severely by A-HC, and EHC reduced the extent of the damage. The results suggested that EHC can mitigate HS effects against broiler growth performance and oxidative stability of breast meat, leading to possibly reducing meat quality defects.

## **Effects of strain and stocking density on broiler carcass yield and meat quality**

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Consumer concern for fast growth on the welfare and meat quality of broiler chickens has increased interest in products from slower growing broilers raised at lower stocking densities. However, this will lower chicken production and the relationship between stocking density and indicators of broiler meat quality remains unclear. Our study used broilers from strains selected to reach market weight (2.8 kg) at 42 days (CONV) and at 63 days (SG) and birds were exclusively raised in 16 pens at stocking densities of either 29 kg/m<sup>2</sup> or 37 kg/m<sup>2</sup>. Birds were provided the same starter, grower and finisher diets with phase changes occurring when SG birds matched CONV birds' body weight. At their respective market weights, birds were processed and the live weight, carcass weight and part weights of the wings, quarters, breasts, tenders and rack were recorded to calculate carcass and part yield. The color, cook loss, and shear force of breasts and thighs were measured and recorded. There was a strain effect on yield and meat quality. CONV birds had 3.3%, 13.0%, and 2.8% higher carcass, breast and tender yield ( $P \leq 0.002$ ), respectively, while SG had 2.7%, 5.0%, and 7.5% higher wing, quarter and rack yield ( $P < 0.0001$ ). SG thigh meat had higher L\* (lightness) and a\* (redness) values ( $P \leq 0.002$ ). SG breast meat had higher L\* values and CONV had higher b\* (yellowness) values ( $P < 0.001$ ). These results indicate differences in the yield and meat quality of broilers from two divergent growth strains.

# **Live Production**

## **Improvement of Radiant Heater Efficiency by Cleaning Reflector**

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A comfortable thermal environment for young chicks during brooding is critical to the overall performance of a broiler chicken. Warm litter or bedding material on the floor is critical to helping the baby chick maintain its body temperature the first few days after hatch. Radiant heaters do a much better job warming the floor than do forced-air heaters. The radiant heat reflectors direct heat and energy from the radiant tube, down to the floor. The design and cleanness of the reflector is critical to deliver as much of the energy from the heated tube to the floor level. The efficiency of the radiant heater with a dirty reflector decreases when the dirty reflector reflects less heat and absorbs more heat. To achieve maximum radiant efficiency the underside as well as the top of the reflector typically should be thoroughly cleaned twice a year. A study was conducted to demonstrate the effectiveness of cleaning reflector on the improvement of radiant heater efficiency and to compare litter floor temperatures in broiler houses before and after cleaning radiant tube heater reflectors. Three tube heaters in two broiler houses were tested by using an infrared camera. The reflectors were removed from the radiant heaters. An organic based foaming cleaner was used to clean the reflectors and rinsed off with clean water. The results showed that cleaning the reflectors of radiant heater significantly improved heater efficiency by raising floor surface temperature after cleaning ( $P < 0.001$ ). The average surface temperature increased by 2.5 to 5.4 oF.

## **Measuring Extension's Impact: Poultry**

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Commercial poultry growers face a mix of challenges and must make decisions daily that affect the farm, birds and business. Delmarva poultry farmers face some of the most stringent regulations in the United States. In order to support commercial poultry farms, the University of Maryland Extension has created a team of educators and specialists to deliver newsletters, factsheets, workshops, discussion groups, and field days. These programs have evolved over the last ten years to include everything from traditional extension hands-on education, to addressing timely critical issues through multiple media outlets. In order to evaluate the impact of these outreach efforts, a survey was created in 2018 to measure knowledge increase, farm implementation, and educational needs. The survey had a response rate of 27%, with 55% of respondents being poultry growers residing in all three states located on Delmarva. Most growers (95%) found programing beneficial to their farm operation. Additionally, 82% of growers reported improved compliance with government regulations as a result of programing. Other benefits growers reported include: improved biosecurity (75%), better farm safety (61%), and improved animal welfare (43%). Benefits to non-farmers included: better understanding of the poultry industry (67%), better understanding of everyday challenges producers face (63%), better able to serve clientele (59%), better understanding of biosecurity (59%), and helped producers implement Best Management

Practices (43%). This survey shows the importance of effective programing by extension professionals to clientele and using the extension service is a good way to assist farmers in adapting to an ever-changing world.

**Effect of manganese preconditioning and replacing inorganic manganese with organic manganese on performance of male broiler chicks.**

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The effects of manganese (Mn) preconditioning, 96 h post-hatch followed by the replacement of inorganic Mn with different levels of organic Mn (5 to 21 D), on growth, tissue and excreta Mn content were evaluated. A total of 420-day-old male Cobb 500 broilers were divided into 2 groups. One group was fed a corn–soybean meal basal diet containing 17 mg of Mn/kg (preconditioning diet, MnPD); the second group was fed the non-preconditioning diet (NPCD), which was the MnPD supplemented with 60 mg of Mn/kg from manganese sulfate (MnSO<sub>4</sub>). On day 5, each group was divided into 5 subgroups and were randomly assigned to dietary treatments consisting of MnPD alone or MnPD supplemented with 12 or 60 mg Mn/kg Mn as MnSO<sub>4</sub> or Mn proteinate (6 replicate cages of 6 birds). Broiler chicks that were fed the MnPD had lower ( $P \leq 0.05$ ) body weight gain (BWG) and G:F ratio when compared to those that were fed the NPCD at 4 D of age. Birds that were fed MnPD (1 to 4 D) and switched to MnPD supplemented with 60 mg/kg Mn (5 to 21 D) had lower ( $P \leq 0.05$ ) BWG compared to those that were fed NPCD (1 to 4 D) and switched to MnPD supplemented with 60 mg/kg Mn for 21 D. Excreta, tibia ash, liver, and heart Mn levels were increased ( $P \leq 0.05$ ) by supplemental Mn. These results confirmed that feeding marginally deficient Mn diets to broiler chicks post-hatch does affect growth rate and tissue Mn concentration.

# **Poultry Health**

## **Early-life thermal conditioning alleviates effects of heat stress in broilers**

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Two trials were conducted to determine the efficiency of thermal conditioning (TC) on alleviating heat stress (HS) in broilers. Firstly, 900 1-d old male broiler chicks (Ross 708) were brooded in floor pens. At d 3, chicks were weighed and divided into 2 pens in each of 3 rooms. Half of the chicks were subjected to TC (37.8oC) for 24h while the remaining half were not TC (NTC) (33oC). On d 24, chicks were allocated into 4 grower batteries. On d 31, half of the birds were HS (35oC) for 8h, half were not (22oC). During HS, birds were euthanized, and blood collected and analyzed for circulating metabolites. Subsequently, plasma was obtained and assayed for levels of T3, T4, and corticosterone. Secondly, 1,440 1-d old chicks were placed in 12 floor pens for TC. On d 3 half of the birds were TC for 24h. At 4 d, chicks were distributed into 60 pens in the broiler house. Blood samples were collected at d 13, 29 and 35 for plasma corticosterone, T3 and T4 analysis. On d 31, all the birds were subjected to HS for 8h. Data were analyzed using a mixed model ANOVA (SAS v9.4, Cary NC). In the battery trial, TC birds had a lower (p<0.05) FCR, plasma glucose and corticosterone compared to NTC birds. In both studies, NTC birds had a higher (p<0.05) mortality rate than TC birds.

## **University of Delaware Avian Bioscience Center International Poultry Health Training Certificate Programs**

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Helping other countries control avian influenza and virulent Newcastle disease in-country helps reduce the risk of these diseases reaching the US. The University of Delaware Avian Biosciences Center, in collaboration with USDA, have developed three certificate programs. Emergency Poultry Disease Response (EPDR), is a technically oriented, five-day course concentrating on building the skills and knowledge required to respond to a disease outbreak. Poultry Disease Outbreak Management and Regionalization (PDOMR), is a three-day, policy-oriented course focusing on how the US uses disease management techniques and regionalization to improve international trade. Veterinary Diagnostic Laboratory Quality Assurance Symposium (VDLQA), is an intensive five day technically oriented course that provides an overview of Quality Assurance System requirements, the implementation and upkeep of a Quality Assurance System, document control, creating and revising standard operating procedures, corrective actions, audits and management reviews. All three courses use a slightly different mixture of seminars, discussions, hands-on technology demonstrations, written exercises and site visits. All utilize the “Delaware model,” which emphasizes close cooperation between government, industry and educational institutions to operate laboratories and manage disease outbreaks utilizing best management practices. These programs also seek to improve the educational opportunities for undergraduate and graduate students including program interns in the planning and implementation of the programs, helping to

provide a rich international experience. Since inception, the programs have trained over 235 participants from more than 80 countries.

### **Effects of dietary supplementation of direct feed microbials on performance and intestinal morphology of broiler chickens challenged with coccidiosis**

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Poultry coccidiosis is a major parasitic disease that inflicts significant annual losses to commercial production. With the industry increasingly employing natural feed additives as drug alternatives, our objective was to evaluate the effects of direct feed microbials (DFM) on performance, intestinal lesion scores, and gut morphology of broiler chickens during a coccidiosis challenge. Cobb 500 broiler chicks (n=840) were randomly allocated to 3 treatments (7 floor pens each, 40 birds/pen) including 1) negative control (NC): basal diet without challenge; 2) positive control (PC): basal diet with coccidiosis challenge; and 3) DFM supplemented diet with coccidiosis challenge. At 15 days (d) of age, all birds except the NC group were orally challenged with 10X dose of a commercial live oocyst vaccine. Dietary DFM supplementation significantly reduced d 21 lesion scores in the duodenum and jejunum when compared with the PC group. Body weight gain (BWG) of the DFM birds was significantly higher than that of the challenged birds during d 0-21. The coccidia challenge reduced the cumulative (d 0-42) BWG of the birds regardless of dietary DFM supplementation. No significant differences in FCR were observed among the treatment groups. At d 6 post challenge (d 21), jejunum and ileum villus heights of the DFM birds were significantly higher than birds in the challenge group. Based on these findings, dietary DFM supplementation may help restore broiler performance during the starter and grower period after a coccidiosis challenge by improving intestinal integrity and reducing lesion scores.

### **Controlling necrotic enteritis in broilers in the drug-free era**

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Necrotic enteritis (NE) continues to present major challenges to the poultry industry and the etiologic agent *Clostridium perfringens* is the fourth leading cause of bacterially-induced food-borne illnesses in the US. This research evaluated the effects of various probiotics on performance, intestinal lesion scores, mortality, and RNA expression of tight junction (TJ) proteins during a naturally occurring NE. Two independent experiments involved in-feed supplementation of different commercial probiotics from hatch to 42 days (d) of Cobb 500 male broilers, a group fed the basal diet served as negative control (NC). In both experiments, all birds were challenged one day after placement with a commercial live oocyst coccidia vaccine as a precursor to naturally occurring NE. Performance was measured at the onset of NE (d8) and end of starter (d14), grower (d28), and finisher (d42) periods. On d8, the small intestines of 2-3 birds/pen were examined for NE lesions. In both trials, supplementation of probiotics significantly reduced lesion scores in the duodenum and jejunum compared to NC birds. In addition, mortality was significantly reduced with probiotic supplementation. Also, FCR was significantly improved in the probiotic supplemented groups for the overall experimental period. Expression of TJ proteins was

significantly higher in probiotic-supplemented birds compared to NC. These findings show that supplementation of probiotics during NE challenge helps reduce lesion scores in the duodenum and jejunum. This may partially be by increased expression of the TJ proteins that promote gut integrity as *C. perfringens* toxins disintegrate TJ proteins, increasing the para cellular permeability of the gut.

### **Implications of early antibiotic and probiotic supplementation**

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The global modern poultry industry is facing numerous changes to feeding and supplementation regulations and restrictions. Although these differ greatly across countries and companies, the fastpaced changes in poultry feed supplements are ubiquitous, especially in regard to antibiotics, pro- and pre-biotics. However, few studies investigate the impacts of the use of these products in young, developing birds. In the present study, 1 day old broiler chicks were given water treated with an antibiotic cocktail, probiotic cocktail, or no supplement (control). Treatment water was provided ad libitum for the first week of life and changed daily. Following the treatment week, all birds received untreated water for the remaining 4 weeks of the study. At week 5 of age, tonic immobility (TI) durations were measured in 1 bird per pen, and flight distances from a novel human were measured in all pens (n=12). Body and organ weights were taken. Probiotic-treated birds had reduced TI durations (P=0.1), however, antibiotic-treated birds tended to have a greater heart weight than probiotic-treated birds (P=0.072) and greater spleen weight compared with control birds (P=0.057). Sequencing analysis of the microbiome from fecal samples in this study indicates changes in the gut microflora of the three treatment populations over time. Our data shows that long-lasting behavioral and physiological changes can be achieved by early ingestion of antibiotic and probiotic supplements.

### **Linc-GALMD1 regulates viral gene expression in the chicken**

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A rapidly increasing number of reports on dysregulated long intergenic non-coding RNA (lincRNA) expression across numerous types of cancer indicates that aberrant lincRNA expression may be a major contributor to tumorigenesis. Marek's disease (MD) is a T cell lymphoma of chickens induced by Marek's disease virus (MDV). Although we have investigated the roles of lincRNAs in bursa tissue of MDV-infected chickens in previous studies, the molecular mechanisms of lincRNA functions in T cells remain poorly understood. In the present study, Linc-GALMD1 was identified from CD4<sup>+</sup> T cells and MSB1 cells, and its expression was significantly downregulated in MD-resistant line of birds in response to MDV challenge. Furthermore, loss-of-function experiments indicated that linc-GALMD1 significantly affect the expression of 290 genes in trans. Through integrated analysis of differentially expressed genes (DEGs) induced by MDV and linc-GALMD1, we found that LAC gene expression levels had a positive correlation with the degree of MD infection and could potentially serve as an indicator for clinical diagnosis of MD.

Moreover, an interaction between MDV and linc-GALMD1 was also observed. Accordingly, CEF cells were inoculated with MDV with and without linc-GALMD1 knockdown, and the data showed that linc-GALMD1 could repress MDV gene expression during the course of MDV infection. These findings uncovered a role of linc-GALMD1 as a viral gene regulator, and suggested a function of linc-GALMD1 contributing to tumor suppression by repressing expression of MDV genes and regulating immune responses to MDV infection.

### **Evaluation of a Low-Cost Vehicle Disinfection System**

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Avian influenza and Newcastle disease (NDV) are examples of highly infectious diseases causing significant outbreaks impacting the poultry industry. One way to help prevent these outbreaks is to improve biosecurity. In the Fall of 2016, an engineering student team developed a low-cost, open source and portable vehicle undercarriage decontamination system. The system was improved, and a two-step system was used to evaluate the process. First the coverage of the system was evaluated using litmus paper strips adhered to various locations on the truck exterior and undercarriage and sprayed with a diluted citric acid solution. In the second step, galvanized steel coupons inoculated with NDV were affixed to the truck using magnets and the truck driven through the decontamination system. Three coupon orientations (horizontal, vertical, and complex) were chosen to test the system's ability to target various locations on the truck. One peroxide disinfectant and one detergent were used to determine the differences in decontamination effectiveness between a disinfectant and detergent. The viral material from the steel coupons was pooled by orientation type and inoculated into specific pathogen free eggs. Chorioallantoic fluid was collected from each egg and used in hemagglutination (HA) and hemagglutination-inhibition assays (HI). The horizontal coupon orientation passed both trials with no positive HA results and a neutralizing index above 2.8. The vertical and complex coupon orientations did not pass the disinfectant guidelines, but a decrease in virus titer was observed in both groups. The system was able to achieve approximately a 2 log drop in virus titer.

### **Protection Afforded By Avian Influenza Vaccination Programs Consisting Of A Novel RNA Particle And An Inactivated Avian Influenza Vaccine Against A Highly Pathogenic Avian Influenza Virus Challenge In Layer Chickens Up To 18 Weeks Post Vaccination.**

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The efficacies of an oil adjuvanted-inactivated reverse genetics derived H5 avian influenza virus(AIV) vaccine and an alphavirus replicon RNA particle (RP) AIV vaccine were evaluated in

commercial leghorn chickens. Challenge utilized A/Turkey/MN/12582/2015, an isolate representing the U.S. H5N2 Clade 2.3.4.4 responsible for the 2015 highly pathogenic avian influenza (HPAI) epornitic in commercial poultry the United States. As part of long term, 36-week study, chickens were challenged at seven weeks of age after receiving a single vaccination, at 18 weeks of age after following a vaccine prime-single boost and at 36 weeks of age after a prime-double-boost. All vaccine programs reduced virus oropharyngeal and cloacal shedding and mortality compared to the non-vaccinated control birds however, chickens receiving at least one administration of the RP vaccine generally had diminished viral shed especially from the cloacal swabbing's. A detectable serum antibody response and protection was observed through 18 weeks post vaccination. Our data suggest that in conjunction with a comprehensive eradication, enhanced biosecurity and controlled marketing plan, vaccination programs of commercial layer chickens with novel RP vaccines may represent an important tool for preventing HPAI related mortalities and decreasing viral load during a catastrophic influenza outbreak.

### **Production of cross-protective autogenous bacterin vaccine strains for controlling Escherichia coli infections in poultry**

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*Escherichia coli* is an important opportunist pathogen of poultry, causing significant economic loss. Vaccination has been a moving target for this pathogen because avian pathogenic *E. coli* (APEC) exhibits significant O antigen diversity and protective immunity is O serogroup specific. Despite this antigenic diversity, APEC possess a common core LPS epitope. The O-antigen masks this LPS epitope. An O-antigen deficient APEC was identified that possessed a common core LPS chemotype among poultry *E. coli* isolates screened. This strain was used to formulate a water-oil-water emulsion, bacterin that was used to vaccinate pullet chickens. Chickens were vaccinated with this bacterin; a *Salmonella* water-oil, emulsion bacterin; or *Pasteurella multocida* water-oil-water, emulsion bacterin. Vaccinated chickens were evaluated for gross and histopathological reactivity to the bacterin vaccines, as well as immunological response to various avian *E. coli*, O serogroups and strain types, and *Salmonella*. The *E. coli* killed vaccine produced mild reactivity, comparable to the *P. multocida* bacterin. Chickens immunized with the *E. coli* bacterin produced broadly, cross-reactive, strongly agglutinating antibodies to several *E. coli*, O serogroups and strain types, compared to *Salmonella*-vaccinated group, which produced weak reactivity to few *E. coli* strains. This *E. coli* bacterin is currently being evaluated in the field for its efficacy at reducing disease.

## **Evaluating the Role of Exosomes in Marek's Disease Virus (MDV) Pathogenesis and Vaccine-induced Protection**

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Exosomes are small (50 to 150 nm), membrane-bound vesicles in diameter that are produced by all cell types and are found in all biological fluids at high concentration (10<sup>8</sup> – 10<sup>9</sup>/ml). Exosomes contain surface and transmembrane proteins as well as various RNA species, enzymes, lipids, etc., as cargo. Exosomes mediate their effects on target cells via fusion. In cancer, primary tumors secrete exosomes that target sites of potential metastasis, preparing the tissue microenvironment. During immune responses, exosomes can present antigens via surface MHC-I or -II to antigen-presenting cells with which they fuse. Marek's disease (MD) is a highly-contagious herpesvirus (Marek's disease virus, MDV) -mediated paralytic, immune-suppressive, and oncogenic pathology of chickens. MD is effectively controlled by vaccination either in ovo (broilers) or at day of age (layers, broiler breeders). We examined the transcriptomes and proteomes of exosomes isolated from the serum of MDV-infected, tumor-bearing and vaccinated-and-protected chickens. RNAs (miRNAs, mRNAs, etc.) were mapped to the chicken and MDV genomes and miRNA-predicted targets were mapped to cellular signaling pathways. Our analyses revealed that: (1) tumor-associated exosome (TEX)-borne miRNAs consistently targeted phosphatidylinositol signaling, (2) vaccine-associated exosome (VEX)-borne miRNAs targeted MAP kinase signaling, (3) TEX-borne mRNAs mapped primarily to the oncogene-coding region of the MDV genome, while (4) VEX-borne mRNAs mapped to the entire MDV genome, including most structural genes. In addition, proteomic analysis identified putative biomarkers for immune suppression (TEX) and vaccine-mediated protection (VEX). Our data provide the basis for future experiments to identify key mechanisms of MDV-mediated immune suppression, tumor formation and alternatively, vaccine-mediated protection.

## **A recombinant Newcastle disease virus (NDV) expressing S protein of infectious bronchitis virus (IBV) as a bivalent vaccine against virulent IBV and NDV**

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IBV causes a highly contagious predominantly respiratory and subsidiary renal and reproductive tracts disease in poultry, resulting in substantial economic losses for the poultry industry worldwide. The huge economic losses of disease result from respiratory distress, decreased and poor-quality egg production and carcass weight losses. IBV infects chickens of all ages and breed types. However, compared to adult chickens, the outcomes of infection in young chicks are more severe and more extended. Currently IB in commercial chickens is controlled by the use of live-attenuated and inactivated vaccines. The live vaccines are not safe due to mutation, recombination and reversion to virulence. The inactivated vaccines are not practical in poultry industry due to labor intensive administration method. Therefore, there is a need to develop an alternative IBV vaccine that will not create variant viruses. In this study, we generated recombinant Newcastle

disease viruses' strain LaSota (rNDVs) expressing the S1, S2 and S proteins of IBV using reverse genetics technology. Our results showed that the S protein is the best protective antigen of IBV. Immunization of adult (4-week-old) SPF chickens with the rNDV expressing S protein elicited IBV- specific neutralizing antibodies and provided complete protection against virulent IBV and virulent NDV challenges. We also showed that immunization of young (1-day-old) SPF chicks with rNDV expressing IBV S protein provided protection against virulent IBV and NDV challenges. These results suggest that the rNDV expressing the S protein of IBV is a safe and effective bivalent vaccine candidate for both IBV and NDV.

### **Adaptation of hepatic mitochondrial metabolism and lipogenesis during embryonic to post-hatch transition in chicken**

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Mortality rates are the highest during the first 3-4 days after hatch (0.5 to 3%), and is an economic burden to the broiler industry. During embryonic to post-hatch transition, the chicken embryo derives 90% of the energy from yolk lipid oxidation, and further upregulate lipidsynthesis, immediately post-hatch. We determined the metabolic factors responsible for an efficient post-hatch transition. Fertile chicken eggs ( $64 \pm 3$  g) were incubated at 38°C and 45% relative humidity. At embryonic days (e), e14, e18 and post-hatch days (ph), ph3 and ph7, serum and tissues were collected for metabolic and gene expression analysis. Isolated mitochondrial activity in the liver was determined using U-[<sup>13</sup>C]pyruvate. Serum ketones ( $\mu\text{M} \pm \text{SEM}$ ,  $P < 0.01$ ), were significantly higher during e14 ( $3237 \pm 189$ ) and e18 ( $3944 \pm 503$ ) and reduced after hatch(ph3;  $381 \pm 42$ , ph7;  $322 \pm 60$ ). There was a dramatic increase in hepatic triglycerides ( $\text{mg/g} \pm \text{SEM}$ ,  $P < 0.01$ ) from  $2.3 \pm 0.6$  at e14,  $7.4 \pm 1.2$  at e18 to  $93.6 \pm 11.79$  and  $92 \pm 14$  in ph3 and ph7 chicks respectively ( $p > 0.001$ ). Expression of fat oxidation and lipogenic genes followed trends in ketones and TGs respectively. Higher incorporation of <sup>13</sup>C into the tricarboxylic acid (TCA) cycle metabolites identified higher mitochondrial activity in e18 and ph3. Interestingly, during this transition, the levels of lipotoxic intermediates and inflammatory genes did not increase. These results illustrate optimal coupling between mitochondrial metabolism and lipid accumulation, preventing hepatic oxidative stress. These mechanisms could be targeted to enhance optimal embryonic to neonatal transition and minimize production losses.

**A novel avian paramyxovirus type-3 expressing VP2 protein of Infectious bursal disease virus (IBDV) protects chickens against virulent IBDV challenge**

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Infectious bursal disease (IBD) is an acute, highly contagious, immunosuppressive disease affecting young chickens, resulting in substantial economic losses to the poultry industry worldwide. Although strict hygienic measures and various vaccination strategies have been adopted, IBD still remains as a major problem for poultry industry. Live-attenuated IBDV vaccines are commonly used for the control of IBD. One of the major disadvantages of live-attenuated vaccines is reversion to virulence and generation of new variant viruses. Viral vector vaccines provide an alternative approach to live-attenuated IBDV vaccines. The primary aim of the present study was to engineer recombinant Newcastle disease virus (NDV) strain LaSota and Avian paramyxovirus-3 (APMV-3) strain Netherlands expressing VP2 protein, the immunogenic protein of IBDV and to evaluate their protective efficacies following vaccination of one-day-old specific pathogen free (SPF) chicks. We successfully generated both the recombinant viruses (rLaSota-VP2 P/M & rAPMV-3 VP2 P/M) containing the chicken codon optimized VP2 gene of IBD virus. Our results showed that expression of VP2 protein and in vitro growth characteristics of the recombinant viruses were similar to that of their respective parental virus. Immunization of one-day-old SPF chicks demonstrated that rAPMV-3 expressing VP2 protein elicited IBDV specific neutralizing antibodies and provided complete protection against IBDV challenge. These results suggest that recombinant APMV-3 expressing VP2 protein can be used as a potential vaccine against IBD.