Food Safety: Poultry Aspects


Super-shedders, cattle shedding at least 10⁴ colony forming units (CFU) of _E. coli_ O157:H7, elevate risks of contaminating the food chain and maintaining the organism in cattle populations. As detecting super-shedders in cattle populations is laborious and time-consuming, a study was conducted to evaluate the role of hide and pen-floor contamination by model super shedders (MSS) in transmission of _E. coli_ O157:H7 to penned cattle. Steers (n = 48) negative for _E. coli_ O157:H7 for 3 wks were allocated to 6 pens, with 2 replicate pens per treatment. Treatment A consisted of 3000 g of feces inoculated with 10⁶ CFU spread in artificial fecal pats on the pen floor for d 0 through 4 and 14 through 18 of the study. For treatment B, 100 g of the feces was spread on the perineum of 1 MSS per pen and the remaining feces spread on the pen floor similar to treatment A. Treatment C differed from B in that 50 g of feces was spread on the perineum and 50 g on the brisket of the MSS. Fecal samples, perineal swabs (500 cm² area of the rump), freshly voided fecal pats and manila rope samples were collected during the 56 d experimental period. More positive rope samples were found in treatments B and C as compared with A (P < 0.05) and steers within treatments B and C were 1.3 times more likely (P < 0.05) to shed _E. coli_ O157:H7 in their feces than steers in treatment A. Even though loads of _E. coli_ O157:H7 were similar in pens, results indicate a heightened importance of hide as compared with pen floor contamination for transmission of this organism to cattle. As cattle within treatments B and C were persistently colonized with _E. coli_ O157:H7, this model would be suitable for future studies investigating mitigation of _E. coli_ O157:H7 transmission by super-shedders.

Key Words: _Escherichia coli_, O157:H7, feedlot cattle, super-shedder

440 Feed supplementation with caprylic acid reduces _Campylobacter_ colonization in market aged broiler chickens without altering cecal microbial populations. J. Reyes-Herrera*, F. Solis de los Santos¹, M. Hume², K. Venkitanarayanan³, A. M. Donoghue⁴, I. Hanning¹, M. F. Slavik¹, V. F. Aguilar¹, J. H. Metcalff⁵, P. J. Blore¹, and D. J. Donoghue¹, ¹Dept. Poultry Science, University of Arkansas, Fayetteville, ²Food and Feed Safety Research Unit, Southern Plains Agricultural Research Center, USDA-ARS, College Station, TX, ³Dept. Animal Science, University of Connecticut, Storrs, ⁴Poultry Production and Product Safety Research Unit, USDA-ARS, Fayetteville, AR.

_Campylobacter_ is a leading cause of food-borne illness in the United States and epidemiological evidence indicates that poultry products are a significant source of human infections. Caprylic acid is an 8-carbon medium chain fatty acid which has been reported by our laboratories to reduce _Campylobacter_ colonization in chickens. The mechanism of action of caprylic acid, however, has not yet been determined but may be due to changes in the intestinal microflora. To evaluate this possibility, cecal microbial populations were evaluated using denaturing gradient gel electrophoresis (DGGE) in market age broiler chickens fed caprylic acid. In the first trial (n = 40 per trial) chicks were assigned to 4 treatment groups (n = 10 birds per treatment group): Positive controls (_Campylobacter_, no caprylic acid) with or without a 12 h feed withdrawal before slaughter; 0.7% caprylic acid supplemented in feed for the last 3 d of the trial with or without a 12 h feed withdrawal before slaughter. Treatments were similar for Trial 2, except caprylic acid was supplemented for the last 7 d of the trial. On d 14 of age, chicks were orally challenged with _C. jejuni_ and on d 42, ceca were collected for DGGE and _Campylobacter_ analysis. Caprylic acid supplemented for 3 or 7 d at 0.7% reduced _Campylobacter_ compared with the positive controls (approx. Three log reduction), except for the 7 d treatment with a 12 h feed withdrawal period. DGGE profiles of the cecal content showed that caprylic acid had little, if any, effect on the cecal microbial community. The results of this study indicate that caprylic acid’s ability to reduce _Campylobacter_ does not appear to be due to changes in cecal microflora.

Key Words: _Campylobacter_, caprylic acid, DGGE

441 Evaluating the prevalence and distribution of _Campylobacter_ in newly constructed broiler houses. K. N. Eberle*, J. L. Purswell, J. D. Davis¹, C. D. McDaniel¹, and A. S. Kless, ¹Mississippi State University, Mississippi State, ²USDA-ARS Poultry Research Unit, Mississippi State.

In 2009, the USDA Food Safety and Inspection Service announced the development of new pathogen reduction performance standards for _Salmonella_ and _Campylobacter_ both on-farm and in the processing plant. The objective of this study was to evaluate the prevalence and distribution of _Campylobacter_ in 3 newly constructed broiler houses for the first 4 flocks placed to determine the necessity for on-farm regulation. Litter and fecal samples were collected from each house at 0, 28, and 48 d of production. Samples were serially diluted and spread onto Campy Cefex agar plates. Two 40-mL water samples were collected each production day and filtered through a 0.45 µm membrane before being placed onto a Campy Cefex agar plate. All plates were purged with a microaerophilic gas and incubated for 36 h at 42°C. Individual plates were screened for characteristic _Campylobacter_ colonies and suspect colonies were confirmed using a latex agglutination kit. An additional 50 g of litter was collected from the evaporative cooling inlets, middle, and tunnel ventilation fans to determine litter moisture and pH. Inside and outside temperature and humidity were collected using a weather station. Out of 2300 litter, 900 fecal, and 45 water samples, only 5, 6 and 1 of the collected samples respectively were confirmed _Campylobacter_ positive. Litter moisture was different depending on location: the middle contained a higher moisture level (37%) than the evaporative cooling inlets (33%) and tunnel ventilation fans (34%) (P < 0.05). Litter pH was not different for day, location or flock. Temperature and humidity averaged 26.8°C and 69.3% inside and 27.6°C and 60.6% outside. In conclusion the newly constructed houses did not show a high prevalence of _Campylobacter_. Litter moisture and humidity were at levels conducive for _Campylobacter_ growth. The high litter pH and low temperatures, along with other on-farm management strategies, may have suppressed the ability of _Campylobacter_ to colonize the litter.

Key Words: _Campylobacter_, litter, broiler house

442 Colonization of marker and field strains of _Salmonella_ Enteritidis and Typhimurium in antibiotic pretreated and non-pretreated laying hens. J. F. Hannah*, J. L. Wilson¹, N. A. Cox², L. J. Richardson², J. A. Cason³, and R. J. Buhr², ¹University of Georgia, Department of Poultry Science, Athens, ²USDA, ARS, Richard Russell Research Center, Athens, GA.
A study was conducted to evaluate the effects of a vancomycin (VNC) pretreatment on the ability of marker ( nalidixic acid-resistant) S. Enteritidis (SE), field S. Enteritidis (SEF), or marker S. Typhimurium (STM) to colonize within the intestinal and reproductive tracts and translocate to other internal tissues of laying hens. In each of 3 trials, caged hens (76, 26, and 33 wk-of-age) were divided into 6 groups designated to receive SE, SEF, or STM, and half pretreated with VNC (n = 12). VNC treated hens received 0.5 ml for 5 d to inhibit gram-positive bacteria. On d 6, all hens were challenged orally, intravaginally and intraco longally with S. Enteritidis (SEF), or marker S. Typhimurium (STM) and cultured for S. Enteritidis. Results for the 3 hen ages were combined, and Chi-squared and Fisher’s exact test were used to identify significant differences (P < 0.05) in colonization. Among tissues sampled, there were no significant differences in SE, SEF, or STM colonization between VNC pretreated and non-pretreated hens. For the ceca, spleen, and LGB samples, SE (83, 100, and 100%) and STM (100, 73, and 91%) colonization was significantly greater than SE (8, 0, and 8%) colonization in non-pretreated hens. For VNC pretreated hens, SE (92, 92, and 83%) and STM (100, 75, and 83%) colonization among ceca, spleen, and LGB samples was significantly greater than SE (36, 0, and 0%) colonization. Overall colonization of S. Enteritidis in the LRT samples was relatively low, ranging from 8 to 42% and SEF and STM were isolated from 8 and 18% URT samples, respectively. Only SEF (8–17%) was isolated from the ovarian follicles. In conclusion, the VNC pretreatment had no significant effect on the level of colonization of SE, SEF, or STM in the tissues evaluated, and these results may provide further understanding of S. Enteritidis ecology within laying hens.

**Key Words:** S. Enteritidis colonization, vancomycin, laying hens

### 443 Evaluation of Campylobacter challenge route (in ovo vs. crop) and feed additives to reduce cecal Campylobacter in broilers.

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The objective was to compare challenge route to establish Campylobacter in the ceca of broilers and then the efficacy of additives to reduce this reservoir of contamination. Twelve treatment groups (8 cages of 7 male Ross 308 broilers) were evaluated, representing 2 challenge routes (in ovo E17d; gavage to the crop at 3d post hatch) and 6 additives (None, Antibiotic, Adhesion, Probiotic, MCFA and Organic acid). Based on preliminary experiments the optimum in ovo dose of Campylobacter jejuni was determined to provide cecal colonization; one half of the chicks were thus infected and then randomized in respective cages separated from other cages by solid plastic sheets 30cm in height. The remaining cages were housed with chicks from the same egg source, but hatched in a commercial hatchery to avoid cross contamination. All chicks were given ad libitum access to feed (6 diets) and water. A basal mash diet, formulated to meet or exceed the NRC broiler requirements was divided into 6 groups and remixed with appropriate feed additive, the chicks were maintained on diets to 18d of age. At 3d, a ex creta (collected on paper for 4 h) sample from each cage was used to establish presence of Campylobacter; all cages from in ovo chicks, except those fed an antibiotic were 100% positive; only 3 of the 48 non challenged cages were positive demonstrating low cross contamination. Body weight (18d) was significantly impacted by challenge route (3d Crop > in ovo) and additive (Antibiotic > MCFA = Organic Acid = None = Adhesion = Probiotic), and reflected similar differences in feed intake and FCR. At 11 and 18d of age 4 broilers/cage (32 / treatment) were monitored for presence of Campylobacter by cloacal swab andecal content swab, respectively. There were no significant differences in number of contaminated birds due to challenge route, although this was numerically higher for in ovo treated birds. The Antibiotic diet caused a significant reduction in Campylobacter at 11 and 18d of age, the None treated diet was numerically the highest, but not different from Organic Acid = Adhesive = Probiotic = MCFA.

**Key Words:** Campylobacter, in ovo challenge, broilers

### 444 The efficacy of the natural plant extracts, thymol and carvacrol, against Campylobacter colonization in broiler chickens.


Campylobacter is one of the most common causes of foodborne bacterial gastroenteritis in the US. Case control studies have demonstrated that consumption or handling of raw or under-cooked poultry products can be directly linked to human campylobacteriosis. Incidence of Campylobacter in broiler flocks can range from 70 to 100%, thus a reduction of this pathogen in poultry would greatly reduce the incidence of human disease. Unfortunately, most treatments fail to produce consistent reductions in Campylobacter colonization in chickens. Natural plant extracts, such as thymol and carvacrol, have been tested against pathogens like S. Enteritidis, E. coli, Shigella, Listeria but their ability to reduce Campylobacter in chickens has not been reported. The objective of this study was to determine the efficacy of different concentrations and combinations of thymol and carvacrol in feed to reduce C. jejuni in broilers. To evaluate in vivo efficacy, death of hatch birds were fed 0% (controls) or 0.0625, 0.125, 0.25, 0.5, 1.0 or 2.0% thymol or carvacrol or combinations of both (n = 10 chicks/dose). Birds were orally challenged with 5 different C. jejuni strains at d 3 and at d 10,ecal samples were collected for Campylobacter enumeration. Four different trials were conducted. Data were analyzed by ANOVA using the GLM procedure of SAS and a probability of P < 0.05 was required for statistical significance. Significant reductions of C. jejuni were observed with 0.25% or 2% thymol, and for 1% or 2% carvacrol. A 2-log reduction was observed with the combination of 0.5% thymol and 0.5% carvacrol. However, treatments did not always produce consistent reduction in C. jejuni between trials. These results justify the potential application of these compounds to control Campylobacter in chickens, but additional experiments are required to determine the most consistently effective concentrations and combinations of these plant extracts.

**Key Words:** Campylobacter, natural plant extracts, carvacrol/thymol
isololation, spreadsheet formulas were used to calculate binomial and multinomial probabilities of picking serotypes present at various ratios on plates, assuming that 100% of picked colonies are Salmonella. When 2 serotypes are present in equal numbers, 6 colonies must be picked to have a 95% probability of finding both serotypes. To identify 3 serotypes under the same conditions, 11 colonies must be picked. If a serotype is outnumbered 10 to 1 by another serotype on a plate, a ratio that has been reported in the scientific literature, 32 colonies must be picked to have a 95% probability of finding the minority serotype. Relatively small survival and growth rate differences can produce large changes in the likelihood of picking a colony of a particular serotype even when that serotype was present in the original sample in equal numbers, so picking one colony per plate can give a distorted picture of what serotypes are in samples. Given the labor and expense of isolating and serotyping suspect Salmonella colonies, methods are needed for culturing specific serotypes of interest.

**Key Words:** Salmonella, serotypes, colonies


Poultry processing hang rooms are one of the dirtiest areas of a processing plant. To determine the bioaerosols in the hang room of a particular processing plant, 3 electrostatic polarization light filters utilizing UV light were mounted on 3 different walls of the hang room. Over a period of 24 sampling days, the filters were turned on or off and air and settle plate samples were taken of the air in the room to test for Glucose- and Lactose-fermenting Enterobacteriaceae, and Salmonella spp. Relative humidity, temperature and wind speed were also taken inside and outside the hang room and number of workers in the room and number of fans on were also noted. Samples were taken every 0, 3, 6, or 9 h into the processing shift. Enterobacteriaceae, (Lactose and Glucose) levels were not affected ($P > 0.05$) by filter position. Salmonella was low in counts sampled for at all positions, but were not different from another one another when comparing position location within the bacteria. The filters had no impact on airborne Enterobacteriaceae, (Lactose) during sampling hours 0, 3, and 9 ($P > 0.05$). Results showed a significant decrease for both Enterobacteriaceae, bacteria at the 9th sampling period. These results may be indicative of a shift change occurring in between the 6 and 9th where new workers were introduced to the environment. Salmonella positive counts were not significantly different regardless of filter use or hour. In general, all of the bacterial counts were low, only reaching approximately 2 logs at their highest. The environmental factors accounted for did not attribute to a large amount of variation for any bacteria sampled. Although, position of the filters were highly correlated for both Enterobacteriaceae, types.

**Key Words:** poultry, bioaerosols, Enterobacteriaceae

447 Role of lauric acid-potassium hydroxide concentration on bacterial contamination of spray washed broiler carcasses. A. Hinton Jr.*, J. Cason, R. Buhr, and K. Liljebljelke, Russell Research Center, Athens, GA.

A series of experiments were conducted to examine reductions in bacterial contamination of broiler carcasses washed in a spray cabinet with various concentrations of lauric acid (LA)-potassium hydroxide (KOH) solutions. Fifty eviscerated carcasses and 5 ceca were obtained from the processing line of a commercial poultry processing facility. An inoculated cecal paste was prepared by mixing 5 g of cecal contents with 0.3 mL of a bacterial suspension containing $10^6$ cfu/mL each of antibiotic resistant strains of Escherichia coli, Salmonella Typhimurium, and Campylobacter coli. A 0.1 g portion of the inoculated cecal paste was applied to the skin of each carcass and allowed to dry for 15 min. Inoculated carcasses were then divided into 5 groups of 10 carcasses each, and groups were sprayed with water, 0.25% LA-0.125% KOH, 0.5% LA-0.25% KOH, 1% LA-0.5% KOH, or 2% LA-1% KOH at 80 psi (552 kPa) for 15 s. Washed carcasses were rinsed for 15 s with sterile, deionized water to remove excess LA-KOH before whole carcass rinses were performed for 2 min in 200 mL of sterile phosphate buffer saline. Total plate count bacteria (TPC) and antibiotic resistant E. coli, Salmonella Typhimurium, and C. coli in the rinsates were enumerated, and the pH of the rinsates was measured. Findings indicated that significantly fewer TPC bacteria, E. coli, and Salmonella Typhimurium were recovered from carcasses washed with 2% LA-1% KOH than from carcasses washed in water. Furthermore, significantly fewer C. coli were recovered from carcasses washed in 1% LA-0.5% KOH than from carcasses washed in water, and no C. coli were recovered from carcasses washed in 2% LA-1% KOH. The pH of rinsates from carcasses washed in water, LA-0.125% KOH, 0.5% LA-0.25% KOH, 1% LA-0.5% KOH, or 2% LA-1% KOH was 7.27, 7.41, 7.57, 8.00, and 9.94, respectively. Findings indicate that the concentration of LA-KOH plays an important role in the ability of this antibacterial surfactant to reduce bacterial contamination of broiler carcass.

**Key Words:** lauric acid, potassium hydroxide, spray washing

448 Antimicrobial effect of sodium metasilicate on Salmonella enterica serovar Typhimurium and psychrophots in ready to cook, skin-on chicken breast meat stored at 4 ± 1°C. C. S. Sharma*, S. K. Williams, and G. E. Rodrick, University of Florida, Gainesville.

The objectives of this study were to determine the antimicrobial effects of sodium metasilicate (SMS) against Salmonella Typhimurium and psychrotrophic organisms in fresh ready to cook skin-on chicken breasts, and to ascertain the effects of the treatments on pH. The chicken breasts were inoculated with S. Typhimurium (ATCC 14028), treated with 0% SMS and no inoculum (negative control), 0% SMS and inoculum (positive control), 1% and 2% SMS (w/w), packaged and stored at 4 ± 1°C. All samples were analyzed in duplicate after 0, 1, 3, 5 and 7 d of storage for recovery of S. Typhimurium, psychrotrophic organisms and pH measurements. The whole experiment was repeated 3 times. Treating the breast meat with 1% and 2% SMS resulted in low ($P < 0.05$) Salmonella counts when compared with the positive control. Chicken breasts treated with 1% and 2% SMS resulted in 1.1 to 1.4 and 2.5 to 4.1 log cfu/g reductions of S. Typhimurium, respectively, as compared with positive controls. Psychrotrophic counts for breast meat treated with 2% SMS were lower ($P < 0.05$) than the control samples on all sampling days except d 0. The pH values were higher ($P < 0.05$) for all SMS treatments when compared with the negative and positive controls. This study revealed that SMS could function to control the pathogen S. Typhimurium, and extend the shelf life of poultry by retarding the growth of psychrotrophic bacteria, which are the primary spoilage organisms in fresh poultry.

**Key Words:** sodium metasilicate, Salmonella, psychrophots

The objectives of this study were to determine the antimicrobial effects of sodium metasilicate (SMS) marinades against Salmonella Typhimurium and psychrotrophic organisms in fresh marinated ready to cook skinless and boneless chicken breast meat, and to ascertain the effects of the treatments on pH. The chicken breasts were inoculated with S. Typhimurium (ATCC 14028), marinated in solutions containing either 0% SMS and no inoculum (negative control), 0% SMS and inoculum (positive control), 1% or 2% SMS, packaged and stored at 4 ± 1°C. All samples were analyzed in duplicate after 0, 1, 3, 5 and 7 d of storage for recovery of S. Typhimurium, psychrotrophic organisms and pH measurements. Chicken breasts marinated with 1% and 2% SMS had lower (P < 0.05) Salmonella counts when compared with the positive control at 3 d storage and through 7 d. Chicken breasts treated with 1% and 2% SMS resulted in 0.83 to 0.91 and 1.04 to 1.16 log cfu/g reductions of S. Typhimurium, respectively, after 3 d through 7 d of storage as compared with positive controls. The psychrotrophic counts were similar (P > 0.05) for all treatments. The pH values for 1% and 2% SMS treatments were higher (P < 0.05) when compared with the controls. This study revealed that SMS could function to control the pathogen S. Typhimurium, but had no effect on reducing the spoilage microflora when it was used in the marinade.

Key Words: sodium metasilicate, Salmonella, psychrotrophs

Aviplus treatment improves growth efficiency in broilers and swine but does not affect intestinal populations of experimentally inoculated Salmonella. T. R. Callaway*1, E. Grilli2, T. S. Edrington1, N. Krueger1, R. Anderson1, D. W. Pitta3, W. E. Pinchak3, and A. Piva2, USDA/ARS, Food and Feed Safety Research Unit, College Station, TX, 2University of Bologna, Bologna, Italy, 3Texas A&M University Agrilife Research Station, Vernon.

Organic acids improve growth efficiency in food animals, and can impact the microbial ecology of the gastrointestinal tract. Thus it has been suggested that they could be used to reduce foodborne pathogenic bacterial populations before animals enter the food chain. This study was undertaken to determine the effect of a commercial microencapsulated organic acid product on populations of experimentally inoculated Salmonella populations in swine and broilers. Broiler chicks (n = 192 in 2 replications; 1 d of age) were artificially inoculated with 10^7 CFU S. Typhimurium and randomly assigned to 0g, 0.2 kg, 2 kg, or 10 kg Aviplus/1000 kg feed diets. Feed consumption and weights were measured daily for 7 d. Salmonella populations were analyzed upon sacrifice but no differences in cecal Salmonella populations were found. Aviplus treatment at 0.2 kg/1000 kg feed increased (P < 0.05) pen weight, average body weight and average daily gain across the study. Aviplus inclusion at 0.2, 2, and 10 kg/1000 kg feed reduced (P < 0.05) feed to gain ratios as well. In another study, newly weaned pigs (7 d of age; n = 24) were blocked by sex and were randomly assigned to either 0g, 3 kg, or 30 kg Aviplus/1000 kg feed diets and fed for 14 d and were weighed and feed intake was measured daily. Pigs were artificially inoculated with 10^7 CFU S. Typhimurium and fecal samples were collected daily for 5 d until sacrifice. Intestinal contents from the rectum, cecum and ileum were collected, as well as ileocecal lymph nodes. No differences in Salmonella populations were found in any compartment, though rectal populations were reduced in swine fed 30 kg Aviplus/1000 kg feed. Feed efficiency (feed to gain) in pigs were increased (P < 0.05) by 3 kg Aviplus/1000 kg feed treatment, and ADG and BW was increased by 30 kg Aviplus/1000 kg feed treatment. Collectively, our results indicate that while Aviplus does not affect artificially inoculated Salmonella populations in vivo in these short-term studies, Aviplus treatment increased the feed efficiency of broiler chicks and newly weaned swine.

Key Words: food safety, organic acids

Aviplus treatment reduces E. coli and Salmonella populations in pure and mixed ruminal culture fermentations. T. R. Callaway*1, E. Grilli2, and A. Piva2, USDA/ARS, Food and Feed Safety Research Unit, College Station, TX, 2University of Bologna, Bologna, Italy.

Foodborne pathogenic bacteria can live in the intestinal tract of cattle, swine and poultry and can be transmitted to humans through the food supply or indirectly through animal/fecal contact. Organic acid products have been used as non-antibiotic modifiers of the gastrointestinal fermentation of food animals to improve animal health and performance. However, the impact of these organic acid products on foodborne pathogens remains unknown. Therefore, this study was designed to examine the effects of a commercial organic acid product on populations of the foodborne pathogens, Escherichia coli O157:H7 and Salmonella Typhimurium. Pure cultures (2 × 10^5 CFU/mL) of each pathogen were added to tubes that contained water-solubilized Aviplus added at of 0, 0.1, 0.5, 1, 2, 5, and 10% (w/v; n = 4). Water-solubilized Aviplus reduced (P < 0.05) the growth rate and final populations of E. coli O157:H7 and S. Typhimurium in pure culture at concentrations greater than 2% w/v. In further in vitro studies, E. coli O157:H7 and S. Typhimurium were added to mixed ruminal bacterial fermentations collected from cattle fed a pasture-based diet. The in vitro fermentations contained water-solubilized Aviplus at concentrations of 0, 1, 2, 5, and 10% (w/v; n = 2) and were incubated for 24 h. Aviplus addition reduced (P < 0.05) the final populations of E. coli O157:H7 and S. Typhimurium in ruminal fluid at concentrations >5% w/v. The A:P ratios from the in vitro ruminal fermentations were reduced (P < 0.05) by solubilized Aviplus treatment and total VFA production was not affected, but methane and ammonia concentrations were decreased. Organic acid products, such as Aviplus, can alter the intestinal microbial ecology and enhance animal productivity and health. Under in vitro conditions, solubilized Aviplus can be used to reduce populations of pathogenic bacteria. Intervention strategies to reduce foodborne pathogens that can improve animal performance have the advantage of a food safety intervention that pays for itself financially.

Key Words: food safety, Salmonella, organic acids