The application of cultures of *Lactobacillus* spp. isolates with or without a chitosan coating reduce *Campylobacter jejuni* on chicken wingettes. Komala Arst,1 Ann Woo-Ming,1 Basanta R. Wagle,1 Sandip Shrestha,1 Abhinav Upadhyay,1 Pam J. Blore,1 Ann M. Donoghue,2 Kumar Venkitanarayanan,1 and Dan J. Donoghue1.

The presence of *Campylobacter* on poultry products remains one of the leading causes for foodborne illness in the US Increased consumer preference for more natural and less processed food products has led to an increased focus on alternative forms of improving food safety. The use of lactic acid bacteria (LAB) as a bio-preservative/protective culture in food commodities is an ancient technology that is safe and natural. In this study, 13 *Lactobacillus* spp. isolates were screened by a chicken skin dipping model to evaluate the potential to reduce *C. jejuni* counts. From this screening assay, 4 isolates (isolates 1–4) which produced >1 log reduction in *Campylobacter* counts were chosen for further evaluation in a chicken wingette model. In replicate trials, chicken wingettes were inoculated with *C. jejuni* (~7 Log cfu/mL) and treated with either a *Lactobacillus* broth culture or a BPD control (n = 5 samples/treatment). *Campylobacter* counts were determined at d 0, 1, 3, 5 and 7 post treatment. *Campylobacter* counts were log10 transformed and data were analyzed using ANOVA with the PROC MIXED procedure of SAS. Isolates 2 or 4 were the most effective and consistently reduced *Campylobacter* counts from d 1 through d 7 (P < 0.05). For the follow-up studies, isolates 2 and 4 were subjected to additional testing aimed at assessing potential synergistic activity between the *Lactobacillus* isolates and their combination with a 2% chitosan (CH) solution. Each isolate by themselves or when combined with chitosan significantly reduced *Campylobacter* counts (~1–2.5 log reduction) from d 1 through 7. Even though CH or isolates+CH applied as a coating reduced *Campylobacter* counts on wingettes, they did not demonstrate any additional reduction compared with the isolates alone. Our studies demonstrate the potential use of *Lactobacillus* isolates as a protective culture to reduce *Campylobacter* counts on raw poultry.

**Key Words:** *Campylobacter jejuni*, food safety, poultry, protective culture

Validation of peroxycetic acid, lactic acid, lactic and citric acid blend, and sodium hypochlorite against unstressed- and cold-stress-adapted salmonella on broiler carcasses and wings processed at a small USDA-inspected slaughter facility in West Virginia. Lacey Lemonakis,1 KaWang Li, Jordan Garry, Payton Southall, and Cangliang Shen, *West Virginia University, Morgantown, WV.*

Locally grown and pastured poultry products produced by small processors are of particular food safety concern due to their exemption of USDA-FSIS Poultry Products Inspection Act. This study aims to evaluate the efficacy of commercial antimicrobials to inactivate unstressed- and cold-stress-adapted *Salmonella* on broiler carcasses and wings processed at a small USDA-inspected facility in West Virginia. Fresh chilled broiler carcasses and wings were spot inoculated with a 2-strain mixture of unstressed and cold-stress-adapted (4°C, 5 d) *Salmonella Typhimurium* and Tennessee (5.5 to 6.0 log10cfu/mL of sample rinsate), and then undipped, or dipped into peroxycetic acid (PAA; 1,000 ppm), lactic acid (LA; 5%), lactic and citric acid blend (LCA; 2.5%), and sodium hypochlorite (SH; 70 ppm) for 30 s without (carcasses) or with 2-min drain (wings). Surviving bacteria were recovered in buffered peptone water (60 s shake) and spread-plated onto tryptic soy agar, XLT-4 and HardyCHROM agar for analyzing total microbial population and *Salmonella*, respectively. Data (3 replicates/3–4 samples/replicate) were analyzed using the Mixed Model procedure of SAS. For broiler carcasses, unstressed and cold-stress-adapted *Salmonella* behaved similar (P > 0.05) after treated with antimicrobials, and the reduction ranged from 0.6 to 1.7 log10cfu/mL (unstressed) and 1.0 to 1.9 log10cfu/mL (cold-stress-adapted), respectively, based on the counts recovered on XLT-4 and HardyCHROM agar. For chicken wings, reduction of cold-stress-adapted cells (0.9 to 1.7 log10 cfu/mL) were greater (P < 0.05) than those from the unstressed cells (0.5–1.3 log10 cfu/mL). Reduction of *Salmonella* on carcasses and wings increased in the order SH ≤ LCA < LA < PAA, irrespective of unstressed or cold-stress-adapted cells. Results indicated that applying post-chilling antimicrobial dipping treatments was an effective intervention to reduce *Salmonella* contamination on locally raised and processed broiler carcasses and wings.

**Key Words:** pastured broiler carcass, wing, *Salmonella*, antimicrobial, cold stress

Gut microbiota-mediated suppression of virulence and antibiotic resistance of *Salmonella Typhimurium* DT104 by original XPC in an in vitro poultry model. Victor L. Nseré,1 Tom Weigand1, Steve A. Carlson2, Joan M. Butler1, Don R. McIntyre3, and Mark F. Scott1. *Diamond V, Cedar Rapids, IA.*

*Salmonella* is typically avirulent in poultry; however, it can become a formidable food safety hazard when chicken meat is contaminated during processing. In a recent study, when broilers were challenged with a multiple-antibiotic resistant (MAR) *Salmonella Typhimurium* (ST) strain, feeding Original XPC (XPC) mitigated this risk by reducing fecal shedding, large intestinal colonization, virulence and antibiotic resistance when compared with the control (CON). In view of these findings, we utilized an in vitro model to determine whether the effects of XPC on virulence and antibiotic resistance of ST are: (1) independent of the host animal; and, (2) dependent on the presence of gut microbiota. Fresh excreta obtained from broilers, fed a finisher diet, served as the source of gut microbiota. Under anaerobic conditions, buffered pH 6.8 excreta, or buffer alone; predigested (pepsin, pH 2.0; pancreatin, pH 7.0) finisher diet, with or without XPC (n = 5); and MAR ST strain DT104 (1 × 104 cfu/mL, final concentration) were added to vessels and incubated with continuous mixing (39°C; 24 h). ST was enumerated (XLT4 medium), and then subjected to a human epithelial type 2 cell invasion assay, as a measure of virulence. Antibiotic sensitivity testing was performed using chloramphenicol (32μg/mL). The entire trial was performed 3 times, and the combined data analyzed using JMP. When treatments were incubated in buffer only, ST from CON and XPC had similar (P > 0.05) invasiveness (1.0%); however, antibiotic resistance was marginally lower (P < 0.05) for XPC (84%) than CON (98%). In the presence of gut microbiota, XPC reduced invasiveness of ST (P < 0.05) from 1.1% (CON) to 0.5% (XPC) and antibiotic resistance (P < 0.05) from 90% (CON) to 35% (XPC). Feeding XPC suppressed virulence and re-established antibiotic sensitivity of ST, at least in part, via
its influence on gut microbiota. Furthermore, the observed effects were not dependent on extra-intestinal host factors.

**Key Words:** Salmonella, food safety, virulence, antibiotic resistance

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**474P** Microbiological quality assessment, Salmonella and Campylobacter prevalence in broiler ceca and ready-to-cook carcasses. KaWang Li*SC, Lacey Lemonakis, Brian G. Glover, Jordan Garry, Payton Southall, Joseph S. Moritz, and Cangliang Shen, *West Virginia University, Morgantown, WV.*

Foodborne pathogens are concerns for ready-to-cook carcasses produced by small scale and mobile processing units. The objective of this study was to evaluate the microbiological quality, Salmonella, and Campylobacter prevalence in broiler ceca and ready-to-cook carcasses. Straight-run Hubbard × Cobb broilers were reared for 38 d on either clean shavings or built-up litter. A total of 64 carcasses (30 from clean shavings and 34 from built-up litter) were processed at the West Virginia University pilot processing facility that mimics a mobile poultry processing unit. Aerobic plate counts (APC), *E. coli*/coliforms, and yeast/molds of carcasses were analyzed on petrifilms. For *Salmonella*, ceca and carcasses were pre-enriched in buffered peptone water (BPW), second-enriched in Rappaport-Vassiliadis medium and streak-plated onto XLT-4 and HardyCHROM-agar, and further confirmed by API-20E kits and qPCR (InvA gene). For *Campylobacter*, ceca and carcass samples were harvested in Bolton broth and on modified Campy-Cefex agar under microaerophilic conditions (5.0% O$_2$, 10% CO$_2$, and 85% N$_2$) at 42°C for 48–72h, and further confirmed using *Campylobacter* Latex Test Kit and Gram-Staining. Data were analyzed using the t-test and Chi-Square of SAS. APC, coliforms, and *E. coli* were 3.4–3.5, 2.2–2.5, and 2.1 log$_{10}$cfu/mL, respectively on clean-shavings and built-up litter carcasses. Carcasses of broilers raised on built-up litter contained greater ($P < 0.05$) yeast/molds population (2.2 log$_{10}$cfu/mL) than those reared on clean-shavings (1.8 log$_{10}$cfu/mL). *Salmonella* was not detected in any ceca samples, while 6% (2 of 34 carcasses) of the carcasses from the built-up litter were present with *Salmonella*. The prevalence of *Campylobacter* decreased ($P < 0.05$) in ceca (28% vs. 60%) and carcasses (77% vs. 88%) obtained from broilers reared on clean shavings compared with those reared on built-up litter. These data suggest that raising broilers on clean shavings as opposed to built-up litter may decrease the presence of foodborne pathogens.

**Key Words:** broiler, *Salmonella*, *Campylobacter*, ceca, carcass

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**476P** In vitro evaluation of four commercially available *Bacillus* spp. probiotic supplements and their effect on an antibiotic resistant strain of *Salmonella* Heidelberg. Claudia D. Castañeda*WSC, Omar Gutierrez2, Christopher D. McDaniel1, and Aaron S. Kiess1, 1Mississippi State University, Mississippi State, MS, 2Huvepharma Inc., Austin, TX.

Bacterial resistance to antibiotics is a worldwide issue, however probiotics have also been found to reduce pathogens associated with poultry. Therefore, the objective of the current study was to evaluate 4 commercially available *Bacillus* spp. probiotic supplements for their ability to reduce an antibiotic resistant strain of *Salmonella* Heidelberg (S), in vitro. Treatments included: an antibiotic resistant strain of S (control), 4 probiotics (P1, P2, P3, P4), and the combination of S with each probiotic (S+P1, S+P2, S+P3, S+P4). A 12 h S stock culture was diluted 10-fold to provide the S control of 10$^8$ cfu/mL. Probiotics were weighed in an attempt to provide 10$^6$ cfu/mL of bacteria. For combinations, 1 mL of S stock culture was added to 9 mL of nutrient broth containing 10$^6$ cfu probiotic/mL. After treatments were made, 100 µL of each treatment was serially diluted at 0, 12, and 24 h of incubation, spread plated onto the appropriate agar (Mannitol Egg Yolk Polymixin (MYP) agar for probiotics and Tryptic Soy (TSA) agar containing Nalidixic acid for S) and incubated at 37°C for 24 h under aerobic conditions. Log-transformed counts were analyzed using a randomized complete block design with split plots over incubation times. The means were separated using Fisher’s protected LSD when $P \leq 0.05$. A treatment by time interaction was detected for S growth ($P = 0.05$). Only at 0 h was S growth reduced by the addition of each probiotic. However, by 12 h of incubation, S+P3 increased S growth as compared with the S control and S+P2. Probiotic growth was also affected by the presence of S and the length of incubation. The main treatment effect revealed that S+P1 had less probiotic growth when compared with P3 and P4 (6.5 vs 7.3 and 7.2 log cfu/mL, respectively; $P = 0.03$). However, a treatment by time interaction ($P = 0.03$) indicated that S alters probiotic growth over incubation. In conclusion, all these probiotics can reduce S immediately after incubation, aerobic and anaerobic bacteria in the cultures were enumerated on agar media composed of the same lactate concentration in which the cultures had been grown and 1.5% Bacto agar. Inoculated agar plates were incubated aerobically or anaerobically at 37°C for 48 h. Colony-forming units were counted, and isolated colonies were selected for identification using the Biolog Bacterial Identification System. Results indicated that lactate concentration of the media produced no significant difference in the number of aerobic or anaerobic bacteria recovered from the cecal cultures. Furthermore, there were differences in the bacterial flora of the cecal cultures from the 3 experiments, and different incubation atmospheres selected for different cecal bacteria. In addition to *Escherichia coli*, *Enterococcus* spp. was among the major antibiotic isolates recovered in all trials, while anaerobic isolates included *Veillonella* and *Propionibacterium* spp. Findings also indicated that the composition of cultures from media supplemented with lactate differ from cultures from media supplemented with succinate. However, other media supplements may be required to select beneficial bacteria from different cecal cultures.

**Key Words:** broiler, cecal bacteria, lactate, aerobe, anaerobe

Antibiotics use in the poultry industry as therapeutics or growth promoters have been reported to increase sensibility to some pathogens. In the present study, 2 independent experiments were conducted with one-day-old broiler chickens. Chicks were allocated in electrical batteries into one of 4 groups with n = 25 birds: 1) control; 2) Enrofloxacin (EN) 25 mg/kg; 3) EN 50 mg/kg; and 4) EN 75 mg/kg. EN treatment was administered in the drinking water from d 1 to 5. At d 7, all chickens were oral gavage with 10^6 cfu Salmonella Enteritidis (SE). Twenty-four hours post SE inoculation chickens were euthanized and liver and ceca-cecal tonsils (CCT) were cultured. In Exp. 1, a significant increased (P < 0.05) Log10 cfu/g of SE in CCT was observed in groups 3 (EN 50 mg/kg) and 4 (EN 75 mg/kg): 1.23b; 0.21b; 4.46b and; 5.61a with an incidence of 41, 50, 100 and 100% SE among control and EN levels of 25, 50 and 75 mg/kg respectively. Log10 cfu/g of SE in liver were 0a, 0.90a, 1.25b and 2.70a, with an incidence of 0, 33.3, 41.7 and 100% between control and EN levels of 25, 50 and 75 mg/kg respectively. In Exp. 2, Log10 cfu/g of SE in CCT was 0.25b, 4.94b, 4.61b and 5.53a of cfu, with an incidence of 0, 33.3, 75, and 100% between control and EN levels of 25, 50 and 75 mg/kg respectively. Log10 cfu/g of SE in liver was 0.22b, 1.12b, 0.90b and 2.25a with an incidence of SE: 83.3, 33.3, 50 and 83.33% between control and EN levels of 25, 50 and 75 mg/kg respectively. These data support and extend previous investigations involving other salmonellae and indicate that selected antibiotics may increase the severity and frequency of SE colonization and invasion rate in broiler chicks.

Key Words: Salmonella Enteritidis, enrofloxacin, broiler, antibiotic, infection


Bacillus species are at the forefront of probiotic research due to their ability to produce secondary metabolites, which inhibit the growth of many pathogenic bacteria. The aim of this study was to measure the inhibition ability of Bacillus spp. against Escherichia coli, Salmonella spp., and Clostridium perfringens. To accomplish this, 10 strains each of E. coli, Salmonella spp. and C. perfringens were grown overnight in brain heart infusion broth (BHIB) under the appropriate conditions. Each of the 30 cultures was then spread on 4 trypticase soy agar (TSA) plates. Three sterile discs were placed on each inoculated plate, to achieve 3 replicates per plate. The discs were inoculated with Bacillus amyloliquefaciens strains AB01 and AP183, and Bacillus subtilis strains AP206 and AP302 (30 plates/Bacillus strain). Each strain was grown for approximately 96 h in a BHIB. After which, they were centrifuged, supernatants removed, and 10 microliters (mL) of supernatant were added to the sterile discs onto their designated plates. Plates were incubated for 24 h at either 37°C in aerobic conditions (E. coli and Salmonella spp.), or 40°C in anaerobic conditions (C. perfringens). Any zones of inhibition (ZI) present were measured in centimeters (mm) with the diameter of a zone 15 mm being considered significant. No E. coli and Salmonella spp. inoculated plates were affected by the presence of Bacillus spp. supernatant. Of the C. perfringens inoculated plates, 3/10 had ZI present against all 4 Bacillus strain’s supernatants. Due to the lack of inhibition on most of the plates, statistical analysis was not conducted on this data. The AP206 supernatant had the largest ZI (24 mm) when values were averaged among these 3 C. perfringens strains, followed by AB01 (24 mm), AP302 (22 mm) and AP183 (20 mm). One additional C. perfringens strain was inhibited by AP206 (16 mm). This study has shown that the secondary metabolites produced by these 4 strains of Bacillus spp. were able to inhibit the growth of C. perfringens in an in vitro setting, providing evidence of their potential to reduce the prevalence of C. perfringens infections in poultry.

Key Words: probiotic, pathogen, inhibition, Bacillus, Clostridia

479P  Effect of feeding Original XPC on Salmonella enumeration and prevalence in ceca, breast, and ground breast meat in heat-stressed and non-heat-stressed broilers. Christine Z. Alvarado*, Jiyang Fang1, Gerardo Casco1, Gregory S. Archer1, James A. Byrd2, Paul T. Price4, Douglas P. Smith3, and Hilary O. Pavlidis4, 1Texas A&M University, College Station, TX, 2Southern Plains ARS-USDA, College Station, TX, 3Diamond V, Cedar Rapids, IA.

A study was conducted to evaluate the effects of feeding Original XPC to reduce Salmonella prevalence and numbers in ceca, breast fillet and ground breast fillets when broilers are reared in ambient or heat stress temperatures. A total of 320 Ross 708 broilers were fed either a control diet (CON) or a diet containing Original XPC (XPC) at 1.25 kg/MT. Half the birds in each dietary treatment were subjected to either no heat stress (70°F constant) or heat stress (95°F:70°F for 18.6 h daily) from 28 to 42 days. Two pens (16) were housed in environmentally controlled rooms with treatments replicated in each room. On d 3, Salmonella free chicks were orally challenged with 0.25 to 0.5 mL of 10^5 cfu/mL of antibiotic marked strains of Salmonella Typhimurium. To ensure Salmonella colonization, 5 birds per pen were euthanized on d 21. On d 42, 8 birds per pen from both the heat-stress and non-heat-stress environments for CON and XPC were processed following industry practices. Individual ceca (C), right breast fillet (BF) and ground left breast fillet (GBF) were collected for Salmonella prevalence and enumeration. No significant 2-way interactions between dietary treatment and environment were observed so data were pooled for analysis. The prevalence data were analyzed by chi-squared and enumeration data by GLM in SAS with dietary treatment as the main effect. The prevalence of Salmonella for CON-fed birds for C (25.76%) and BF (75.76%) were similar (P > 0.05) to the XPC-fed birds (28.99% and 79.71%; respectively). However, in the GBF a significantly lower (P = 0.046) Salmonella prevalence was observed for the XPC-fed birds (15.94%) versus CON-fed birds (30.03%). The Salmonella counts (log10 cfu/mL) for the XPC-fed birds in the C (0.91 ± 0.08), BF (1.47 ± 0.09) and GBF (1.13 ± 0.12) were similar (P > 0.05) to the counts observed on the CON-fed birds (0.96 ± 0.10, 1.32 ± 0.06, 1.40 ± 0.13; respectively). In conclusion, XPC has the potential to reduce the prevalence of Salmonella in broiler ground breast meat.

Key Words: XPC, Salmonella, ground breast, broiler ground breast meat

480P  Ice slurry to reduce processing intensity in poultry sanitation. Stephanie Richter*, Ebony Rowe, Daniel Sabo, and Comas Haynes, Georgia Tech Research Institute, Atlanta, GA.

In 2013, the United States processed 8.6 billion chickens, according to the US Department of Agriculture. This large volume promotes the development of new environmentally friendly and cost-effective food safety technologies for poultry processing. Conventional poultry
processing methods thus far have used chilled water or cooled air treatments for pathogen reduction. We used ice slurry to test its ability to more rapidly reduce cooling time and pathogen levels. Ice slurry is known to have greater heat capacitance than water due to its 2-phase coolant properties. We hypothesized that ice slurry would provide an antimicrobial scrubbing effect. Each trial tested 20 birds, where half were the control (pre-chiller) and half were the chiller intervention (chilled water or ice slurry). All birds were inoculated onto the center of the breast skin with Salmonella enterica serovar typhimurium, a nalidixic acid antibiotic-resistant strain. All birds were heated for 2 h to ~35°C to simulate core temperatures after evisceration. The chiller treatment went into a 250-gallon air-agitated auger chiller filled with 125 gallons of water with a set ppm (parts per million) of peracetic acid (PAA) for 20 min. The peracetic acid acted as the antimicrobial processing condition. All birds were then processed for microbial analysis. For processing, the inoculated breast area was excised and treated with a solution containing 100 ppm of nalidixic acid antibiotic. The antibiotic treatment was used to ensure specific growth of the target organism. All samples were plated onto 3M Petrifilm and incubated for 24 to 48 h at 37°C. Chilled water temperatures averaged 4°C, while ice slurry media averaged ~1°C in 20-min trials. Results showed a decrease in thermal cooling times with ice slurry versus chilled water. At 50 ppm of PAA and 2.0% salinity, ice slurry showed twice the log-scale reduction when compared with chilled water. We believe ice slurry’s 2-phase mixture provides a physical abrasive phenomenon that adds to carcass disinfection and holds promise as an alternative poultry chilling medium.

**Key Words:** ice slurry, antimicrobial, poultry processing

**481P**  
Evaluating methods for experimental contamination of Salmonella on eggs. Andrew C. Rehkopf*, James A. Byrd, Craig D. Coufal, and Tri Duong, 1Department of Poultry Science, Texas A&M University, College Station, TX, 2USDA-ARS, Southern Plains Agriculture Research Center, College Station, TX.

Salmonella contamination on the surface of eggs is an important critical control point for improving microbial food safety of poultry and poultry products. The recovery of Salmonella from experimentally contaminated eggs is important for studies investigating the horizontal transmission of Salmonella during incubation and hatch and is dependent on the methods used for inoculation. In this study, we evaluated the effect of various methods and media for the experimental contamination and recovery of Salmonella on the surface of broiler hatching eggs. Eggs were inoculated with a suspension of Salmonella in phosphate buffered saline (PBS) using a pipette, a gauze pad, a cotton ball, or a sponge. At 1 h post-inoculation, recovery of Salmonella was similar from eggs inoculated using a pipette, gauze pad, and a sponge. At 1 h post-inoculation, recovery of Salmonella was similar from eggs inoculated using a pipette, gauze pad, and a sponge and was approximately 4 log_{10} cfu egg^{-1} greater than from eggs inoculated using a sponge (P < 0.05). At 24 h post-inoculation, recovery of Salmonella from eggs inoculated using a pipette was approximately 2.4 to 3.4 log_{10} cfu egg^{-1} greater than from eggs inoculated using a gauze pad, cotton ball, or sponge (P < 0.05). Using a pipette, eggs were inoculated with a culture of Salmonella suspended in tryptic soy broth (TSB), PBS, buffered peptone water (BPW), or 10% suspension of feces in PBS and incubated for up to 14 d. At 24 h post inoculation, recovery of Salmonella from eggs inoculated using TSB was approximately 1.5 - 3.5 log_{10} cfu egg^{-1} greater than from eggs inoculated using BPW, PBS, or a fecal suspension (P < 0.05). Also, the number of Salmonella positive eggs was greater when inoculated using TSB (20%) then when inoculated using PBS (0%) or fecal suspension (0%) (P < 0.05). However, no difference in the number of Salmonella positive eggs was detected when eggs were inoculated using BPW (10%) as compared with the other treatments. Of those evaluated, pipetting a culture suspended in TSB on to the surface of eggs was determined to be the most effective method for experimental contamination with Salmonella. These results will inform future studies investigating Salmonella contamination of hatching eggs during incubation and hatch.

**Key Words:** Salmonella, experimental contamination, horizontal transmission, egg

**482P**  
Growth of Salmonella in four enrichment broths at 37 or 42°C. Douglas E. Cosby*, Nelson A. Cox, and Mark Berrang, The US National Poultry Research Center, USDA, Athens, GA.

No single enrichment broth or temperature is used consistently throughout the research, regulatory or industry laboratories for the detection of Salmonella. This lack of a single methodology leads to confusion and possible bias both for and against Salmonella serotypes. The objective of this experiment was to evaluate 4 selective enrichment broths [selenite cystine (SC), tetrathionate Hajna (TT), GN broth (GN) and Rappaport-Vassiliadis (RV)] and 2 temperatures (37°C and 42°C) to determine the one best able to allow growth of 4 Salmonella serovars [Enteritidis (SE), Heidelberg (SH), Kentucky (SK), and Typhimurium (ST)]. Four Salmonella serovars were inoculated individually (10^8 cfu) into duplicate tubes containing 10 mL of each of 4 enrichment broths at each temperature. After overnight enrichment, serial dilutions were prepared and plated onto brilliant green sulfa (BGS) agar plates for enumeration. Counts were made and recorded after 24 h incubation. Three replicates were conducted. All 4 enrichment broths were significantly (P < 0.05) more effective for recovery of Salmonella at 37°C than at 42°C. TT was the least effective at recovering the 4 serotypes at 42°C with only one serotype (SK) recovered. In SC, recovery was log_{10} 4.4, 7.7, 7.6 and 7.5 for SE, SH, SK, and ST, respectively; in GN, recovery was log_{10} 8.2, 8.4, 8.5 and 8.4 for SE, SH, SK, and ST, respectively; and in RV, recovery was log_{10} 8.1, 8.2, 8.2 and 7.9 for SE, SH, SK, and ST respectively when incubated at 37°C. At 37°C, significant differences were observed between TT and GN; TT and RV, SC and GN; and SC and RV and no difference was observed between SC and TT or between GN and RV. At 42°C, no significant difference was observed between the 3 broths, SC, TT and RV, where Salmonella was recovered. Pre-enrichment is used to elevate the numbers of Salmonella allowing detection even with losses due to the stresses of temperature and broths designed to reduce background. Recovery of Salmonella strains can unintentionally be biased by the temperature selected and the enrichment broths used which are often selected simply because of laboratory preference or regulatory protocol.

**Key Words:** selective enrichment broth, Salmonella, recovery

**483P**  

Many different selective enrichment broths can be used to recover Salmonella spp. from multiple sample types associated with poultry and poultry products. This experiment was designed to evaluate the efficacy of 4 enrichment broths, selenite cystine (SC), tetrathionate Hajna (TT), Gram Negative broth (GN) and Rappaport-Vassiliadis (RV) at 2 temperatures, 37 and 42°C for reduction of extraneous microbes on brilliant green sulfa agar. Two ecometric methods (EM1 and EM2) were compared with serial dilution to determine the level of background microflora from whole carcass rinses. Absolute growth indexes were
determined by the 2 ecometric techniques and counts recorded. Three replicates were conducted, data combined and analyzed by t-test to determine significance. No significant differences (P < 0.05) were noted between the 2 ecometric techniques at either 37 or 42°C respectively. TT broth was significantly more effective at reducing the background microflora at 42°C and 37°C than other media. GN and RV broths had the most bacterial growth (<log₁₀ 7.5 cfu/mL) for both temperatures. Significant differences were observed between TT and both GN or RV and between GN and both SC or RV at 42°C. Significant differences were noted using EM1 for SC between 37 and 42°C and using EM2 for GN between 37 and 42°C. As expected, incubation at 42°C reduced the background bacteria for all of the selective enrichment broths with TT being the most effective reducing the bacteria count by log₁₀ 1.3, 2.6 and 3.1 cfu/mL when compared with SC, RV and GN respectively. By determining the most effective broth for reducing background microflora, researchers should be able to more effectively isolate and identify Salmonella spp. from poultry samples.

Key Words: Salmonella, enrichment, ecometrics, recovery

484P  Inactivation of Salmonella Enteritidis on shell eggs by coating with phytochemicals. Indu Upadhyaya*, Hsin Biao Yin, Meera Surendran Nair, Chi-Hung Chen, Rebecca Lang, Michael J. Darre, and Kumar Venkitanarayanan, University of Connecticut, Storrs, CT.

Salmonella Enteritidis (SE) is a major foodborne pathogen that causes human infections largely by consumption of contaminated eggs. The external surface of eggs becomes contaminated with SE from multiple sources, highlighting the need for effective egg surface disinfection methods. This study investigated the efficacy of 3 GRAS-status, phytochemicals, namely carvacrol (CR), eugenol (EG) and β-resorcylic acid (BR) applied as pectin or gum arabic based coating for reducing SE on shell eggs. White-shelled eggs, spot inoculated with a 5-strain mixture of nalidixic acid (NA) resistant SE (8.0 log cfu/mL) were coated with pectin or gum arabic solution containing each phytochemical (0.0, 0.25, 0.5 or 0.75%), and stored at 4°C for 7 d. Three eggs per treatment at every sampling point for each temperature were included in all 3 replicated experiments (n = 756, n = 9). SE on eggs was enumerated on d 0, 1, 3 and 7 of storage. Approximately 4.0 log cfu/egg of SE was recovered from inoculated and pectin or gum arabic coated eggs on d 0. However, all coating treatments containing CR and EG, and BR at 0.75% reduced SE to undetectable levels on d 3 (P < 0.05). Results suggest that the aforementioned phytochemicals could effectively be used as a coating to reduce SE on shell eggs, but detailed studies on the sensory and quality attributes of coated eggs need to be conducted before recommending their use.

Key Words: Salmonella Heidelberg, pectin, gum arabica, broccoli, trans-cinnamaldehyde, β-resorcylic acid, in-feed supplementation

486P  Direct and indirect transmissibility of attenuated and virulent Mycoplasma gallisepticum strains among caged layers. Jeffrey D. Evans*, Scott L. Branton, Joseph L. Purswell, Spencer A. Leigh, and Stephanie D. Collier, USDA-ARS Poultry Research Unit, Mississippi State, MS.

Mycoplasma gallisepticum (MG) is a major pathogen of avian species and is considered the most economically important Mycoplasma species affecting poultry. Means of control against MG are limited to bio-security and bio-surveillance across the poultry industries and live attenuated vaccines with commercial table egg layers. MG has been characterized as readily transmissible by direct and indirect measures and therefore further characterization of MG’s transmissibility is necessary. To evaluate the ability of MG strains to pass from infected to non-infected poultry, naïve Hy-Line W-36 pullets were obtained at 1 d of age. At 10 weeks of age (woa), pullets (n = 240) were singly caged among 4 identical rooms of a commercial-type layer facility each containing 4 rows of vertically offset cages (20 cages/row). At 12 woa, pullets in 2 rooms were inoculated with an F strain-derived attenuated MG (FMG) vaccine. At 7 d post-inoculation, naïve sentinel pullets (n = 16) were co-housed with inoculated pullets or were placed in cages separated from inoculated pullets by at least 1 foot. After 10 wk of exposure, inoculated pullets and sentinels were assessed for MG via serum plate agglutination (SPA). To assess the effect of virulent MG challenge on transmissibility, 1 room each of naïve and FMG-vaccinated pullets (21 woa) were inoculated with a virulent challenge strain of MG. Sentinels were placed and maintained as previously described and following 10 wk of exposure, MG status was assessed for all MG-inoculated pullets and sentinels. Results indicated that following the initial inoculation, FMG was transmitted to 37.5% of the co-housed sentinels, but no evidence of FMG transmission to birds separated by at least 1 foot was found.
Following the MG challenge of naïve pullets (21 woa), the virulent MG strain was transmitted to 68.75% of co-housed sentinels, but not to sentinels separated from challenged pullets by at least a foot. Virulent MG challenge of pullets previously inoculated with FOG demonstrated transmission rates of 37.5% and 18.75% among co-housed and separated sentinels, respectively.

**Key Words:** Mycoplasma gallisepticum, vaccine, horizontal transmission

487P  Microbial changes in young turkeys after transportation stress. Samantha M. Anderson*, Evan Hutchison, Rebecca Kangas, Josh Rehberger, Alexandra Smith, Eric Vang, and Thomas Rehberger, Agro Biosciences Inc., Milwaukee, WI.

Early colonization of beneficial bacteria in the gastrointestinal tract has been shown to play a vital role in the overall health and performance of commercial turkeys. Other factors, like stress, may have a negative effect on the early colonization of these key bacteria. In this study, we investigated the effect that early stress can have on the homeostasis and pathogen levels of newly hatched turkeys that were transported long distances and experienced elevation changes. Bacterial diversity by terminal restriction fragment length polymorphism (TRFLP) as well as enumeration of lactic acid bacteria (LAB) and avian pathogenic E. coli (APEC) of gastrointestinal tracts (GITs) were used to compare treatment groups within 2 studies: (1) 20 day-of-hatch (DOH) GITs and 20 day-old GITs from flocks that were transported; and (2) 20 DOH GITs, 20 day-old GITs transported and 20 day-old GITs that were not transported; day-old birds received a Bacillus/Lactococcus-based direct-fed microbial (DFM). The results from both studies showed that the level of APEC was below detectable levels in the DOH birds while the day-old birds that were treated with DFM had lower APEC levels compared with the untreated post transit birds. Pre and post transit LAB levels were not different in the first study but LAB levels were significantly higher in DFM treated day-old birds compared with DOH birds. TRFLP indicates that Enterobacteriaceae was the most abundant taxa in post-transit birds, but this trend was not observed in the day-old transported chicks that received the DFM (P ≤ 0.05). TRFLP also showed that day-old birds treated with the DFM had lower proportions of Enterococcus and higher proportions of Bacillus, a component of the DFM, when compared with untreated day-old birds (P ≤ 0.05). In conclusion, the DFM treatment of young turkeys seemed to mediate the negative microbial shifts associated with the stress caused by transportation.

**Key Words:** microbiota, terminal restriction fragment length polymorphism (TRFLP), turkey, direct-fed microbial, stress

488P An investigation of microbial differences between commercial breeder flocks that historically produce high and poor preforming progeny. Rebecca Kangas*, Samantha Anderson, Evan Hutchison, Joshua Rehberger, Alexandra Smith, Eric Vang, and Thomas Rehberger, Agro Biosciences Inc., Wauwatosa, WI.

Research has shown that the intestinal microbial composition established in early life of poultry helps to foster immune development and create a positive microbial progression, which directly affects overall bird performance. To evaluate the effect of microbial composition on commercial turkey performance, this study investigated the microbial differences of 60 birds across 3 different flocks, 2 flocks contained hens that produce historically high-performing progeny (HPP) and their corresponding day-of-hatch (DOH) chicks and 1 flock that contained hens that historically produce poor-performing progeny (PPP) and their corresponding DOH chicks. HPP hens and PPP hens were defined by the feed conversion rate and 7 d mortality of their offspring. Terminal restriction fragment length polymorphism (TRFLP) analysis of microbial community in HPP hens revealed greater Bacillus spp. proportions than PPP hens, while PPP hens had greater Campylobacter and Mycobacterium/ Rhodococcus proportions than HPP hens (P ≤ 0.05). DOH chicks from HPP hens had greater Enterococcus proportions, while DOH chicks from PPP hens had greater Clostridium proportions (P ≤ 0.05). These results indicated that there is a difference in microbial composition between HPP and PPP hens and that these differences may contribute to differences in overall performance of the corresponding chicks.

**Key Words:** microbiota, terminal restriction fragment length polymorphism (TRFLP), community, bacteria, performance

489P Analysis of the vertical transmission of gut microbiota from hen to chick in commercial broiler systems. Eric Vang*, Samantha Anderson, Rebecca Kangas, Josh Rehberger, and Tom Rehberger, Agro Biosciences, Wauwatosa, WI.

Studies have shown the vital role the early colonizing gut microbiota have in the overall health and performance of chickens through the development of a healthy immune system, improved digestion and nutrient absorption and reduction of enteric pathogens. It has been reported that in natural settings much of the early colonizing gut microbiota is maternally derived; however, little is known about the extent of vertical transmission of the gut microbiota in a commercial broiler system. This study investigated microbial diversity of the gastrointestinal tracts (GITs) of day-of-hatch (DOH) broilers and their respective maternal hens for evidence of vertical transfer of microbiota from hens to chicks. GITs from hens (n = 43) and DOH (n = 141) birds across 3 different US broiler companies were examined in this study. Terminal restriction fragment length polymorphism (T-RFLP) with lactic acid bacteria (LAB) specific primers was used to evaluate the LAB diversity in hen and DOH gut samples. UPGMA cluster analysis with Dice similarity coefficients indicated 2 distinct clusters: one cluster grouping mainly the DOH and the other cluster grouping the hens. Pediococci, enterococci, Lactobacillus, Enterococcus crasatus, L. salivarius, and L. johnsonii peaks were present in the hens. Most DOH birds had a pediococci peak, while enterococci were present in some DOH birds from 2 out of the 3 companies. Peaks for L. salivarius, L. crasatus, and L. johnsonii were present in the hens and largely missing in DOH birds. These findings were consistent with genotypic comparisons of individual strains of LAB isolated from these samples. These results suggest a limited vertical transmission of the maternal LAB from hens to DOH birds. The loss of maternal microbial diversity in the commercial broiler system may have a negative effect on bird health and performance.

**Key Words:** vertical transmission, terminal restriction fragment length polymorphism (T-RFLP), lactic acid bacteria

490P Efficacy of octenidine hydrochloride in reducing Salmonella Enteritidis on chicken. Hsinbai Yin*, Chihung Chen, Michael J. Darre, and Kumar Venkitanarayanan, University of Connecticut, Storrs, CT.

Salmonella Enteritidis (SE) is a foodborne pathogen commonly transmitted to humans by consumption of contaminated poultry meat. Effective disinfection of chicken carcasses is critical to reduce SE on poultry meat.
and control human illnesses associated with the pathogen. We investigated the efficacy of octenidine hydrochloride (OH), a new generation disinfectant used in human mouth rinse, as an antimicrobial wash treatment in rapidly inactivating SE on chicken. Commercial chickens wings, spot inoculated (~5.0 log cfu/wing) with a 5-strain mixture of nalidixic acid-resistant SE, were washed with sterile water containing 0, 0.05, 0.1, or 0.2% OH at 25°C for 30 or 60 s, followed by a sterile water rinse to remove OH residue from wings. An untreated group of chicken wings subjected to no water and OH wash treatments was included as baseline. All samples were stored at 4°C, and surviving SE populations on wings were enumerated on d 0, 1, and 2 by serial dilution and spread plating on xylose lysine deoxycholate agar. Duplicate samples of each treatment and baseline were included, and the experiment was replicated 3 times. Results revealed that all OH wash treatments for 30 and 60 s were effective in reducing SE on chicken wings as compared with the baseline ($P < 0.05$). Treatment containing 0.1% OH for 60 s reduced SE by ~3.5 log/wing, whereas 0.2% OH wash decreased the pathogen to undetectable levels ($P < 0.05$). However, substantial pathogen populations (~4.0 log cfu) survived on wings washed with sterile water. In addition, no SE was detected in OH wash solution. The results indicate that OH could potentially be used as a wash treatment to reduce SE on chicken carcasses, however, consumer acceptability of OH-washed wings needs to be determined before recommending its use.

Key Words: Salmonella Enteritidis, octenidine hydrochloride, chicken wings, wash treatment


The objective of this study was to identify and describe antimicrobial resistance (AMR) in Salmonella isolated from poultry farms. Four farms were sampled in 2 different seasons; each farm had 4 houses, for a total of 16 houses. Each house was divided into 4 quadrants and drag swabs, cloacal swabs and litter samples were collected from each location. Antimicrobial resistance (AMR) was tested in 50% of the isolates from each sample. The Salmonella isolates were confirmed by PCR and subjected to 14 different antimicrobials using the NAMRS panel. A total of 403 isolates were tested for AMR and 100% of the isolates showed resistance to at least 2 classes of antimicrobials. Out of 403 isolates, 99% of the isolates exhibited resistance to more than 2 classes of antimicrobials. Out of the 403 isolates showing antimicrobial resistance, 179 were confirmed to be Salmonella by PCR, resulting in 44% of prevalence of Salmonella with antimicrobial resistance on these poultry farms. More than 60% of the isolates were resistant to azithromycin, cefotiofur, streptomycin and sulfisoxazole, which belong to macrolides, cephems, aminoglycosides and sulfonamides class, respectively. Some of the antimicrobial classes are being used for veterinary medicine, human medicine or both. Azithromycin and sulfisoxazole are antimicrobials that are being used for human medicine and could pose a threat when used to treat human salmonellosis. Bacteria with resistance to antimicrobials used for human medicine result in public health issues due to the difficulties in treatments for infections with antimicrobial resistant bacteria since it limits the treatments or make them less effective.

Key Words: Salmonella, antimicrobials, poultry, multidrug resistance