TABLE OF CONTENTS

ASSOCIATION
1 USPOULTRY and Foundation Approve $599,099 in New Research Grants
2 USPOULTRY and Foundation Approve $243,166 in New Board Research Initiative Grants
14 Dr. Sarge Bilgili Receives 2015 Charles Beard Research Excellence Award

RESEARCH
2 Development of Best Practices for Shell Egg Disinfection Based Upon Efficacy, Egg Quality and Economics
3 Studies on Newly Emerging Reassortant Very Virulent Infectious Bursal Disease Viruses
4 Energy Balance Analysis of a Poultry Processing Plant
5 Ultrasonics and its Synergy for Poultry Water Disinfection
6 Salmonella and Campylobacter Contamination of Turkeys, from Breeders to Processed Carcasses
7 Blackhead Disease (Histomoniasis): Reducing Losses through Molecular Tracking and Immunization
8 Enriched Colony Cages: Stocking Density on Laying Hen Well-being
9 The Effect of Body Weight Restriction During the Rearing Period on Carcass Composition and Early Production Traits in Commercial Turkey Breeder Hens
10 Studies on the Efficacy of Recombinant HVT-IBD Vaccines
11 Development of Campylobacter jejuni Proteins as in ovo Vaccines for Broiler Chickens
12 Role of Distillers Dried Grains with Solubles (DDGS) in Necrotic Enteritis Development
13 Live Performance, Carcass Yield and Breast Meat Discoloration of Broiler Chickens Fed Diets Supplemented with Different Levels of Zinc, Copper, and Iron

2014 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.

Send Comments to:
U.S. Poultry & Egg Association, 1530 Cooledge Road, Tucker, GA 30084-7303
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USPOULTRY and the USPOULTRY Foundation approved a total of $599,909 for 11 new research grants at seven institutions. 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 Association’s research program dates back to the early 1960s, when funds were first approved for poultry disease research. It gradually grew into a comprehensive program incorporating all phases of poultry and egg production and processing. Since the inception of the research program, USPOULTRY and the USPOULTRY Foundation have reinvested more than $25 million into the industry in the form of research grants, with the International Poultry Expo as the primary source of funding. In recent years, earnings from the USPOULTRY Foundation have supported half of the research grants. More than 50 universities and federal and state facilities have received grants over the years.

“Research is a crucial component of USPOULTRY’s and the USPOULTRY Foundation’s service to the industry,” said Elton Maddox, Wayne Farms, and USPOULTRY chairman.

The research grants are as follows:

### Combined Heat Recovery and Ammonia Control System for Broiler Brooding
North Carolina State University (research grant made possible by an endowing gift from Case Farms)

### Factors Contributing to Superficial Pectoral Myodegeneration and Sclerosis (Wooden Breast) in Broilers
North Carolina State University (research grant made possible by an endowing gift from Ozark Mountain Poultry)

### Branched-chain Amino Acid Requirement and Their Role in Protein Synthesis and Growth Performance in Female Broiler Chickens
University of Arkansas (research grant made possible by an endowing gift from Simmons Foods)

### Identifying Amino Acids in the Spike Protein Critical for Arkansas-DPI Vaccine Binding
University of Georgia

### Generation of the Bivalent Vaccine against Newcastle Disease (ND) and Infectious Laryngotracheitis (ILT)
USDA ARS Southeast Poultry Research Laboratory (research grant made possible by an endowing gift from Claxton Poultry)

USPOULTRY and Foundation Approve $243,166 in New Board Research Initiative Grants

USPOULTRY and the USPOULTRY Foundation approved a total of $243,166 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 USPOULTRY Board Research Initiative was created by the Boards of USPOULTRY and the USPOULTRY Foundation to address current issues facing the poultry industry. The USPOULTRY Board Research Initiative operates concurrently with the USPOULTRY research program and augments the great success of the existing program by focusing additional resources toward defined areas of research.

The research grants are as follows:

### Determining the Dose, Time and Route of Challenge and the Eventual Sites of Colonization of Two Salmonella Serovars
Auburn University (research grant made possible by an endowing gift from Cargill)

### Characterizing Thermal Micro-Environment during Poultry Transportation
University of Arkansas (research grant made possible by an endowing gift from Prestage Farms)
Development of Best Practices for Shell Egg Disinfection Based Upon Efficacy, Egg Quality and Economics

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Salmonella contamination of shell eggs and subsequent recalls in 2010 resulted in economic losses to the egg industry and increased consumer concerns about the safety of eggs. In addition, HACCP regulations for egg processing are probable in the near future. Treatment of eggs with chlorine or quaternary ammonium (QAC) sprays as a final disinfection step following washing has been the standard for egg processing in the US for decades. The use of ultraviolet light (UV) as a final disinfection step for shell egg processing has been approved by the USDA but has not gained widespread use in the US egg industry. Previous research has indicated that all the egg sanitization processes listed above do not completely disinfect the surface of shell eggs during processing. As a result, more effective eggshell sanitization technologies are needed to help assure the safety of shell eggs and egg products.

The overall goal of this project was to develop and evaluate improved egg sanitation processes for shell eggs to enhance food safety. The specific objectives were to: (1) survey egg processors across the US to determine current practices and costs of shell egg sanitization; (2) conduct a microbial survey of egg processing facilities to evaluate current sanitization of shell eggs; (3) evaluate the effectiveness of prewash egg disinfection procedures; (4) determine efficacy and quality parameters of current methods of egg sanitization compared to alternative technologies; and (5) conduct an economic analysis to compare current and alternative methods for the sanitization of shell eggs.

The survey of egg processors across the US indicated that egg sanitization practices are quite standardized across the industry. This is not surprising since 77 percent of respondents indicated they were processing eggs under inspection (presumably USDA) and must, therefore, follow set guidelines. Eighty-three percent of egg processors are using a chlorine solution rinse in the final disinfection step. Results also indicate that few processors apply a sanitation process prior to egg washing or conduct microbiological monitoring. Eggs sampled from six egg packing plants in Texas verified that currently used egg sanitization methods significantly reduce the microbial load on eggshells but usually leave a low level of bacteria remaining on the eggshell surface (2.1 log10 cfu/egg).

Four trials were conducted to evaluate the use of hydrogen peroxide (H2O2) in combination with UV light to treat eggs prior to washing. Results indicated that treatment prior to washing resulted in fewer dirty eggs following washing, and visibly clean eggs after washing had lower microbial counts if they were treated prior to washing.

Several experiments were conducted to compare the effectiveness of eggshell disinfectants currently used in the egg industry to alternative methods of peracetic acid (PAA), PAA in combination with UV light, and H2O2 in combination with UV light. Peracetic acid was found to be more effective than chlorine but less effective than QAC. Hydrogen peroxide with UV light was the most effective sanitizer, resulting in zero microbial counts on most eggshells. Sensory panel evaluation indicated that eggs treated with H2O2 and UV light were perceived by consumers to be equal to untreated eggs and eggs treated with QAC, but eggs treated with chlorine received the highest scores for taste and texture. While H2O2 with UV light was found to be a superior eggshell disinfectant compared to methods currently used in the egg industry, the cost is greater. In addition, experiments conducted to evaluate the effectiveness of various disinfectants to reduce Salmonella inoculated on eggshells found that all the disinfectants, including those currently used in the egg industry, reduce Salmonella on eggshell surfaces to levels below detection by rinse and plate sampling technique.
Studies on Newly Emerging Reassortant Very Virulent Infectious Bursal Disease Viruses

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In December 2008, the first very virulent (vv) infectious bursal disease virus (IBDV) was isolated in California, and in 2009, we identified the reassorted viruses, K785 and D495. Genome segment A of these reassortants was typical of vvIBDV, but genome segment B was most closely related to serotype 2 IBDV (A/B = vv/serotype 2). Since that time we have identified several genome reassorted viruses that represent combinations of genome segments from vv, classic virulent (cv) and serotype 2 IBDV. The problem is, we know very little about the virulence of these newly emerging reassorted viruses for chickens and turkeys.

OBJECTIVES:
Objective 1: The ability of three reassorted vvIBDV from California to infect and cause disease in chickens and turkeys will be determined.

Objective 2: The ability of anti-IBDV maternal immunity to block infection and disease of pathogenic reassorted viruses will be determined in chickens.

RESULTS AND DISCUSSION
Objectives 1 and 2 were expanded to include more than three IBDV strains. All the viruses were isolated from California. The K669, D495 and K785 viruses are interserotypic vv/serotype 2 reassortants. The D2712 (serotype 2/cv) virus was isolated from commercial turkeys in January 2012. The 7741 strain is a vv/cv reassortant. It was identified in commercial layers in August 2010 and its genome segment B aligned with cvIBDV strains from Argentina and Australia. The cv/vv reassorted virus D6337 was isolated in 2012 from a backyard chicken flock. A non-reassorted turkey isolate, T3599 (serotype 2/serotype 2) was isolated in 2013 from the small intestine by Dr. P. Woolcock (CAHFS) and was included in this study, because it was a recent isolate from a region where the vv/serotype 2 viruses were discovered.

Objective 1. In vivo virulence of reassortant IBDV was assessed relative to the virulence of other IBDV pathotypes: vvIBDV (rB strain), classic virulent (cv) (STC strain) and subclinical variant (sc) (Del E strain). Morbidity and mortality in 4 week old SPF leghorns indicated that reassortant IBDV with a vv genome segment A and non-vv segment B were less pathogenic than the vv/vv rB strain but more pathogenic than the cv/cv STC strain. The cv/vv IBDV strain D6337 was comparable to the STC strain in pathogenicity. Viruses with a serotype 2 genome segment A, regardless of the type of genome segment B, did not cause clinical disease in SPF chickens or turkeys. Reassorted viruses did not cause morbidity, mortality or gross lesions in SPF turkeys. Histopathologic lesions in the bursa of turkeys were not observed in any group except OH, which had a mild lymphocytic depletion.

Objective 2. No mortality was observed in maternally immune broilers inoculated with any of the IBDV pathotypes at 1, 2, 3 and 4 weeks of age. No bursal lesions were observed in any of the experimental groups at 1 week of age except for the D2712 inoculated birds that had mild lymphocyte depletion. In broilers challenged at 2 weeks of age, the K669 virus broke through the maternal immunity while the STC, Del-E, rB, D2712 and 7741 viruses did not. All viruses broke through maternal immunity at 3 weeks of age except the Del-E and D2712 viruses. At 4 weeks of age, maternal antibodies were very low, and bursal lesions were observed in all broilers challenged with these viruses.

IMPACT OF THE RESULTS
Genome reassorted IBDV appear to be less pathogenic than vvIBDV. However, these viruses can still cause morbidity and mortality in SPF chickens, and they were able to break through maternal immunity produced using commercial classic and variant vaccines. This suggests that current breeder vaccination programs may not adequately protect against the reassorted vv/serotype 2 and vv/cv IBDV strains.
The objective of this project was to characterize the energy uses in a broiler processing facility and to identify specific measures to improve energy efficiency. The process of quantifying power requirements and energy usage was separated into two levels, referred to as a high level and a detailed level. The high level included analysis of 12-month energy usage records for the plant and available 15-minute electrical interval data from the utility company, in order to gain an overview of total energy consumption. Electricity was used for refrigeration, conveyors, lighting, air conditioning, pumps, compressed air and other mechanical drives. Natural gas was used for production of steam, and further used to generate hot water for processing and sanitation, as well as for space heating. The production process was split into individual unit operations, which were monitored in the detailed level.

The major processes include RKP (receiving, killing, and picking), Evis (evisceration), Offal (collection and removal of non-edible parts), 2nd process (sizing, cutting and deboning), pack and ship, utilities (chilled ammonia, compressed air, waste water) and boilers (steam production). Electrical energy for each of the individual unit operations was monitored over three separate data collection intervals, each consisting of two-week periods, between November 2012 and January 2013. Natural gas energy was measured with some novel non-invasive data collection methods during April 2013.

Recorded sub-metered power consumption data on weekdays from individual unit operations matched the 15-minute interval data within 3%, indicating that sub-metering captured the major consumption of electrical usage. Utility usage, including the production of chilled water and compressed air, was the largest energy user of electricity, followed by Offal, RKP, and Evis operations. On total energy (MMBtu) basis, natural gas usage for steam generation by boilers was higher than site electricity consumption.

Energy efficiency recommendations included insulating steam pipes and valves, installing an automatic blowdown system to improve boiler efficiency, adding variable speed motors and drives to the cooling towers of the refrigeration system, upgrading to energy efficient lighting, upgrading to a more efficient air compressor for the plant air system, etc. These measures have guaranteed payback periods of two years, with some leveraging of utility rebate programs. Several additional energy saving measures were investigated during the course of this project. Although most of the measures in this category represent viable technologies, they are either too costly to implement or are more difficult to estimate the energy savings due to uncertainty. The addition of temperature or occupancy sensors to the cooling shed will allow the automation of the cooling fans with reduced running hours. This measure may warrant further investigation to better determine the magnitude of running hour reduction.

Because of the amount of hot water used for scalding and cleanup, it will be more efficient to generate hot water directly and avoid the intermediate step of producing steam. It was difficult to estimate the efficiency improvement of this measure due to the existing boiler system deficiencies. Recognizing that this is a costly system change, it should be considered when a boiler change-out is required. Results from this project demonstrated that cost-effective energy saving opportunities are available in poultry processing plants and warrant investigation.
Ultrasonics and Its Synergy for Poultry Water Disinfection

ultrasound experiments were conducted in isothermal conditions to eliminate disinfection via indirect heat input from the ultrasonic probe.

The data from the Salmonella inoculated water tests showed a correlation between an increase in ultrasonic energy, increase in exposure time, and decrease in volume that led to better disinfection of Salmonella. The data demonstrates that ultrasound can be used to inhibit Salmonella growth. However, the extent of disinfection from the ultrasonic energy magnitude was not significant enough to be used as the only means of disinfection.

This study also evaluated the effectiveness of ultrasound and chemical disinfection agents (chlorine or peracetic acid (PAA)) in Salmonella inoculated water, simulated chiller water, and actual poultry chiller water. Based on previous results, a volume of half liter and a single ultrasonic energy were selected to determine the synergistic effects in most tests. The synergy effect for chlorine (1.66, 3.32 and 4.98 ppm) and PAA (0.75, 1.5 and 2.25 ppm) in water was evaluated by assessing the disinfection agents with approximately 40kJ of ultrasonic energy (65W for 10 minutes) in Salmonella inoculated water. When comparing the data of chemical disinfection with the combination of ultrasonic and chemical, the disinfection was greater for the combined system than the disinfection observed with chemicals only.

Simulated chiller water (5 g of chicken skin and fat per litter of water) was treated with combined chlorine (16.6 ppm) and ultrasound and compared to chlorine treatment alone. This experiment was also repeated using peracetic acid concentrations of 0.75, 1.5, and 2.25 ppm. A similar trend of log reduction was observed for combination ultrasound and chemicals treatment for all concentrations of chlorine and PAA.

Actual poultry chiller water was found to be more challenging to evaluate. Since the chiller from a poultry plant has chemical residue, more work was needed to characterize and evaluate the efficacy of the chemical residue. Poultry chiller water (TS=6.34g/L and chemical residue of 10 ppm total chlorine, and 0 free chlorine) was used to evaluate the disinfection of ultrasonic treatment and its synergy with chemicals. The Salmonella inoculated poultry water test showed no inactivation from residue chemical. The ultrasonic disinfection trend in poultry chiller water was found to be similar to that of the Salmonella inoculated water experiments. Furthermore, the poultry chiller water with additional chemicals (16.6 ppm chlorine and 2.25 ppm PAA) and ultrasonic were also tested. The data showed that the combined disinfection system performed better than the chemical alone. However, more work is needed to characterize the actual chiller water.

In all cases, samples treated with combined ultrasound and chemicals exhibited better disinfection than samples treated with chemicals alone.
Salmonella and Campylobacter Contamination of Turkeys, from Breeders to Processed Carcasses

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Current USDA FSIS regulations require turkey processing companies to not exceed established low limits of Salmonella and Campylobacter in processed products, as monitored by testing programs. A number of methods including chemical interventions are used to control these pathogens in the plants. However, reducing or eliminating both pathogens from birds prior to processing is potentially a more cost effective method than excessive plant interventions. The entry, transmission, and overall prevalence of both pathogens in the production chain must be understood in order for appropriate interventions to be instituted. Although these pathogens are frequently found colonizing the intestinal tract of poultry, they have also been isolated from the avian reproductive tract, indicating a possible source of contamination of hatching eggs and resulting progeny. These mechanisms have been studied in broilers, but less so in turkeys.

The objectives of the project were to: 1) determine routes of transmission for Salmonella and Campylobacter throughout turkey production and processing; and, 2) determine effects of selected interventions on prevalence and numbers of Salmonella and Campylobacter.

The project included growing and monitoring of turkey breeder hens and toms through 65 weeks of age, artificial insemination and collection of fertile eggs for hatching a 2nd generation meat bird flock, and then monitoring these progeny (meat bird flock). Intervention assessments included washing fertile eggs with sanitizer and feeding probiotics to both breeder hens and meat bird progeny.

Monitoring results showed that Campylobacter spread rapidly and cross-contaminated turkeys throughout the growout house. For both Salmonella and Campylobacter, wild strains that appeared seemed to outcompete marker strains after a few weeks and persist in the flock. The most common wild strains were Campylobacter jejuni (tetracycline resistant), Campylobacter coli (kanamycin resistant), and Salmonella Agona. Pathogens were also isolated from pest vectors (flies, beetles and a rodent) in the houses, confirming the importance of proper pest control and biosecurity to control the spread of the bacteria. The same wild Campylobacter strains and the marker C. jejuni TSKQ (contrasted by PFGE) were also isolated from other houses at the farm during other studies. PFGE revealed that they were the same strains.

Vertical transmission of these pathogens through hatching eggs was not demonstrated in this study, however, marker Salmonella inoculated in semen was found in the upper reproductive tract of breeder hens. Furthermore, wild strains of both pathogens were isolated from semen and wild Campylobacter strains were also isolated from the upper reproductive tract of breeder hens, indicating a potential route of transmission to progeny.

Results from the interventions indicate a positive effect of washing and sanitizing eggs that may decrease the prevalence of Salmonella detected on the eggshell. The application of a feed probiotic treatment (Primalac®) at high dosage for two weeks prior to slaughter was not found to significantly reduce the prevalence of Salmonella and/or Campylobacter in ceca for either breeder hens or meat bird progeny tested.

This study demonstrates the potential role of poultry house pests in the spread of both Campylobacter and Salmonella in turkeys. Further, it indicates a possible route of transmission of these pathogens via the insemination of contaminated semen. Results indicate that sanitizing hatching eggs will reduce the levels of eggshell contamination by Salmonella. In this study, use of a probiotic in breeders or meat turkeys did not reduce the levels of Salmonella or Campylobacter detected.
Blackhead Disease (Histomoniasis): Reducing Losses through Molecular Tracking and Immunization

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Molecular techniques were used to track outbreaks of Blackhead Disease, to correlate these outbreaks with specific isolates of *Histomonas meleagridis*, and to identify genes associated with the virulence of specific isolates. Additionally, the project tested whether a vaccination approach could be used to protect turkeys from this disease. Isolates from outbreaks in turkeys, broiler breeder chickens, layer chickens and bobwhite quail were tested for virulence and categorized by molecular markers.

The objectives of the research were to:
1. Survey *H. meleagridis* field isolates from turkeys, broiler breeder pullets, and other wild birds for variation in virulence. Correlate virulence to molecular markers that classify *H. meleagridis* stains.
2. Investigate the epidemiology of field isolates obtained from *H. meleagridis* outbreaks using molecular markers. Identify potential disease reservoirs and modes of transport of *H. meleagridis* between farms.
3. Establish and validate a molecular diagnostic test for *H. meleagridis*.
4. Establish a vaccination protocol to stimulate protective immunity in turkeys.

Our data confirm that there are multiple disease reservoirs and that most outbreaks of blackhead arise from distinct isolates of *H. meleagridis*. Isolates varied considerably in virulence, sensitivity to nitarsone (Histostat-50) and expression of virulence genes. Variations in mortality and morbidity in outbreaks in the field was a result of the virulence of the associated isolate, particularly in chickens. Based on our data, litter from breeder or layer pullets is likely to contain cecal worm (*Heterakis gallinarum*) eggs, the known vector for *H. meleagridis*, is the most likely source of infection in turkeys. Although this confirms earlier work, it emphasizes the increasing infection pressure resulting from overlapping areas of broiler breeder chicken farms and turkey farms. One strain of *H. meleagridis* isolated from layer pullets resulted in 17 percent mortality in chickens in the lab, emphasizing the potential of this parasite to devastate chicken flocks as well as turkeys.

We have designed a new molecular-based method to diagnosis *H. meleagridis* in samples obtained from tissue. This PCR-based method is specific to *H. meleagridis* and allows quick diagnosis without costly DNA sequencing. Vaccination approaches using attenuated live and killed preparations tested by our group failed to produce adequate timely protection in turkeys. Although we saw a delay in the onset of the disease in birds given live attenuated *H. meleagridis*, complete protection was never attained. While this approach was reported as successful in the literature, our results do not support the use of a vaccination to prevent blackhead.

This study is the first of its kind funded in the recent years. It is clear from our results that a significant part of the biology of *H. meleagridis* and its interaction with chickens and turkeys is poorly understood. Based on our data, there are many local reservoirs of infection in the environment, increasing the difficulty in preventing blackhead outbreaks. Our data suggests that careful consideration should be taken when spreading litter from chicken farms near turkey facilities. More research is needed to identify insects or other mechanical carriers responsible for survival and spread of the blackhead. Identification of strains with high virulence in chickens suggests we should use caution in spreading litter from these farms near other poultry operations.
Consumers are expressing a greater interest in knowing that laying hens have good well-being. The consumers’ perception is that the issue can be easily resolved with alternate housing systems for laying hens. One potential housing system becoming more popular is the enriched colony cage. As the commercial laying hen industry begins to phase out the conventional cage moving toward alternative housing systems, research studies need to populate data that can provide guidance on management practices relative to the housing system. The overarching objective was to investigate laying hen space allocation in enriched colony systems. The long-term goal was to provide a better understanding of how production and well-being are entwined to aid producers in making sound decisions and provide information to address issues raised by the consumer. The specific objectives for this grant were to 1) evaluate performance of a single strain of laying hens at different stocking densities in enriched colony cages and 2) assess impacts of different stocking densities on laying hen well-being using measures of health, stress, and behavior.

W-36 laying hens were housed in the enriched colony cage at 464 cm², 580 cm², 651 cm², 748 cm², 799 cm², and 929 cm² from 17 to 69 weeks of age. Production measures including egg production, body weight, egg weight and feed disappearance were collected. Hen-day production was similar across the various densities. Hens with greater than 748 cm² of space per hen had slightly higher production compared to hens with less space allowance. Egg production declined over time with all treatments ending around 79 percent, with the exception of the 929 cm² treatment which ended around 82 percent. Egg weight, feed consumption and body weights were similar across all treatments.

The other aspect of the trial was to evaluate the health, stress and behavior of the birds using the European Union Welfare Quality® (WQ) Assessment Protocol for Poultry (Welfare Quality® Consortium, 2009). The Avoidance Distance Test, which assesses the hen’s response to humans, was not practical as the hens interacted with farm staff daily during egg collection and were habituated to human presence. The fear response of the hen was therefore only assessed using the Novel Object Test. The hen’s responses to the novel object, a colorful rod, were not different by density. According to the WQ protocol hen health can be assessed through observation of hens with labored breathing/sneezing and visual examination of feces to identify enteric infections. Based on these procedures, hen health was deemed not to be of issue. The lack of health concerns was confirmed by an avian pathologist who conducted necropsies of mortality. Due to lower than expected numbers of pullets at placement, the sacrificing of pullets was not possible, and the adrenal weights of the pullets, an indicator of stress, were not evaluated. The welfare quality measures of comb abnormality, comb wound, keel deformation or fracture, skin lesions, toe damage, foot condition, and plumage damage were assessed bi-monthly in 10 percent of each enriched colony population. Keel deformation or fracture increased over time and was similar amongst all treatments over time (20 to 30 percent). The plumage damage was assessed on seven different areas of the hen. All areas became worse with time (increased feather loss or feather breakage), and the areas of the head, abdomen, and back were impacted by density with the proportion of hens having worse plumage quality in 464 cm². Further research should additionally focus on finding the density between 651 cm² and 748 cm² at which improvements to feathering occurs.
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The commercial breeding sector of the turkey industry has been very successful at continually improving the commercial traits (i.e. body weight, breast muscle yield) that are the economic drivers of the industry. It has been recognized for many years that the continual selection for traits of economic importance may have negative correlated effects on reproductive efficiency. The turkey industry has partially countered this via the use of artificial insemination to maintain high levels of fertility, but there is no simple, identified management tool for optimizing production efficiency in commercial breeder hens. It is accepted that body weight control of turkey hens during rearing is essential for optimizing egg production, but physical restriction of hens as practiced in broiler breeders is not an accepted option. Thus, there is a need for studies whose objective is to control body weight in replacement hens via ad libitum, controlled feeding of low nutrient dense diets.

The primary objective of the research reported herein was to create different body weight groups during rearing via dietary manipulation. This was followed by individual weighing of hens at 24 weeks and allocating them into replicate pens of Heavy, Medium and light body weight hens. These body weight treatments were designed to bracket the target body weights recommended by the primary breeder, Hybrid. In addition to hen-day egg production and egg weight determinations, a sample of hens from each treatment were euthanized for carcass, reproductive organ and selected skeletal measurements at one week (30 weeks) and three weeks post-photostimulation (32 weeks). The dietary rearing treatments resulted in hens that weighed 24.45 and 23.0 lbs at 24 weeks, 26.6 and 25.6 lbs at 30 weeks and 24.8 and 24.1 lbs at 42 weeks. The Heavy, Medium, and Light hens weighed 27.6, 26.3, and 24.3 lbs at 30 weeks and 25.6, 24.6, and 23.0 lbs at 42 weeks.

After the onset of hen-day egg production, the Light treatment hens had significantly better hen-day egg production than either the Medium or Heavy hens. The Heavy hens had approximately a 2 gram improvement in egg weight at 4, 8, and 10 weeks of egg production compared with the Light hens. The Medium weight hens were intermediate. At 30 weeks of age, hens in the Heavy, Medium and Light body weight groups had corresponding differences in carcass weight, but there were no significant effects on shank length, follicle number or reproductive tract weight. At 32 weeks, there were also no body weight treatment effects on follicle number or reproductive tract weight, but there was a progressive decrease in femur, tibia and shank length in the sampled hens as body weight declined. Total carcass lipid (percent DM) increased from 42 percent to 51 percent between 30 and 32 weeks of age, but there were no significant differences between body weight groups. The greatest increase in carcass lipid occurred in the Light body weight group (41.7 percent to 53.5 percent), and this resulted in a marginal age by body weight interaction (P < .087). Across all three body weight groups, there was a significant decline in body weight between 30 and 42 weeks of age.

In conclusion, the lower plane of nutrient intake during rearing significantly reduced body weight at 24 and 30 weeks. Carcass lipid increased between 30 and 32 weeks, particularly in the Light hens. At 32 weeks, there was a decline in femur, tibia and shank length with each decrease in body weight among the Heavy, Medium, Light groups, and this may have reduced maintenance energy needs and allowed for increased carcass lipid deposition.
Studies on the Efficacy of Recombinant HVT-IBD Vaccines

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The efficacy of commercially available recombinant herpes virus of turkeys-infectious bursal disease virus (rHVT-IBD) vaccines in broiler chickens derived from an IBDV-vaccinated breeder flock at 30-weeks of age (Trial 1) and 60-weeks of age (Trial 2) was studied. Specific-pathogen-free (SPF) white leghorn chickens, free of maternal antibodies against IBDV, were used as controls. Broilers and SPF leghorns were vaccinated subcutaneously in the neck at one-day of age with Company A rHVT-IBD or Company B rHVT-IBD vaccines and were placed in isolators. On day post vaccination (DPV) 10, 14, 18, 22 and 26, vaccinated and unvaccinated broilers and SPF leghorns were bled and challenged via the conjunctiva sac route with reference strains ST-C, Delaware variant E (DelE), or contemporary field isolates DMV/5038/07 or FF6.

IBDV serum antibodies were detected to varying degrees depending on the commercial ELISA kit used. ELISA A and C kits more readily detected rHVT-IBD vaccine induced (active) serum antibodies compared to ELISA kits B and D. Bursa/bodyweight ratios were not consistently useful as a tool for assessing IBDV challenge in broiler chickens with IBDV maternal antibodies. Bursa/bodyweight (B/BW) ratios were more useful for assessing protection against IBDV challenge in SPF leghorns. Microscopic lesion assessment of the bursa was useful for assessing IBDV challenge in both rHVT-IBD-vaccinated broiler and SPF leghorn chickens. rHVT-IBD vaccines, in general, induced greater protection with increasing age of the chicken.

Based on bursal microscopic lesion assessment, Company A rHVT-IBD vaccination of SPF leghorns induced protection by 18 DPV and continued to protect on 22 DPV and 26 DPV in Trials 1 and 2. Company B’s rHVT-IBD vaccine induced protection of SPF leghorns by 18 or 22 DPV in Trial 1, depending on the IBDV used for challenge. However, the onset of protection was delayed until 22 or 26 DPV in Trial 2. rHVT-IBD vaccination of broiler chickens with either commercial vaccine was not as effective as was observed in SPF leghorns, based on bursal microscopic lesion assessment. However, Company A rHVT-IBD vaccination protected broilers following challenge with ST-C in both Trial 1 (30-week-old breeder progeny) and Trial 2 (60-week-old breeder progeny). Partial protection vs FF6 (Trial 1) and DMV/5038/07 (Trial 2) challenges were observed. Company B rHVT-IBD vaccination protected broilers vs. FF6 challenge in Trial 1. In Trial 2, the vaccine did not offer protection on the basis of microscopic lesion assessment. Overall bursal lesion scores in challenged broilers appeared to be lower in Company A vaccinated chickens vs. those receiving Company B rHVT-IBD vaccine.
Development of *Campylobacter jejuni* Proteins as *in ovo* Vaccines for Broiler Chickens

Hung-Yueh Yeh  
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*Campylobacter jejuni*, commonly associated with poultry, causes human campylobacteriosis. Although many strategies for reducing *C. jejuni* contamination in poultry have been examined, there are currently no practical intervention means available for the poultry industry to effectively reduce the contamination of *C. jejuni* during production and processing. Therefore, research for practical on-farm interventions is an urgent need. In this proposal, we attacked this problem by development of *C. jejuni* subunit proteins as potential vaccines for broilers.

The hypothesis of the proposed study was that expression of a battery of *C. jejuni* proteins involved in colonization has the potential for discovery of novel antigen(s) that can be used for *in ovo* vaccination to reduce the bacterium in broiler chicken gastrointestinal systems. The specific objectives of this proposal are to: (1) construct a system for expressing large amounts of important *C. jejuni* proteins, (2) produce and purify the recombinant *C. jejuni* proteins, (3) assay the immune response in broiler chickens against these *C. jejuni* proteins, and (4) conduct vaccination experiments with the *C. jejuni* proteins.

**Objective 1:** Fifty-seven *C. jejuni* genes potentially involved in colonization were identified. Twenty-eight genes were successfully over-expressed to allow purification of proteins.

**Objective 2:** The proteins were purified using chromatography.

**Objective 3:** Two recombinant proteins -- flagellar capping protein (FliD) and methyl-accepting chemotaxis protein (Cjj0473) reacted strongly to sera from broilers older than six weeks of age in our preliminary experiments, indicating that these were antigens which the broiler immune system was exposed to and responding to in the field. Next, we evaluated whether sera collected from other areas in the U.S. contained antibodies against the FliD protein. Sera from layer breeders at 44-52 weeks of age showed 100 percent positive, while sera from broilers at 4-6 weeks of age from 22 premises showed 7-100 percent positives. These results suggest that *C. jejuni* was widespread in these poultry populations, and chickens had been exposed to this microorganism. It appears that prevalence of *C. jejuni* in these poultry populations was age-related.

**Objective 4:** Three in ovo experiments were conducted. The results showed very poor hatchability, suggesting that the adjuvant used was toxic to chicken embryos. Next, one-day-old maternal antibody-positive broiler chickens were vaccinated with 100 mg/chicken of the FliD protein prepared with an equal amount of incomplete Freund's adjuvant. The broiler chicks responded by producing antibody to the protein. These results suggest that the FliD protein is immunogenic in broilers and that this protein has potential as a vaccine candidate. The results also indicate that maternal antibodies may not affect immunization.

Our results have the following potential impacts for the industry. We found that this protein is present in all of our 21 *C. jejuni* isolates and is immunogenic in broilers. Therefore, this protein will be an excellent candidate for further evaluation as a vaccine to reduce *Campylobacter* in poultry. In addition, antibodies to this protein may be used as a tool to monitor the *Campylobacter* status during poultry production. Currently, *C. jejuni* is regarded as a commensal in the chicken gut intestine, which does not harm chickens. Our studies, however, demonstrated that broiler sera reacted to a variety of *Campylobacter* proteins, suggesting that chickens had been exposed to or infected with this microorganism, and consequently developed antibodies against it. Further, we showed antibodies against the FliD protein were widespread in poultry populations. Therefore, control of *Campylobacter* exposure through vaccination of chickens is a logical approach to *Campylobacter* control, and the FliD protein is a good vaccine candidate. Further studies must be done to evaluate the use of this protein by *in ovo* application to determine its potential practical use and efficacy.
Necrotic enteritis is a serious issue for the poultry industry. In the near future it may become even more of a problem with reduced use of antibiotics/anticoccidials in poultry feed. The use of dried distillers grains with solubles (DDGS) in poultry diets has increased as the availability of this reasonably priced feed ingredient has risen over the past several years. Preliminary research conducted in our laboratories had implied that feeding DDGS might lead to an increase in necrotic enteritis development.

The objective of this proposal was to determine the role, if any, of DDGS in necrotic enteritis development. To accomplish this, two experiments were performed. In the first experiment birds were fed a standard corn soy diet that had either 7.5 or 15 percent DDGS included in the diet. Birds were then challenged, first with a coccidia cocktail and then four days later with Clostridium perfringens over three consecutive days. Ten days after the coccidia cocktail was administered, the birds were necropsied. During the necropsy necrotic enteritis and coccidiosis lesions were scored, and samples were collected for C. perfringens recovery. In addition, feed conversion, body weight and mortality data were collected. Utilizing the results from the first study, a second experiment was performed using the most detrimental level of DDGS (15 percent) and three different challenge levels (low, medium and high) of C. perfringens. The goal of this experiment was to determine if DDGS influenced the severity or incidence of necrotic enteritis development with these three different levels of C. perfringens. The same experimental measurements were collected in this experiment as in the first.

The first experiment did not clearly define the relationship between feeding DDGS and necrotic enteritis development. What was obvious, though, was that the treatment group fed the 15 percent DDGS diet had a significantly worse 15-28 day adjusted feed conversion ratio (AFCR) than the group fed the diet that did not contain DDGS. Though not significant, the group fed the intermediate DDGS diet (7.5 percent) had six points higher AFCR than the group fed the control diet. This is important because the 15-28 day period is when the birds were challenged with coccidia and C. perfringens. Based on these as well as previous results from our labs, it was concluded that 15 percent DDGS was more likely to have an effect on necrotic enteritis development. In the second experiment similar differences were seen in the 15-28 AFCR, with the groups fed the 15 percent DDGS diet having higher AFCR values than similarly challenged birds fed a diet containing no DDGS. Not surprisingly, the birds fed either diet (0 or 15 percent DDGS) that were not challenged had similar AFCR. Also in that experiment birds that were fed the 15 percent DDGS diet and challenged with the low and medium dose of C. perfringens had more severe necrotic enteritis lesions then those that were not fed a diet with DDGS. Both groups given the high challenge doses had a similar number of cases of severe necrotic enteritis lesions. These results show that high levels of DDGS may lead to decreased bird performance. When the birds have a mild to moderate C. perfringens challenge, this can lead to more severe cases of necrotic enteritis as well as a decrease in bird live performance.

DDGS is commonly being used by the poultry industry. In this report it is demonstrated that high levels of DDGS (15 percent) can amplify mild to moderate cases of necrotic enteritis into more severe cases. It also shows that even with a mild case of necrotic enteritis, bird live performance is more negatively impacted in those birds fed DDGS versus those that were not.
Red discoloration of fully cooked poultry products is a sporadic yet chronic problem. Zinc has been reported to replace iron in myoglobin in an irreversible reaction that creates a red pigment. Many poultry producers add Zn and Cu to feed, sometimes over supplementing 50-200 percent because these minerals are important for broiler performance. Therefore, this project evaluated whether Zn or Cu in broiler feed at normal or high levels caused an increase of red discoloration in cooked product. Two studies were conducted on broilers to determine: 1) the effect of feeding different levels of inorganic dietary zinc (Zn), copper (Cu) and roxarsone (As) on growth performance, carcass and meat quality, and blood zinc protoporphyrin/heme ratio (ZPP/H); and 2) the effect of adding high levels of inorganic dietary zinc (Zn) on growth performance, carcass and meat quality, and blood ZPP/H ratio.

In the first study, 1,152 broilers were sexed and grown in 72 pens by sex and treatment (varied dietary levels of Zn, Cu, and As) to 56 d on litter. Results of the first study showed that males in comparison to females had increased body weight, body weight gain, feed intake and AdjFCR; however, females had lower mortality. Supplementation of 240 mg/kg Zn improved AdjFCR in the starter period in males and grower period in females. Supplementation of 100 mg/kg Cu was beneficial for both males and females in the grower and finisher periods. Roxarsone supplementation improved AdjFCR only in the finisher period. Minerals did not increase muscle redness.

In the second study, 288 broilers were sexed and raised in battery cages (to minimize availability of minerals from litter) to 42 d. Dietary Zn had no effect on feed intake of males, however, body weight gain and AdjFCR measured from 1-42 d was better in males fed diets supplemented with 120 mg Zn/kg. Dietary Zn had no effect on body weight, feed intake, body weight gain or AdjFCR of females. Dietary Zn had no effect on carcass weight and parts yield, however, breast fillet weights were improved in males when Zn was added to the diets. Breast fillet cook yield and tenderness were not influenced by either sex or Zn. Raw breast fillet color was influenced by sex. Breast fillets from males were darker, redder and contained less yellow color. Raw thigh color was also lighter and less yellow compared to females. Raw marrow color measured immediately after harvest was more red and yellow in females. Lightness (L*) and redness (a*) of raw and cooked bone marrow collected from males was influenced by Zn. L* was higher at 120 mg Zn/kg for both raw and cooked marrow, while a* of cooked marrow was higher at 240 mg Zn/kg. Dietary Zn had no effect on the measured ZPP/H ratio except at 28 d, where it tended to be lower at 120 mg Zn/kg. Females had higher ratios at 1, 7, and 42 d. Results indicate that under the current experimental conditions, supplementing high levels of dietary Zn did not further enhance performance of broiler females while 120 mg Zn/kg improved performance in males. Also, supplementing 120 and 240 mg Zn/kg improved breast tender size and increased marrow redness. Fe levels in the body, diet and litter could have been sufficient to prevent replacement of Zn in the myoglobin and hemoglobin. It is also possible that levels of myoglobin and hemoglobin in chicken breast and thigh are not high enough to result in a clear effect. However, the increase in marrow redness from Zn does show the possibility that muscle in contact with bones could be affected with higher redness values.
Dr. Sarge Bilgili Receives 2015 Charles Beard Research Excellence Award

"Dr. Bilgili's research program is a great example of how USPOULTRY research funds can be directed toward important, applied research to find solutions to current problems faced by the poultry industry. The quality of Dr. Bilgili's research is outstanding, and the results have been used by the broiler industry to make improvements in several areas including product quality, food safety and broiler production," remarked Dr. John Glisson, vice president for research programs for USPOULTRY.

Dr. Bilgili received his DVM from Ankara University in Turkey, his MS from Oregon State University and Ph.D. from Auburn University. Dr. Bilgili joined the Department of Poultry Science at Auburn University in 1985 as an assistant professor and extension poultry processing specialist, later attaining the ranks of associate professor (1991) and professor (1996). His scholarly work uniquely bridges live production and processing phases of the broiler industry.

USPOULTRY and the USPOULTRY Foundation are proud to recognize Dr. Sacit “Sarge” F. Bilgili, professor and extension specialist in the Auburn University Department of Poultry Science, as the 2015 recipient of the annual Charles Beard Research Excellence Award. The award is named in honor of Dr. Charles Beard, former director of the Southeast Poultry Research Laboratory and retired vice president of research at USPOULTRY.

The USPOULTRY Foundation Research Advisory Committee selected Dr. Bilgili for this prestigious award based on his exceptional research to enhance efficiency and product quality in the broiler industry. During the course of his research, Dr. Bilgili and his colleagues have received numerous research grants from USPOULTRY and the USPOULTRY Foundation to investigate methods to improve the quality and safety of broiler products and improve poultry production methods. Dr. Bilgili has an impressive record of communicating his research findings to the broiler industry and assisting in implementing innovations.

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. Bilgili received a $1,500 cash prize. The award was presented to him during the International Poultry Scientific Forum, held in conjunction with the 2015 International Production & Processing Expo, by Dr. Beard and Dr. John Smith, Fieldale Farms Corporation, and chairman of the Foundation Research Advisory Committee.

"The U.S. Poultry & Egg Association has been very supportive of our research program over the years, and I am extremely grateful for this support. Dr. Beard's research career is impressive, and I am pleased to receive this award that bears his name," commented Dr. Bilgili.