2020 RESEARCH NEWSLETTER

USPOULTRY®

U.S. POULTRY & EGG ASSOCIATION

The All Feather Association progressively serving poultry and egg members through research, education, communications and technical services.

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2020 USPOULTRY RESEARCH

The U.S. Poultry & Egg Association (USPOULTRY) is the world's largest and most active poultry organization. USPOULTRY represents the entire industry as an "All Feather" Association. USPOULTRY is a nonprofit organization which represents its poultry and egg members through research, education, communication, and technical assistance. Membership includes producers and processors of broilers, turkeys, ducks, eggs, and breeding stock, as well as allied companies. Formed in 1947, the Association has member companies nationwide and affiliations in 28 states. USPOULTRY also sponsors the International Poultry Expo, part of the International Production & Processing Expo.

Send Comments to:
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USPOULTRY and Foundation Approve $750,000 in New Research Grants through Comprehensive Research Program

USPOULTRY and the USPOULTRY Foundation have approved $750,000 for twelve new research grants at eight institutions through the comprehensive research program. The research funding was approved by the boards of directors of both organizations, based on recommendations from the Foundation Research Advisory Committee. The committee evaluates research proposals to determine their value to the industry and then makes recommendations to the boards for funding. Committee members are professional specialists from different segments of the poultry and egg industry who represent a variety of disciplines.

The research grants for each institution include:

The Use of Accelerometers and Artificial Intelligence to Predict Presence of Woody Breast in Live Broilers Throughout Growout and in Broiler Fillets
University of Arkansas (research grant made possible in part by an endowing Foundation gift from George's Inc.)

Automated Tracking of Laying Hens in Cage-Free Aviary Environment Using Affordable RadioFrequency Identification (RFID) Chips
North Carolina State University

Pathogenicity and Genetic Profile of Turkey Hepatitis Reovirus (THRV)
University of Minnesota (research grant made possible in part by an endowing Foundation gift from the Cooper Family Foundation)

Comparative Genomics and In Vitro Screening Approach for the Identification of Vaccine Candidates for Food-Borne Pathogen *Campylobacter jejuni*
Mississippi State University (research grant made possible in part by an endowing Foundation gift from Peco Foods)

Effectiveness of Various Compounds in the Sanitation of Shell Egg Processing Equipment and Facility Surfaces
USDA ARS (research grant made possible in part by an endowing Foundation gift from MPS Egg Farms)

Using Electrostatic Precipitator to Improve Indoor Air Quality in Cage-Free Layer Houses
North Carolina State University (research grant made possible in part by an endowing Foundation gift from Cal-Maine Foods)

Poultry-Safe and Environment-Acceptable Pest-Repellent Paint
University of Kansas (research grant made possible in part by an endowing Foundation gift from Wayne Farms)

Role of Early Incubation Temperature Variation in the Development of the Wooden Breast Myopathy in Broiler Chickens
Auburn University (research grant made possible in part by an endowing Foundation gift from Koch Foods)

Assessing the Impact of Feed Supplements on Selection of Avian Pathogenic *Escherichia coli* (APEC)
University of Georgia (research grant made possible in part by an endowing Foundation gift from MarJac Poultry)

Role of Ratios of Limestone Particle Size and Phytase to Support Late Lay in Single Cycled Laying Hens – Focus on Eggshell Quality
Mississippi State University (research grant made possible in part by an endowing Foundation gift from Centurion Poultry)

Enhancing Immunogenicity and Protective Efficacy of Recombinant Infectious Laryngotracheitis (ILT) Vaccines in Broiler Chickens
North Carolina State University (research grant made possible in part by an endowing Foundation gift from Mountaire Corporation)

High-Resolution Salmonella Serotyping to Improve Surveillance in Turkeys
University of Georgia (research grant made possible in part by an endowing Foundation gift from Cargill)

USPOULTRY and Foundation Approve $230,000 in New Board Research Initiative Grants

USPOULTRY and the USPOULTRY Foundation approved approximately $230,000 in funding for two new research grants at two institutions through the Board Research Initiative program. The research funding was approved by the boards of directors of both organizations, based on recommendations from the Foundation Research Advisory Committee.

The research was made possible in part by donations to the USPOULTRY Foundation. The donations were from a wide range of poultry and egg companies, individuals and families to support the Foundation's mission of funding industry research and recruiting students into poultry careers.

The research grants for each institution include:

Methods for Preventing Blackhead Disease in Poultry
North Carolina State University (research grant made possible in part by an endowing Foundation gift from Prestage Farms)

Effect of Variable Light Intensity Program on Broiler Gait Score, Stress and Central Positive Welfare in Commercial Broiler Farm
University of Arkansas (research grant made possible in part by an endowing Foundation gift from Simmons Foods)

Research Examines the Impacts of Cage-Free Layer Housing

Dr. Darrin M. Karcher

Purdue University, West Lafayette, Indiana

Research has shown that Salmonella persistence and transmission in poultry flocks can be influenced by various housing facility characteristics and flock management practices. However, the impact that different housing system options for egg-laying hens have on food safety remains unresolved. Further, the appearance of ectoparasites in cage-free housing systems has created challenges that impact bird welfare and egg quality. One of the challenges for cage-free production is the control of northern fowl mites. The impact of northern fowl mite infestation on Salmonella shed is not known.

The objectives of these projects were 1) to assess horizontal transmission of infection and invasion of internal organs after experimental *S.* Enteritidis and *S.* Kentucky infection of laying hens in indoor cage-free housing, and 2) to examine the role and impact of northern fowl mites (NFM) on laying hen performance, welfare and egg safety in a cage-free environment.

The results of this study for Objective 1 suggest that Salmonella infection can spread rapidly and extensively among hens in cage-free indoor housing, including a high frequency of internal organ involvement for invasive serovars such as *S.* Enteritidis. Vaccination reduced but did not prevent either internal organ colonization or horizontal transmission of infection. This confirms prior research which established that Salmonella vaccination can be a useful tool within comprehensive risk reduction programs but may not unilaterally provide complete protection against pathogen infection.

The differing results of this study for Objective 2 (Experiments 1 and 2) indicate that management decisions will have a dramatic impact on the ability of northern fowl mites to establish within a cage-free flock. The intact beak of pullets can play a role in mitigating ectoparasites in cage-free environments, yet, contributes to the exacerbation of cannibalism and large amounts of feather loss. The reduction in egg production and decrease in feather coverage may translate to decreased product for sale and an increase in cost as extra heat may need to be provided to account for the loss of feather cover. Moreover, pullets with trimmed beaks allow the northern fowl mite population to increase over the course of their production, resulting in a long-term infestation that can cause depressed egg production, influence egg quality measures and impact overall hen welfare.

In summary, these projects uncovered some of the challenges with cage-free laying hens and the long-term financial impacts that a company may experience.

The research was made possible in part by endowing Foundation gifts from Cal-Maine Foods and Centurion Poultry.

Research Examines Novel Next Generation Sequencing Techniques

Dr. Naola Ferguson-Noel

University of Georgia, Athens, Georgia

In order to quickly identify and control poultry respiratory diseases, a single, rapid and affordable test is advantageous to pinpoint the pathogens involved in multifactorial respiratory infections. Nanopore sequencing is a relatively novel technology that is accessible and cost effective for labs.

The aim of this study was to develop next generation nanopore sequencing techniques that would not only identify relevant respiratory pathogens quickly, but also differentiate between vaccine and field strains as well as subtype when necessary. The specific objectives of this research were 1) to compare this novel sequencing technology with respect to speed, cost and quality of data obtained from tracheal swabs from infected and non-infected chickens; 2) to develop targeted enrichment protocols for avian respiratory pathogens and vaccines; and 3) to compare broad sequencing analysis to pathogen-specific (Mycoplasma, Infectious Laryngotracheitis virus, etc.) targeted enrichment protocols.

To achieve objective 1, the cost and time of sequencing using current diagnostic protocols were compared to the targeted enrichment protocol developed in objective 2. Overall, the turn-around time for results were similar for both protocols assuming the sequencing step was started immediately. However, the cost of the assays differed substantially. The flexibility of being able to have the needed devices and equipment in house as well as implementation of multiplex amplification greatly contributed to the disparity between costs.

The second objective of this research project was achieved by developing a targeted enrichment protocol for detection of Mycoplasma synoviae (MS) and Mycoplasma gallisepticum (MG), with specific designs that would allow additional pathogens to be included in a respiratory panel as research continues. A multiplex of seven targets was able to identify both MS and MG from DNA extracted from cultures. Added improvements in the speed and accuracy of the bioinformatic workflow will contribute to the ability of implementing this assay in a diagnostic setting, where rapid turnaround time of reliable results is of great importance.

Broad sequencing analysis was found to be either ineffective or too time consuming for use in rapid diagnostics.

Overall, the novel nanopore sequencing assay developed in this study exhibits three vitally important factors for success of diagnostic assays: low cost, fast turn-around time and high accuracy. Further work on streamlining library preparation and the bioinformatic workflow, as well as developing additional targets for viral respiratory pathogens that can be used flexibly, would allow this assay to be used effectively as a respiratory screening panel.

The research was made possible in part by endowing Foundation gifts from Elton and Claire Maddox and the Georgia Poultry Federation.

Researchers Examine the Role of APEC in Turkey Cellulitis

Dr. Catherine Logue

University of Georgia, Athens, Georgia

Colibacillosis is considered one of the leading bacterial causes of economic loss in the turkey industry worldwide. Turkey cellulitis is also among the top health issues in turkey producers nationally, ranking as #3 in 2019 according to the United States Animal Health Association turkey industry survey. Avian pathogenic Escherichia coli (APEC) is one of the pathogens implicated in the disease after Clostridium spp. Cellulitis (also known as clostridial dermatitis) is characterized by locally extensive inflammation of subcutaneous tissues of the inguinal, tail and/or breast regions, often striking production toms at or near market age, resulting in increased mortality and carcass condemnation at slaughter and leading to multi-million dollar losses for the turkey industry.

In turkey production, losses as a result of cellulitis associated with Clostridium species are known. However there is considerably limited information as to the role of E. coli in cellulitis-associated disease in the Midwest, where turkey production is greatest and potential risk for losses are high. This project sought to assess the potential impact of the microbiome of the turkey production environment (including litter) and a Clostridium cellulitis project to assess the role of litter and its quality on E. coli-associated cellulitis in turkey production in Iowa.

Some of the key objectives included in the study aimed to: 1) identify bacterial species associated with cellulitis in turkeys and detect the colonizing species of poultry litter; 2) compare E. coli recovered from cellulitis cases with strains recovered from poultry litter; 3) identify management factors that affect the prevalence of APEC and colibacillosis; and 4) identify potential effective prophylactic measures that do not employ antimicrobial agents to reduce carcass condemnation rates.

Comparative analysis of APEC from tissues with strains in litter found that the majority of APEC were well defined pathogens capable of causing disease. A total of 333 isolates were assessed for the presence of virulence and antimicrobial resistance associated genes. Strains of E. coli detected were found to harbor multiple resistance traits including antimicrobial resistance and metals.

Overall data suggest that the primary cause of cellulitis in turkeys is likely still linked with clostridia. However, the role of APEC in the disease process should not be overlooked, as well as how the organism interacts with the host and clostridia present. The detection and presence of APEC in tissues and in litter also presents a second pathogen with a significant role in the disease process and the role of litter as a source of these organisms. Genetic profiles reflect the disease in the birds and dominant flora associated with disease in the litter. Likely, the role of APEC as a gateway organism for clostridia warrants further investigation and how it can synergistically act in the presence of the clostridia. Ongoing analysis beyond this study consists of information gathering of genetic data to determine how the profiles observed are associated with management factors that have the potential to impact poultry health.

The research was made possible in part by an endowing Foundation gift from the Cooper Family Foundation.

Researchers Characterize Selected Variant Avian Reovirus Strains

Dr. Rodrigo A Gallardo

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Avian Reoviruses (ARV) are distributed worldwide in chickens, turkeys and other bird species and are ubiquitous in poultry farms. The detection of reoviruses in cases where pathology is present does not confirm the disease etiology. Confirmation requires rigorous diagnosis and sometimes challenge studies. The inherent variability of the ARV genome makes them mutate and recombine at high rates. Since 2011, the poultry industry has been facing consequences of the emergence of ARV variants. The variants of ARV have been linked to a rise in clinical cases of tenosynovitis in poultry in the United States. Hundreds of clinically relevant avian reoviruses (ARV) associated with a history of leg problems, poor performance and lack of uniformity have been isolated from broiler chickens and their breeders.

To date, the detection of ARV has been performed by focusing on sequencing a specific ARV gene and serotyping. While sequencing of that gene helps understand the epidemiology of ARV variants, it does not provide antigenic and pathological insights. In addition, an increased number of viruses cannot be typed by serological methods because of their variability. A new, fast and more reliable characterization method of ARV is needed to perform more accurate detection and aid in vaccination and disease control in the field. If successful, this tool can reduce the gap between the molecular and clinical understanding of the virus and provide a tool for veterinarians to expand their knowledge.

The objectives of this study were three-fold: (1) characterize isolates of variant reoviruses associated with pathology, (2) select the most relevant viruses for a full molecular characterization using Next Generation Sequencing (NGS), and (3) perform controlled challenge studies in order to pathologically and antigenically characterize selected reoviruses.

Completion of objective 1 provided a better understanding of the diversity of reoviruses and their epidemiology. Findings indicated that ARV are moving targets and that a good virus choice for autogenous vaccine production is crucial.

For objective 2, 53 different ARV isolates were selected, and their full genome sequenced. The sequences made it clear that some virus isolates were a mix of different reoviruses, and some were contaminated with other viruses. Thus, a different strategy is needed to characterize the viruses immunologically and pathogenically. The sequences were used to study the genome of reovirus variants and compare them with classical strains.

The pathobiology of five ARV variants were studied in objective 3, focusing on viral replication in different tissues (heart, tendons, bursa of Fabricious and intestines), horizontal transmission and immunosuppression. The virus was prone to replicate and cause gross and microscopic lesions in tendons and heart. Depending on the variant, the virus could locate more in intestines, heart or tendons. Moreover, different degrees of horizontal transmission presented from absent to highly effective, while it was previously assumed transmission was only horizontal. Finally, it was confirmed that variant strains induce lymphoid depletion in bursa and thymus during the first week of infection. The lymphoid depletion suggested immunosuppression and was caused not only by the variant strains but also by the vaccine strain.

The results of this research project provide valuable knowledge needed to better strategize diagnostics and surveillance for avian reoviruses affecting the broiler industry. These new diagnostic and surveillance methods are currently being implemented. This information may allow one to streamline the selection of ARV variants for the formulation of inactivated autogenous vaccines. Further, this knowledge will possibly provide insights and basic information for new intervention strategies such as the design of new live or recombinant vaccines stimulating all the immune compartments in the chickens and able to protect against reovirus variants.

The research was made possible in part by endowing Foundation gifts from Jerry and Cherie Moye and Todd and Shelley Simmons.

Researchers Explore Nutritional Strategies to Reduce the Incidence and Severity of Wooden Breast

Dr. Sandra G. Velleman

The Ohio State University, Wooster, Ohio

Wooden breast costs the United States poultry industry more than $200 million each year due to reduced processing yields and product loss. The cause of wooden breast remains unknown, and there are few effective management strategies to reduce the myopathy without sacrificing breast yield. The objective of the proposed research was to increase dietary omega-3 fatty acids and antioxidants to reduce the incidence and severity of wooden breast. Since wooden breast muscle is under extreme oxidative stress and inflamed, diets were used to reduce oxidative stress and inflammation after hatch to decrease the incidence and severity of wooden breast. Omega-3 fatty acids and the antioxidant, vitamin E, reduce inflammation and oxidative stress in many tissues and improve gut health and nutrient absorption. The research conducted explored how these dietary components affect the breast muscle and intestinal health when administered immediately after hatch. The first week after hatch is a very critical timepoint for the development of muscle.

Three objectives were undertaken in the study: (1) investigate the anti-inflammatory properties of omega-3 fatty acids to reduce breast muscle inflammation, improve muscle structure and reduce the incidence of wooden breast; (2) determine if increasing dietary vitamin E reduces oxidative stress and wooden breast; and (3) study how supplementing with both omega-3 fatty acids and vitamin E affects the onset of wooden breast.

For objectives 1 and 2, supplementation of vitamin E, omega-3 fatty acids or a combination of both was performed during the starter phase or grower phase. Growth performance, meat yield, meat quality and wooden breast scores were obtained. There was not a significant difference in final body weight and meat yield when vitamin E was increased. Furthermore, supplemental vitamin E reduced the severity of wooden breast when added to the starter diets. In contrast, omega-3 fatty acid supplementation in the starter diets significantly decreased final body weight, hot carcass weight and chilled carcass weight. This data was suggestive that supplementation with vitamin E may reduce the severity of wooden breast and promote breast meat quality without adversely affecting growth performance and meat yield that allows the live chickens to regulate heat by their metabolism and remain comfortable.

To augment the vitamin E effect, objective 3 was modified to include supplementation with α lipoic acid instead of omega-3 fatty acid, which is an antioxidant with anti-inflammatory effects. Immediate post hatch development through 3 weeks of age was the focus, as this is when the muscle pattern is laid down and wooden breast begins to occur. Diets were supplemented with vitamin E and α lipoic acid independently or in combination during the first 3 weeks of age. Supplementation with vitamin E and lipoic acid independently, or in combination at 2 weeks and 3 weeks of age, reduced the severity of wooden breast and expression of genes associated with muscle fiber repair

Results taken together from all the objectives suggest that early diet intervention with antioxidants will decrease the severity of phenotypic wooden breast at market age. In the long-term, studies maximizing the dietary effect will need to be conducted with multiple broiler lines, but if pursued, implementation of a diet with antioxidants could significantly reduce economic losses from wooden breast.

The research was made possible in part by an endowing Foundation gift from Mar-Jac Poultry.

Researchers Evaluate Novel Method to Vaccinate for Infectious Bronchitis Viruses

Dr. Brian Jordan and Dr. Mark Jackwood

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Infectious bronchitis virus (IBV) is an economically important respiratory virus of chickens. Control of the disease caused by IBV is achieved through vaccination with live-attenuated virus strains. However, many serotypes of IBV exist, and there is minimal cross-protection between variants. Disease surveillance and vaccine development is thus a constant, on-going effort to keep up with new and emerging IBV strains. To date, there are dozens of identified serotypes and hundreds of variants circulating in commercial poultry worldwide. Current control strategies rely on isolating a new IBV variant, determining the serotype through correlation with the spike protein genotype, taking the isolated virus and passaging it through embryonated eggs until the virus has become adapted to embryos and therefore less pathogenic to live chickens (typically over 100 passages), performing efficacy studies, and finally, licensing through governmental agencies for use in commercial poultry. This process often takes more than two years to complete, wherein during that time the virus is uncontrolled in poultry flocks causing significant disease and economic losses.

The goal of this research project was to evaluate the potential for pseudotyped vesicular stomatitis virus (VSV) particles carrying IBV spike proteins on their surface to be used as a vaccine for emerging variant IBVs. The objective was to determine if a novel vaccine production method could be used to shorten the variant-to-vaccine timeline and be efficacious at protection against the new IBVs. Since VSV is non-replicating and would therefore act like a killed vaccine, the strategy was to vaccinate broiler chickens with GA08 IBV spike pseudotyped particles alone or in combination with a live Mass-type IBV vaccine and then challenge with pathogenic GA08 IBV to assess the level of protection developed from vaccination.

The GA08 pseudotyped VSV (GA08-pVSV) particles were created in the laboratory. Two initial studies using an inoculation dose of 16 or 32 HA units were given to specific pathogen free (SPF) chicks via intraocular/intranasal routes or via intramuscular (IM) injection. The chicks were then bled to measure antibody levels. The chicks given 32 HA units of GA08-pVSV via the IM injection route showed a small antibody response, but production of GA08-pVSV at 32 HA units was not continually achievable in the laboratory, so an inoculation dose of 16 HA units and IM route was chosen to proceed with the full vaccine challenge experiment.

In the vaccine challenge experiment, multiple groups were included to encompass several vaccine combinations. The GA08-pVSV particles were either given via in-ovo injection, by IM injection at 1 day of age, by IM injection at 14 days of age, or a combination of these timepoints with or without a live Mass-type IBV vaccine. All vaccinated groups (including the control group) were challenged at 28 days of age and were assessed 5 days post challenge for clinical signs, viral load and antibody development. The group that only received GA08-pVSV had higher clinical sign scores, higher viral loads and no antibodies detected post challenge. There was no statistical difference between any of the other groups, all of which received a live Mass-type IBV vaccine. This indicates that any protection seen in any of the groups was due to the Mass-type IBV and not from the GA08-pVSV.

Data showed that the SPF chicks did not immunologically respond to the GA08-pVSV particles. If a higher hemagglutination unit dose of vaccine could have been given, there may have seen a better response. However, this was not achievable due to the limited scalability in the GA08-pVSV production process in the lab. Furthermore, preliminary studies showed that the best (albeit small) response to these particles came when applied via IM injection with adjuvant. This inoculation route would not be feasible for the broiler industry, for which this vaccine was targeted, also making this vaccine candidate non-viable.

The research was made possible in part by an endowing Foundation gift from Fieldale Farms.

Researchers Investigate Broiler Response to Phytase Enzymes

Dr. Kelley Wamsley

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Phytase is an exogenous enzyme that is commonly incorporated into commercial poultry diets to increase the digestibility of phytate phosphorus. This is significant because a large majority of the ingredients used for poultry feed are plant-derived and contain phytate. Phytate hinders the nutritional potential of diets and bird growth performance, leading to unreached maximal economic gain. Phytase inclusion in diets provide broiler production advantages, such as improved growth performance and better nutrient digestibility. Due to the expense associated with feed, it is common for poultry nutritionists to formulate diets on a least-cost basis to maximize profit. However, lower priced ingredients typically contain higher amounts of antinutrients, like phytate.

The objective of this research project was to determine the effects of feeding combinations of phytases with varying optimal pH ranges. The researchers theorized that if combinations of different phytases are more effective than singular use, the nutritive quality of feedstuffs would be enhanced; thereby, improving broiler production, lowering feed costs and reducing environmental excretion of phosphorus. Two experiments were conducted. In experiment 1, broilers were reared in raised wire cages from 0-14 days to investigate the potential synergy of three different phytase enzymes of varying biochemical properties when fed alone or in combination with low phytase activities (120 or 240 FTU/kg). Data obtained demonstrated a potential synergy with the supplementation of two phytases combined at a higher phytase activity level (240 FTU/kg), as identified from ileal IP6 lower ester concentration, increased digestibility (calcium, phosphorus, and select amino acids) and tibia ash (indicating greater phytate degradation). In experiment 2, the two higher performing phytases from experiment 1 were used to address limitations recognized in experiment 1, including: 1) utilizing a broader and more practical range of phytase activity (250 or 1500 FTU/kg); 2) implementing three diets varying in calcium and available phosphorus; and 3) employing an entire grow-out of broilers within experimental floor-pen facilities.

These data demonstrated that feeding diets lowest in calcium and available phosphorus along with 1500 FTU/kg of a single phytase resulted in improved broiler performance, tibia ash (mg/chick and concentration of select minerals) and nutrient digestibility (calcium, phosphorus and select amino acids). This strategy also demonstrated improved thigh weight at processing and indicated greater phytate degradation. Additionally, there was some indication of synergy for the use of combined phytase at 250 FTU/kg within diets of medium calcium and available phosphorus levels (relative to reduced nutrient diets alone); however, performance was not maximized.

Overall, data do not indicate that feeding multiple phytase enzymes will contribute to significant improvements for the commercial broiler industry; however, these data do suggest that more attention towards calcium and available phosphorus ratios can further the efficacy of phytases. Further research on multiple enzyme use in broiler diets is warranted and could provide valuable economic and environmental insight on the strategic use of exogenous enzymes for the commercial poultry industry.

The research was made possible in part by an endowing Foundation gift from Peco Foods.

Researchers Investigate Biological Control Agents to Decrease House Fly Populations

Dr. Erika Machtinger

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Control of house flies (*Musca domestica)* poses a significant challenge to poultry producers, especially those involved in managing layer facilities. Egg production occurs in facilities where the accumulation of manure in an enclosed space creates the perfect development habitat for fostering large numbers of flies. Historically, pest flies have been controlled with chemical insecticides. However, regulatory restrictions, house fly resistance to current active ingredients in commercial pesticides, and the lack of options labeled for pest control in poultry facilities make management of house fly populations difficult.

To control house flies, the poultry industry is estimated to spend $20 million dollars annually on pesticides alone. This estimate does not include the cost of animal loss due to house fly vectored pathogens causing disease, the cost of labor for pesticide application, or litigation that can be taken by residents living near production facilities due to increased fly numbers affecting their property values.

One potential option to control pest flies is the integration of biological control agents into a layer facilities' integrated pest management (IPM) program. Many natural antagonists of the house fly are found within the environment of the manure pit. Augmentative releases of these antagonists and introduction of pathogenic organisms can help lower house fly populations to manageable levels. The most promising of these biological control agents on a commercial level are parasitoid wasps and the entomopathogenic fungi Beauveria *bassiana*. Parasitoid wasps are commercially available and commonly released into poultry systems as a form of biological control, but more research needs to be done to determine which species or combination thereof would be the most effective in different geographic locations. The fungi, *B. bassiana*, has been widely studied as a form of biological control for house flies in layer facilities as well but has not been widely adopted by the industry. Further research into improving its effectiveness and ease of use could change this trend.

The three objectives of this research project were: (1) to collect new fungal isolates from flies in poultry facilities and screen them to identify strains with fast kill times, (2) to test the most promising strains and subject them to selection for further improvements in kill times, and (3) to ensure their compatibility with the most important natural enemies of flies (three species of parasitic wasps and the beetle predator *Carcinops pumilio*).

Objective 1 results included the collection of five new isolates of *B. bassiana* that had mean survival times under eight days, an improvement from currently marketed *B. bassiana* products. For objective 2, researchers identified which strain was consistently the most virulent and produced the highest numbers of conidia on cadavers in fly-to-fly passages. Selection for faster-killing strains shortened the average time until death by three days, from 7.6 to 4.7 days, after nine generations of selection. Final analysis of the selected strains was postponed when the USDA laboratory was closed due of the COVID-19 pandemic. For objective 3, *Spalangia endius* was the most resilient to the *B. bassiana* applications, whereas *Spalangia cameroni* and *Muscidifurax raptor* had decreased survival when *B. bassiana* was applied.

Overall, the *B. bassiana* strains isolated from house flies killed greater numbers of flies than the negative control. In parasitoids, all strains had a more limited effect than was observed in the house flies, except for in *S. endius*, in which there was no effect. The susceptibility of these house flies to the treatments and the lack thereof in all parasitoid species is a good indicator of the usefulness of field collected strains of *B. bassiana* and their use as a biological control tool. Given that the strains each demonstrated different traits in their infection of house flies, further research should be done to see the extent of each of these traits and if they could be useful for biological control.

The research was made possible in part by an endowing Foundation gift from MPS Egg Farms.

Researchers Investigate Novel Method for Enumeration and Characterization of Coccidia in Poultry

Dr. Rocio Crespo

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An accurate, speedy and reliable diagnosis of Eimeria at the species level is both challenging and necessary. Presently, there are many approaches to distinguish between species of these protozoa, including oocyst morphology, pre-patent period and site of infection. However, these methods are labor intensive, time consuming and unreliable, particularly in cases of mixed Eimeria infection. Furthermore, these methods do not easily lend themselves to high throughput applications. Numerous molecular approaches have been tried and tested with some degree of success. Quantitative polymerase chain reaction (qPCR) assays, capable of speciating and enumerating Eimeria, have been validated. But, due to the relatively high reagent cost their practical utility in field applications and laboratory settings, are questionable. Flow cytometry (FCM) is an automated method for measuring features of single cells in suspension. Many parameters may be assessed simultaneously and objectively at high speed. FCM has the potential to be an excellent diagnostic tool for speciation and enumeration of Eimeria species by exploiting Eimerian oocyst morphology (size and granularity).

The objective of this project was to develop a non-antibody flow cytometry-based diagnostic method for simultaneous enumeration and speciation of coccidian protozoa. To achieve this goal, the project was divided into three specific objectives: (1) characterize each of the Eimeria populations at the species level, (2) define distinct population coordinates of each Eimeria sp. in order to speciate and quantitate mixed cultures of Eimeria, and (3) evaluate the effectiveness of the method with field samples.

Researchers were able to characterize the four Eimeria species (E. acervulina, E. mitis, E. maxima, and E. tenella) on a BD LSRII flow cytometer analyzer by using simple light scatter data plotting. No special staining or antibody labeling procedures were necessary towards resolving these populations. Sorting of mixed populations was successful for the four Eimeria sp. using the MoFlo-XDP cell sorter. The sorted populations aligned with the LSRII data. Purity of these populations was validated by PCR. For enumeration of oocysts, a vaccine and fecal/litter samples were used from poultry flocks mixed with a known number of counting beans. Speciation and quantification of oocysts was performed using the flow cytometer unit. Manual enumeration and speciation by flotation and PCR analysis was performed to validate the counts as well as the purity of each Eimeria sp. identified. Although, additional field samples need to be tested and analyzed, preliminary results showed the method to be reliable for counting and speciating four common Eimeria sp. of chickens.

Flow cytometry application can provide a useful, accurate, rapid and automated diagnostic method for the simultaneous enumeration and characterization of coccidia in poultry. In the long term, this research may lead to the development of other industry assays, such as a more accurate identification of shedding patterns of specific Eimeria sp., differentiation between sporulated and non-sporulated oocysts in the samples, and even recognition of Eimeria strains in vaccine preparations.

The research was made possible in part by an endowing Foundation gift from Perdue Farms.

Researchers Investigate Effect of PAA Solutions in Poultry Processing Wastewater Treatment Systems

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Food safety and sanitation is of paramount importance to the poultry processing industry. Sanitizers, both inorganic (e.g., chlorine-based products) and organic (e.g., quaternary ammonium compounds), have been widely and effectively used by the poultry processing industry to control foodborne agents. Recently, peracetic acid (PAA) has been an effective antimicrobial agent in the poultry industry, used predominantly in chillers, while its use has recently been expanded to other unit processes. Poultry processing personnel refer to upsets of biological wastewater treatment processes and whole effluent toxicity (WET) test failures potentially caused by an excessive use of PAA, thus making it hard to meet effluent discharge requirements. In spite of the potential negative impacts on effluent quality and the rapidly increasing use of PAA in poultry processing plants, no detailed information has been available relative to the fate and effect of PAA in poultry processing wastewater treatment systems.

The goal of this research project was to systematically assess the fate and effect of PAA solutions in poultry processing wastewater biological treatment processes typically used by the poultry processing industry and develop an effective methodology to mitigate any negative effects. This research project accomplished the following tasks: 1) Quantification of hydrogen peroxide (H_2O_2) decay rates under various conditions encountered in poultry processing wastewater (i.e., pH, wastewater strength, and initial H_2O_2); and 2) Bench-scale, batch assessment of the long-term effect of PAA/H_2O_2 solution on the treatment efficiency of aerobic, nitrifying and denitrifying processes, as well as on the diversity and stability of microbial communities.

Researchers identified several key findings during the project. Overall, the results of this study suggest that aerobic and anoxic organic matter degradation, as well as nitrification and denitrification with poultry processing wastewater, will not be affected during the normal plant operation when residual PAA and H_2O_2 are not present in the wastewater. However, nitrification and denitrification will be affected when wastewater with high residual PAA concentrations are expected at the end of the plant operation shift due to emptying of chiller tanks or in the case of accidental PAA solution spills.

This study quantified H_2O_2 decomposition kinetics in dissolved air flotation (DAF) effluent, as well as the long-term effect of PAA/H_2O_2 on the treatment efficiency of biological aerobic, nitrifying and denitrifying degradation processes. Such information enhances our understanding relative to the fate and effect of PAA solutions in poultry processing wastewater treatment systems, information crucial for the rational design and operation of biological treatment processes, especially those related to biological nutrient removal. The outcome of the present study provides systematic information to the poultry processing industry to develop a sound methodology and employ other measures that will ensure the continuous use of PAA solutions to achieve pathogen-free products, while avoiding upsets of biological processes used for the treatment of PAA-bearing wastewater. A more in-depth assessment of the long-term potential effect of PAA solutions on biological treatment processes is currently ongoing.

The research was made possible in part by an endowing Foundation gift from the Stanley & Dorothy Frank Family Foundation.

USPOULTRY and the USPOULTRY Foundation Sponsor $200,000 for FFAR-Funded Research That Benefits the Poultry Industry

USPOULTRY and the USPOULTRY Foundation announced $200,000 in sponsorship funding for two poultry research projects to benefit the U.S. poultry industry. The sponsorship is in partnership with the Foundation for Food and Agriculture Research (FFAR) and will leverage research dollars for two projects that improve poultry welfare and efficiency. The funds are being matched by FFAR for a total $400,000 investment.

The first research project, the SMART Broiler Research Initiative, is a collaboration research program supported by FFAR and McDonald's that is developing automated broiler welfare monitoring technologies for commercial poultry farms. These technologies may also be applicable to the turkey industry. Existing methods for assessing animal welfare rely on human observation and subjective scoring. This initiative is identifying automated solutions to provide objective and comprehensive information about poultry welfare across the supply chain.

The second research project, the Egg-Tech Prize, is a FFAR and Open Philanthropy initiative that is developing new technologies for accurate, high-speed and early stage in ovo sex determination of layer chicks. Only female chicks are used for producing layer hens; male chicks do not lay eggs and are unsuitable for consumption.

The Egg-Tech Prize incentivizes companies and individuals to develop a technology that accurately determines the sex of an egg before it hatches and allows hatcheries to divert male eggs to the food or animal-feed supply chains or use them in vaccine production. This would solve the issue of male chick culling and improve the sustainability of egg production worldwide.

The six winners of Egg-Tech Prize Phase I were announced by FFAR in November 2019. The winner of the $4.5 million Egg-Tech Prize will be announced in 2021.

"We are pleased to have the opportunity to actively collaborate with FFAR as a platinum sponsor for both of these projects. We anticipate that these innovative, unique and cutting-edge technologies will allow the poultry industry to further enhance producer viability, ensure animal wellbeing and meet the changing needs of the industry," said John Prestage, senior vice president for Prestage Farms and USPOULTRY Foundation chairman.

"The Egg-Tech Prize and SMART Broiler are both cutting-edge research initiatives that seek to address global animal welfare concerns and revolutionize on-farm practices while enhancing production," said FFAR's Executive Director Dr. Sally Rockey. "We are thrilled to partner with the U.S. Poultry & Egg Association to fund research that benefits the U.S. poultry industry and U.S. consumers."

Additional Research Information Available on Our Website, www.uspoultry.org/research

Dr. Maricarmen Garcia Receives 2020 Dr. Charles Beard Research Excellence Award

USPOULTRY and the USPOULTRY Foundation are proud to recognize Dr. Maricarmen Garcia as the 2020 recipient of the annual Charles Beard Research Excellence Award. Dr. Garcia is a professor in the Department of Population Health at the University of Georgia Poultry Diagnostic and Research Center. The award is named in honor of Dr. Charles Beard, former director of the Southeast Poultry Research Laboratory and former vice president of research at USPOULTRY.

The USPOULTRY Foundation Research Advisory Committee selected Dr. Garcia for this award based on her exceptional research on infectious laryngotracheitis virus (ILTV), avian mycoplasmas and retroviruses. Dr. Garcia has received five grants on ILTV since 2000. She used the research funding to develop a genotyping system for ILTV, to evaluate the use of recombinant ILTV vaccine, and to study the epidemiology of ILTV. She received three additional grants that were utilized to develop a system for subtyping pathogenic mycoplasmas that led to the development of a national library of avian mycoplasmas. Another grant from USPOULTRY made it possible for Dr. Garcia to develop an improved RT-PCR for avian leukosis virus subgroup J.

"The U.S. Poultry & Egg Association has been very supportive of my research over the years, and I am extremely appreciative for the research grants that I have been given. Without the Association's backing, researchers like me would not be able to conduct some of the vital and essential studies needed throughout the poultry industry," commented Dr. Garcia.

"Dr. Maricarmen Garcia is most deserving of this award. The advancements that she has made through her research have provided invaluable knowledge and resources for the poultry industry," remarked Dr. John Glisson, former vice president for research programs for USPOULTRY. Dr. Garcia received a BS degree from the University of Puerto Rico and her MS and PhD degrees from the University of Georgia. She joined the faculty at the University of Georgia in 1997.

The goal of the Charles Beard Research Excellence Award is to recognize outstanding completed research projects, funded by USPOULTRY or the USPOULTRY Foundation, which have made a significant positive impact on the poultry industry. As the recipient of the award, Dr. Garcia received a $1,500 cash prize.

John Prestage and Dr. Maricarmen Garcia