# Food Safety: General Aspects

**1085 C-di-GMP signaling pathways are critical for acid resistance of *E. coli* O157:H7.**
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Surviving the acidic environment of gastrointestinal tract is important for *E. coli* O157:H7 pathogenesis. However, factors contributing to acid resistance remain poorly understood. The second messenger cyclic diguanosine monophosphate, c-di-GMP, affects various aspects of bacterial physiology. To test its role in acid resistance, strain O157:H7 and selected mutants in c-di-GMP metabolism were exposed to pH 3.5 for 15 min. Cell survival and mRNA levels (measured by qRT-PCR) of selected genes involved in c-di-GMP metabolism and acid resistance were analyzed. In the wild type, acid challenge resulted in increased mRNA levels of the newly identified c-di-GMP receptor gene, *ydiV* (7-fold) and the major c-di-GMP phosphodiesterase gene, *yhjH* (13-fold). Deletion of *ydiV* or *yhjH* impaired acid challenge survival by 600 and 35 fold, respectively. Consistent with this observation, expression of several genes responsible for acid resistance, *asr*, *dsbA* and *katP*, were decreased in the *ydiV* and *yhjH* mutants, compared with the wild type. Interestingly, overall expression profiles of the *ydiV* and *yhjH* null mutants were not identical. In the *ydiV* mutant, mRNA levels of *katG* were lower, whereas those of *gad*, *oxyR* and *cysB* were higher, compared with the *yhjH* mutant and wild type. Under neutral pH conditions, expression of all listed genes was not different among tested strains. In conclusion, in *E. coli* O157:H7, (i) acid stress strongly affects expression of genes involved in c-di-GMP metabolism; (ii) c-di-GMP signaling pathways are critically important for acid resistance, and (iii) c-di-GMP apparently affects acid resistance by several pathways.

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**Key Words:** *E. coli* O157: H7, c-di-GMP, acid resistance

**1086 Monensin level, supplemental urea, and administration of ractopamine on fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle.**
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Inclusion of distiller's grains (DG) in cattle diets has been shown to increase fecal shedding *E. coli* O157. Therefore, factors affecting ruminal fermentation of DG may impact fecal shedding of *E. coli* O157. The effect of feeding monensin at the new maximum limit 44 mg/kg of feed on fecal shedding of *E. coli* O157 in cattle has not been determined. The objectives of the study were to evaluate the effects of monensin level (33 or 44 mg/kg DM), supplemental urea (0, 0.35, or 0.70% of DM; administered with the final diet of the step-up program), and ractopamine (0 or 200 mg/steer daily; administered during the last 42 d of the finishing phase) in a steam-flaked corn-based diet containing 30% wet sorghum DG on fecal shedding of *E. coli* O157. Seven-hundred and 20 crossbred beef steers (initial BW 453 ± 23.1 kg), housed in 48 pens (15 steers/pen), were assigned to dietary treatments in a randomized complete block design with a 2 × 3 x 2 factorial treatment arrangement. Fresh pen floor fecal samples (10 per/pen) were collected every 2 wk for 14 wk and cultured for *E. coli* O157. Fecal prevalence data were analyzed with repeated measures negative binomial regression (PROC GENMOD) to examine effects and interactions of sampling day, urea, monensin, and ractopamine. Cumulative fecal prevalence of *E. coli* O157 was 7.6%, and ranged from 1.6 to 23.6%. Cattle fed monensin at 44 mg/kg had lower ($P = 0.05$) *E. coli* O157 prevalence than cattle fed 33 mg/kg (4.3 vs 6.8%). Supplemental urea that could potentially alter ruminal fermentation had no effect on fecal shedding of *E. coli* O157 ($P = 0.87$). The effect of ractopamine was not significant ($P = 0.89$), but the power to detect an effect was low due to low *E. coli* O157 prevalence in the final phase of the study. Additional research is needed to confirm the reduction in fecal shedding of *E. coli* O157 in cattle fed 44 mg/kg monensin and to assess the effect of ractopamine on fecal shedding of *E. coli* O157.

**Key Words:** *E. coli* O157:H7, distillers grains, monensin

**1088 Effect of feeding rumen undegradable intake protein on gut *Campylobacter* concentrations in fed cattle.**
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*Campylobacter* are a leading bacterial cause of human foodborne illness worldwide, causing more than 2 million infections in the United States alone. These bacteria readily colonize the gut of food animals, but because they lack 6-phosphofructokinase, they do not ferment sugars and thus must derive a substantial proportion of their energy from amino acid catabolism. To test our hypothesis that diets promoting amino acid flow to the lower gut may increase intestinal carriage of *Campylobacter*, 10 ruminally and duodenally cannulated Angus steers, averaging 431 kg, were adapted (n = 5/diet) to diets formulated to achieve 0 or 30% dried distiller′s grains with solubles (DDG; DM basis). Control steers were maintained on the basal diet containing cracked corn, supplemental fat and cottonseed meal. Steers receiving DDG were adapted via incremental increases (every 14 d) to treatment diet and remained on the 30% WDGS diet for 7 d. Duodenal and fecal samples collected before the start of each step up period (on d 0, 14 and 27) and at the end of the final period (d 33) were enumerated for *Campylobacter* spp. via viable cell count and $\log_{10}$ transformations of resultant bacterial colony forming units (CFU) were analyzed for effects of diet, period and their interaction by a repeated measures ANOVA. Fecal *Campylobacter* concentrations ranged from 1.6 to 3.0 $\log_{10}$ CFU/ml (SEM = 0.5) but did not differ ($P > 0.05$) due to diet, period or their interaction. Similarly, main effects of diet or period were not observed ($P > 0.05$) on duodenal *Campylobacter* counts but in this case a diet × period interaction was observed, due to higher recovery of duodenal *Campylobacter* from the WDGS-fed steers during the 2nd (3.1 $\log_{10}$ CFU/ml) than the 1st or 3rd periods (1.4 and 1.3 $\log_{10}$ CFU/ml, respectively). No other differences in duodenal *Campylobacter* concentrations were observed, with values ranging from 1.6 to 2.3 $\log_{10}$ CFU/ml (SEM = 0.5). These results do not support our hypothesis that diets high in rumen undegradable intake protein will increase proliferation of *Campylobacter* in the bovine gut.

**Key Words:** *Campylobacter*, distillers grains, pathogen

**1089 Development of a broader spectrum phage cocktail to decrease *Salmonella* shedding in livestock.**
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*Salmonella* shedding in many livestock species can increase significantly following transport and lairage. These increases in shedding can

amplify the amount of *Salmonella* that enters the processing facility and the likelihood of end product contamination. We previously produced an anti-*Salmonella* phage cocktail that reduced colonization in swine when the pigs were exposed to an environment heavily contaminated with *Salmonella* similar to what might be seen in a holding pen. The purpose of the current study was to increase the efficacy of the phage treatment by: 1) expanding its spectrum of activity; and 2) developing a more cost-effective microencapsulation technique. We collected samples from wastewater treatment facilities and isolated 20 distinct phages that were lytic against *Salmonella*. From this library we identified 10 phages that lysed *Salmonella enterica* Typhimurium, Enteriditis and Kentucky (3 serovars commonly associated with meat and poultry products). We characterized each phage by morphology and electron microscopy. The phages were microencapsulated using a sodium-alginate based method with or without poly-L-lysine which only reduced the cocktail titer by approximately one log (pre-microencapsulation: 10.4 $\log_{10}$ PFU/mL; post-microencapsulation with poly-L-lysine: 9.2 $\log_{10}$ PFU/mL; post-microencapsulation without poly-L-lysine: 8.9 $\log_{10}$ PFU/mL). Microencapsulated phages remained stable at both 4C and 22C with no appreciable drop in titer for up to 14 d (mean titer: 8.9 $\log_{10}$ PFU/mL). Taken together, these data indicate that multi-valent phage cocktails are easily produced and a cost-effective microencapsulation process adequately protects the phages over an extended period of time. Therefore, it may be possible to simultaneously treat large numbers of animals with phage therapy through feed or water.

**Key Words:** *Salmonella*, phage therapy, food safety

**1090 Use of a biophotonic *E. coli* XEN-14 to determine time of contamination in the life cycle of the house fly, *Musca domestica* Linnaeus (Diptera: Muscidae).** G. Schuster*[3], K. E. Moulton[1], P. R. Broadway[4], S. Willard[2], J. Behrends[4], and T. B. Schmidt[1], [1]*Department of Animal, Mississippi State University and Dairy Sciences*, *Mississippi State*, [2]*Department of Biochemistry, Mississippi State University*, *Mississippi State*, [3]*Agronomy, Texas A&M University-Kingsville*, *Kingsville*, [4]*Food Science, Nutrition, and Health Promotion, Mississippi State University*, *Mississippi State*.

Researchers have reported that the house fly (HF), *Musca domestica* Linnaeus (Diptera: Muscidae) is capable of carrying *E. coli* O157:H7, and thus serve as mechanical cross contamination vector. There is limited data identifying when during the life cycle that *E. coli* ingestion occurs. The objective of this trial was to utilize *E. coli* transformed with the XEN-14 gene cassette (BP-E.coli) to monitor the progression of *E. coli* contamination through the life cycle of the HF. HF larvae were incubated in 100-mL cup containing 50-g of sterile manure (72% moisture) inoculated with BP-E.coli at $1 \times 10^4$, $10^5$, or $10^9$ CFU for 24 or 48-h (larva), 7 d (pupae), and 10 d (fly). Post-incubation, larvae, pupae, and flies were imaged intact and macerated to determine uptake of BP-E.coli. After photonic imaging, larvae, pupae, and flies were serially diluted to quantify total CFU's of BP-E.coli ingested. Serial dilution of larvae exposed to BP-E.coli for 24 and 48-h revealed that 86% and 80% of larvae had ingested $3.1 \times 10^5$ and $1.9 \times 10^6$ CFU of BP-E.coli, respectively. There was no difference ($P > 0.05$) in terms of total CFU ingestion between the 2 incubation periods (24 vs. 48 h) or the inoculation concentration of BP-E.coli for larvae. Serial dilution of pupae 7-d post incubation revealed that 53% of pupae were positive for BP-E.coli ($6.9 \times 10^2$ CFU), there was no difference ($P > 0.05$) in ingestion of BP-E.coli by pupae between the 3 inoculation concentrations. Serial dilution of adults 10 d post-incubation revealed that 13.8% of adults emerge from the pupae stage contaminated with $3.9 \times 10^2$ CFU of BP-E.coli, no difference ($P > 0.05$) in the BP-E.coli retained by the adult between the 3 concentrations. Results of this trial suggest that HF can ingest *E. coli* before pupating and emerging with high concentrations of *E. coli*.

**Key Words:** *E. coli*, house fly, biophotonic

**1091 Effect of crust freezing on the survival of *Escherichia coli* and *Salmonella* Typhimurium in raw poultry products.** B. D. Chaves*, I. Y. Han, and P. L. Dawson, *Clemson University*, *Clemson, SC*.

*Escherichia coli* and *Salmonella* spp. are ubiquitous to the poultry production environment and hence their transmission to poultry products is a concern. Industry has widely used freezing as a strategy to halt pathogen growth and more recently, crust freezing has been claimed to improve operations, quality, and even safety of poultry products. Purpose: To determine the effect of crust freezing and the presence of skin on the survival of *E. coli* and *S.* Typhimurium in raw poultry products. A completely randomized experiment was designed. Ampicillin-resistant *E. coli* JM 109 and nalidixic acid-resistant *S.* Typhimurium were used in the trials. A set of cultures was subjected to cold-shock stress by storage at 4 °C for 10 days. Commercial chicken breasts without skin and chicken thighs with skin were inoculated with each bacterium in separate experiments being either cold-shocked or non-cold-shocked prior to inoculation. Samples were crust frozen at −85 °C for 20 min or completely frozen at −85 °C for 60 min. *E. coli* and *S.* Typhimurium were recovered in duplicated plates of appropriate selective (Violet Red Bile Glucose Agar and BG Sulfa, respectively) and non-selective media (Tryptic Soy Agar) containing the corresponding antibiotic. ANOVA of the log reductions and injury extent values from three replicates were performed. No significant differences ($p>0.05$) were observed in the reduction of cold-shocked or non-cold-shocked bacteria on products that were crust- or completely frozen, with or without skin. Reductions tended to be greater for *S.* Typhimurium than for *E. coli*, although none of the final reductions were greater than the desired target (1 log). Bacterial cell injury was not significantly different ($p>0.05$) among any of the treatments. The treatments did not show practical significance for initial reduction of these pathogens thus freezing nor crust freezing should not be considered strategies for the reduction of these pathogens on poultry. However, additional studies are underway to compare crust freezing to refrigeration for inhibition of bacteria on raw poultry products.

**Key Words:** crust-freezing, poultry, pathogens

**1092 Heating wash water for shell eggs...Is it necessary?** S. L. Christian*[1], P. A. Curtis[1], L. K. Kerth[1], M. T. Musgrove[2], and K. E. Anderson[3], [1]*Auburn University*, *Auburn, AL*, [2]*UDSA-ARS*, *Athens, GA*, [3]*North Carolina State University*, *Raleigh*.

Current egg washing regulations state that wash water should be at 32.2°C or higher, shall be at least 6.7°C warmer than the internal temperature of eggs to be washed, and approved cleaning compounds should be used in the wash water (Voluntary Grading of Shell Eggs 7 CFR § 56.76 (f)). These regulations were made when eggs were immersed in water. Studies have shown that immersion washing leads to an increase in microbial load, not the temperature of the wash water. Also, previous research has proven that cool wash water temperatures utilized in an in-line operation did not add to the internal microbial content of the egg. Therefore, the objective of this research was to evaluate the effectiveness of using detergent formulated for cool water in a commercial in-line shell egg processing facility with a temperature of approximately 20°C versus an identical system utilizing a traditional detergent and temperatures. Samples were gathered during normal processing hours over the course of 3 consecutive days and for 3 separate weeks (9

replicates). Egg samples (15 eggs/treatment) were randomly selected from the collector belt before the eggs were washed from both lines and after they were washed but before they reached the packer belt. Eggs were also gathered at the beginning, middle and end of each shift over the course of 2 shifts; therefore, eggs were collected 6 times per day per line (12 treatments total). Water samples (50 mL) were collected from each wash tank at the same time egg samples were taken. Exterior egg shells, egg contents, and wash water samples were evaluated using Aerobic Count Plates and *Enterobacteriaceae* Petrifilms to determine the aerobic microorganisms and Enterobacteriaceae loads. A storage study was also performed in which the eggs were stored at 4°C for 0, 2, 6 and 10 weeks and the microbial load of the exterior egg shells and contents were determined. Cool water washing of shell eggs did prove to be a viable solution for processing shell eggs.

**Key Words:** shell eggs, cool water, *Enterobacteriaceae*

**1093 Multiplication of *Salmonella* Enteritidis in egg yolks after inoculation outside, on, and inside vitelline membranes and storage at different temperatures.** R. K. Gast*, R. Guraya, J. Guard, and P. S. Holt, *Egg Safety and Quality Research Unit, USDA-ARS*, *Athens, GA*.

Prompt refrigeration to restrict bacterial growth can reduce the risk of egg-borne transmission of *Salmonella* Enteritidis to consumers. A recently published federal rule for *S*. Enteritidis control requires eggs to be refrigerated within 36 after they are laid, but allows ambient temperature storage until this time. Although the nutrient-rich interior of the yolk is a relatively infrequent location for initial *S*. Enteritidis deposition in naturally contaminated eggs, migration across the vitelline membrane can result in rapid bacterial multiplication inside eggs stored at warm temperatures. The objective of the present study was to measure the multiplication of *S*. Enteritidis in egg yolks after introduction at 3 different locations and subsequent storage at a range of temperatures. Using an in vitro egg contamination model, approximately 100 cfu of a phage type 13a strain of *S*. Enteritidis were inoculated either inside yolks, onto the exterior surface of vitelline membranes, or into the adjacent albumen. After storage of samples from each inoculation group at 10°, 15°, 20°, and 25°C for 24 h, *S*. Enteritidis was enumerated in yolks. For all 3 inoculation locations, the final *S*. Enteritidis levels in yolks increased significantly with increasing storage temperatures. At all storage temperatures, significant differences in *S*. Enteritidis multiplication were observed between inoculation sites (yolk inoculation > vitelline membrane inoculation > albumen inoculation). At 25°C, final $\log_{10}$ *S*. Enteritidis concentrations of 7.76 cfu/ml (yolk inoculation), 2.01 cfu/ml (vitelline membrane inoculation) and 0.76 cfu/ml (albumen inoculation) were attained in yolks after storage. These results demonstrate that, even when the initial site of *S*. Enteritidis deposition is outside the egg yolk, substantial multiplication supported by yolk nutrients can occur during the first day of storage and the risk of bacterial growth increases at higher ambient storage temperatures. This reinforces the value of rapid refrigeration for protecting consumers from egg-transmitted illness.

**Key Words:** *Salmonella* Enteritidis, eggs, multiplication

**91 Genome-wide analysis of cecal gene expression in *Salmonella*-challenged and probiotic-treated neonatal chicks.** S. E. Higgins*[1], A. D. Wolfenden[2], G. I. Tellez[2], B. M. Hargis[2], and T. E. Porter[1], [1]*University of Maryland*, *College Park*, [2]*University of Arkansas*, *Fayetteville*.

*Salmonella* spp. often cause no clinical signs in infected poultry flocks, however, it is the most common food-borne pathogen in human infections. While some probiotics have been proven to be effective for improvement of health and to reduce enteric pathogens of poultry, the mechanisms of action of these beneficial microflora are not completely understood and have been postulated to involve elicitation of innate host defense mechanisms. Presently, we evaluated global gene expression in the cecae of neonatal chicks following *Salmonella* challenge and probiotic treatment to determine gene expression and potential gene networks involved in reduction of *Salmonella* by probiotic treatment. In this study, day-of-hatch chicks were challenged with *Salmonella enterica* ssp. Enteritidis (SE), and treated one h later with a poultry-derived, *Lactobacillus*-based probiotic culture (FloraMax-B11, B11). Twelve and 24h post-treatment, cecae were collected for *Salmonella* detection and RNA isolation. Cecal RNA samples were then analyzed using long oligonucleotide microarrays containing probes for 21,120 genes. At both 12 and 24h, SE was significantly reduced by 4 or 3 $\log_{10}$ respectively in the B11-treated chicks as compared with the challenged chicks ($P < 0.05$). Microarray analysis revealed gene expression differences among all treatment groups. At 12h, 170 genes were expressed at significantly different levels ($P < 0.05$), with a minimum difference in expression of 1.2 fold. At 24h, the number of differentially regulated genes with a minimum 1.2 fold change was 201. Pathway analysis revealed that at both time points, genes associated with the NFκB complex were significantly regulated, as well as genes involved in apoptosis, such as *growth arrest-specific 2* (*GAS2*) and *cysteine-rich, angiogenic inducer, 61* (*CYR61*). Probiotic-induced differential regulation of the genes *GAS2* and *CYR61* may result in increased apoptosis in the cecae of chicks. Because *Salmonella* is an intracellular pathogen, we suggest that increased apoptosis may be a mechanism by which B11 reduces *Salmonella* infection.

**Key Words:** *Salmonella*, probiotic, microarray

**1094 Microbiological difference of eggs from traditional cage and free range production.** D. R. Jones*[1], K. E. Anderson[2], and M. T. Musgrove[1], [1]*Egg Safety and Quality Research Unit, USDA-ARS*, *Athens, GA*, [2]*Department of Poultry Science, North Carolina State University*, *Raleigh*.

Eggs from alternative production systems are a growing market share in the US. Meeting consumer requests for greater diversity in retail egg options has resulted in some unique challenges such as understanding the food safety implications of eggs from alternative housing practices. A study was conducted to determine what, if any, differences exist between nest run cage and free range produced eggs. A flock of hatch mate brown egg layers were maintained in traditional caged and free range production with egg and environmental sampling every 6 wks from 20 to 79 wks of age. Aerobic, coliform, and yeast and mold populations were monitored. Traditional caged (TC) egg shells had the highest aerobic levels compared with free range nest box (FRNB) and free range floor eggs (FRF) (3.90, 3.55, and 3.48 log cfu/mL, respectively). FRNB and FRF egg shell coliform levels were greater than TC (1.64, 1.40, and 0.25 log cfu/mL, respectively). FRF egg shell yeast and mold levels were greatest (2.49 log cfu/mL). Range grass (RG) microbial levels were greatest for all populations monitored compared with cage swabs (CS) and nest box swabs (NBS). CS maintained the lowest levels of coliforms and yeast and molds throughout the study but had elevated levels of aerobic bacteria. Seasonal effects were also seen for all monitored populations with summer and fall having the highest levels. Understanding the differences in microbial populations present on traditional cage and free range produced eggs can lead to the development of effective cleaning procedures for free range eggs thus enhancing food safety.

**Key Words:** egg, cage, free range