357 Does pre-slaughter stress affect pork safety risk? M. H. Rostagno*, S. D. Eicher, and D. C. Lay, *USDA-ARS-LBRU, West Lafayette, IN.*

Salmonella is the top food safety priority for the pork industry. Although contamination of pork occurs along the slaughter and processing line, infected live pigs entering the abattoir constitute the original *Salmonella* contamination source. However, the extent of carcass contamination is not only determined by the number of pigs infected, but also by the levels of *Salmonella* entering the abattoir in the intestinal tract of slaughtered pigs. Therefore, a series of experiments was conducted to determine if common stressors occurring before slaughter affect the prevalence and levels of *Salmonella* in market pigs. Initially, a field study was conducted to determine the effect of transportation and lairage on the frequency of *Salmonella* shedding in market pigs. A follow up study was conducted under controlled conditions to determine the effect of feed withdrawal and transportation on the levels of *Salmonella* in the intestinal tract of infected market-weight pigs. Finally, a third study was conducted to determine the effect of transportation and mixing with unfamiliar pigs on the susceptibility of market-weight pigs to *Salmonella* infection. In the first study, *Salmonella* shedding increased ($P < 0.05$) from pre-transport (11.3%) to post-transport (20%), and from post-transport to post-lairage (42%). In the second study, feed withdrawal by itself or combined with transportation caused increased levels of *Salmonella* in the ileum ($P < 0.05$), whereas only the combination of feed withdrawal and transportation caused increased levels of *Salmonella* in the cecum ($P < 0.05$). In the third study, pigs subjected to transportation and/or mixing were colonized by higher ($P < 0.05$) levels of *Salmonella* in the ileum, whereas only pigs subjected to both stressors combined were colonized by higher ($P < 0.05$) *Salmonella* levels in the cecum. It is concluded that pre-slaughter stressors, such as transportation, feed withdrawal and mixing, affect pork safety risk by increasing frequency and levels of *Salmonella* in the intestinal tract of market-weight pigs.

Key words: *Salmonella*, stress, swine

358 Salt and nitrite at concentrations relevant to meat processing enhances Shiga toxin II production by *E. coli* O157:H7. S. M. Harris*, S. A. Olsen, J. Hu, M. Du, and M. J. Zhu, *Department of Animal Science, University of Wyoming, Laramie.*

Escherichia coli (O157:H7 is a major food safety threat. It has ability to produce several virulence factors. Shiga toxins (Stxs) are the key virulence factors of *E. coli* O157:H7 that are responsible for hemorrhagic colitis and serious renal failure. Despite the extensive study of *E. coli* O157:H7 survival during meat processing, the production of Stxs by *E. coli* O157:H7 during these processes has not been studied. This becomes a very important question since Stx2 is resistant to heat treatment. The objective of this study is to elucidate the effect of 2 essential additives in processed meats, salt and nitrite, on Stx2 production by *E. coli* O157:H7. *E. coli* O157: H7 (86–24) was treated with different concentrations of salt (1%, 2%, and 3%, W/V) or sodium nitrite (0, 100, 200, 300 ppm) solutions for 6 h. The Stx2 production and cfu were analyzed. After 6 h incubation, the number of *E. coli* O157:H7 in nitrite-100 ppm was not different from that of control (0 ppm). However, the number of *E. coli* O157:H7 in nitrite-200 ppm ($P < 0.1$) and nitrite-300 ppm ($P < 0.05$) were lower than control. Western blotting analysis indicated that adding 100 ppm and 200 ppm of nitrite in LB medium increased ($P < 0.1$) Stx2 production. Similarly, including 2% and 3% of salt decreased ($P < 0.05$) the final *E. coli* O157:H7 population, but supplementing 2% salt increased ($P < 0.05$) Stx2 production per cfu compared with that of control (1% salt), while including 3% salt decreased ($P < 0.05$) Stx2 production. Since 2% salt and 100–200 ppm nitrite are commonly used in processed meats, the current study indicated that salt and nitrite at these concentrations promote Stx2 production. Therefore, not only the survival of *E. coli* O157:H7 during meat processing is important, Stxs production by *E. coli* O157:H7 is another critical control point for producing safe meat products. (USDA AFRI 2009–65203–05716, 2010–65201–20599, Agricultural Experiment Station at University of Wyoming, NIH-INBRE P20RR016474).

Key words: *E. coli* O157:H7, Stx2, nitrite

359 Detection of major serotypes of Shiga-toxin producing *E. coli* in bovine feces by multiplex PCR. Z. Paddock*, X. Shi, T. G. Nagaraja, and J. Bai, *Kansas State University, Manhattan.*

Shiga-toxin producing *E. coli* (STEC) serotypes, particularly O157, are major food borne pathogens. Recently, non-O157 STEC serotypes have also become a major public health concern. Unlike O157, isolation and detection procedures for non-O157 have not been fully developed. Confirmation of non-O157 strains is generally based on agglutination with serotype-specific antisera, which is labor intensive and sometimes nonspecific. We have developed a multiplex PCR, based on O-specific antigen coding genes, rfbE of O157, and wzx and wfqB of non-0157, to distinguish the 7 major STEC serotypes, O26, O45, O103, O111, O121, O145, and O157. The specificity of the procedure was confirmed with pure cultures of STEC strains (n = 138). Our objectives were to evaluate whether the procedure could be used to detect STEC in feces and screen fecal samples before subjecting them to detection by cultural procedures. Fecal samples spiked with different concentrations of a mixture of 7 STEC strains were tested before and after 6 h enrichment in *E. coli* (EC) broth. Fecal samples (108 from feedlot and 108 from dairy cattle) were collected, enriched in EC broth, tested by the multiplex PCR. All samples (n = 216) were cultured for O157, while a subset of samples (n = 24) were cultured for the non-O157 STEC. All 7 serotypes were specifically amplified in spiked feces with detection limits of 6.7×10^5 cfu/g before enrichment and 8.0×10^1 after 6 h enrichment in EC broth. The multiplex PCR revealed a high prevalence of STEC, except O45 serotype, in cattle feces (Table). All samples that were culture positive for STEC were also positive by the multiplex PCR. The multiplex PCR may be used to identify feces positive for the 7 STEC before subjecting the samples for cultural methods, however, additional studies are needed to validate the procedure.

Table 1. Prevalence of Shiga-toxin producing *E. coli* (STEC) in cattle feces

STEC	mPCR positive (n=24)	Culture positive (n=24)	mPCR positive (n=192)	O157 culture positive (n=192)
O26	14	5	165	-
O45	2	0	106	-
O103	10	4	140	-
O111	0	0	34	-
O121	5	4	98	-
O145	0	0	8	-
O157	16	14	149	51

Key words: Shiga-toxin producing *E. coli*, multiplex PCR, cattle feces

360 Microbial contamination rates and antimicrobial resistance patterns in "no antibiotics added" labeled chicken products. J. Zhang*[1], A. Massow[1], M. M. Stanley[1], M. Papariella[1], X. Chen[3], B. Kraft[2], and P. D. Ebner[1], *[1]Purdue University Department of Animal Sciences, West Lafayette, IN, [2]Purdue University College of Veterinary Medicine, West Lafayette, IN, [3]University of Illinois at Urbana-Champaign Department of Animal Sciences, Urbana-Champaign.*

In the United States, poultry product labels can contain "no antibiotics added" if the animals were raised without the use of antimicrobials. In this study we compared microbial community structures of conventional chicken products (CONV; n = 201) with those of chicken products labeled as coming from birds raised without antimicrobials (NON; n = 201). Both CONV and NON products were equally likely to contain Enterococcus spp., (CONV: 17.4%; NON: 21.3%) or *Escherichia coli* (CONV: 25.9%; NON: 22.3%). CONV samples contained higher concentrations of coliforms (CONV: 3.0 $\log_{10}$cfu/mL; NON: 2.5 $\log_{10}$cfu/mL; $P < 0.05$). The number of samples positive for Salmonella was low in both groups, but statistically higher ($P < 0.05$) in NON (5.0%) vs. CONV (1.5%) samples. *E. coli* isolates from CONV samples were more frequently resistant to at least one antimicrobial (CONV: 61.3%; NON: 41.2%; $P < 0.05$). Four multidrug resistance patterns appeared twice or more in *E. coli* isolates obtained from both CONV and NON products. The most common patterns in both groups were tetracycline-sulfasoxazole followed by tetracycline-sulfasoxazole-trimethoprim/sulfamethoxazole and tetracycline-ampicillin. Enterococcus spp. isolates from both groups were equally likely to be resistant to at least one antimicrobial, but Enterococcus spp. isolates from CONV samples were more likely to be resistant to erythromycin, kanamycin or gentamicin ($P < 0.05$). Five multidrug resistance patterns were detected twice or more in Enterococcus spp. isolates from CONV products while 2 multidrug resistance patterns were detected in Enterococcus spp. isolates from NON products. In both groups the most common resistance pattern was erythromycin-tetracycline-tylosin tartrate. Taken together, these data indicate that CONV and NON products have similar contamination characteristics, however, bacteria isolated from CONV products may be more frequently resistant to some antimicrobials.

Key words: antimicrobial resistance, poultry, "no antibiotics added"

361 Antimicrobial activities and comparing bacterial membrane interactions of porcine lactoferricin derived peptides. F. Han*, Y. Liu, Y. Xie, Y. Gao, and Y. Wang, *Institute of Feed Science, Hangzhou, Zhejiang, China.*

Abstract: Antibiotic treatment of microbial infections is under scrutiny because of increasing conventional antibiotic resistance, and discovery of new classes of antibiotic agents is warranted. Antimicrobial peptides are part of innate defense system found almost in all organisms. Porcine lactoferricin (LFP-20) is an antimicrobial peptide identified in N terminus of porcine lactoferrin. To develop novel antimicrobial peptides with improved antimicrobial specificity as compared with LFP-20, we designed analogs LF-2, LF-4 and LF-6 with substituted alanine, serine, or tryptophan residues at the different positions of the molecule. Broth microdilution, hemoglobin release, WST-1 and $DiSC_35$ methods were used in this study. Statistical significance among independent groups was determined using one-way ANOVA. Analogs displayed a 2~16-fold higher antimicrobial activity than LFP-20 but did not induce increased hemolytic activity significantly ($P > 0.05$) to porcine erythrocyte below 32 μg/mL compared with LFP-20. Furthermore, the proliferations of porcine peripheral blood mononuclear cells were not influenced significantly ($P > 0.05$) by LF-2, LF-4 and LF-6 below 50 μg/mL. Except for 8 μg/mL and 16 μg/mL LF-4, the cytotoxicities of LF-2, LF-4 and LF-6 to PBMCs were not increased significantly ($P > 0.05$) below 32 μg/mL compared with LFP-20. To better understand the antibacterial mechanism of LFP-20 and its analogs, we studied their effect on the cytoplasmic membrane of *Escherichia coli*. LFP-20 was not effective in depolarizing the cytoplasmic membranes, whereas 3 analogs could gradually dissipated membrane potential of *Escherichia coli*, demonstrating a correlation between bactericidal activity and membrane depolarization. Compared with LFP-20, 3 analogs enhanced the *Escherichia coli* outer membrane permeability significantly ($P < 0.05$) at 8 ~32 μg/mL. LF-6 led to the most obvious inner membrane permeability of *Escherichia coli*. Collectively, these results suggest that the first target for 3 analogs on *Escherichia coli* may be the cytoplasmic membranes.

Key words: porcine lactoferricin, antimicrobial activity, antimicrobial mechanism

362 Nitrate and nitrite partition in cheese and whey during cheesemaking. F. F. Pinheiro, L. M. Fonseca*, M. O. Leite, M. M. O. P. Cerqueira, R. Rodrigues, C. F. A. M. Penna, and M. R. Souza, *Veterinary School/Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.*

Nitrate is used in the dairy industry as a preservative to prevent cheese blowing due to bacterial growth. However, due to health concerns, concentration limits for usage in countries where it is allowed are usually fixed at 50 mg/kg of nitrate in cheeses of medium and low moisture. The objective of this work was to evaluate the amount of nitrate in the curd and whey after addition to the milk used for Minas cheese production. Five batches of cheese were processed, and for each batch, potassium nitrate was added to the pasteurized milk (LTLT) at the following concentrations: 5g/100L, 15g/100L, and 40g/100L. One treatment was done without any nitrate addition. Nitrate was measured by spectrophotometric determination following modified Jones Method (AOAC 976.14), and raw milk composition was measured by infrared spectroscopy (Combisystem 2300, Bentley). Compositional analysis of cheese and whey were according to International Dairy Federation methods. Differences were evaluated by Duncan Test. Average composition of raw milk was 3.75g/100g, 3.30g/100g, 4.39g/100g, 12.47g/100g and 8,83g/100g for, respectively, fat, protein, lactose, total solids, and solids non fat. Cheese composition was 48.7g/100g of fat in dry matter, and, respectively, 17.2g/100g and 59.1g/100g for protein and moisture. Nitrate concentration in cheese was, respectively, 0 (non detectable), 12.1, 23.3, and 64.93 mg of nitrate/kg of cheese for treatments with addition of 0, 5, 15, and 40 g of nitrate/100L of milk. Fermentation did not affect partition of nitrate in curd and whey phases. Nitrate retained in cheese curd was about 3% of the initial amount, while 97% of the original nitrate was detected in the whey. Results confirm that nitrate addition at levels higher than 20g/100L of milk are risky, since levels close to 30g/100L will result in approximately 50mg of nitrate in the cheese. Further study is necessary to evaluate the impact of this preservative in the whey, since high amounts of nitrate/nitrite will remain in this phase, with serious concerns related to concentrated and dehydrated whey products. Acknowledgments: FAPEMIG, CNPq

Key words: nitrate, cheese, nitrite

363 Prevalence of *Coxiella burnetii* in bulk tank milk and associations with herd characteristics on US dairy operations. J. E. Lombard[1], S. N. Gibbons-Burgener[2], and C. P. Fossler*[1], *[1]USDA-APHIS-VS, Centers for Epidemiology and Animal Health, Fort Collins, CO, [2]University of Wisconsin, Madison, Madison.*

The objectives of this study were to estimate the prevalence of *C. burnetii* and evaluate herd-level factors associated with its presence. During the National Animal Health Monitoring System's Dairy 2007 study, bulk tank milk was collected and tested via PCR for *C. burnetii*, the causative agent of Q fever. Information regarding dairy cow health, reproduction, and productivity was also collected. Samples were collected from 528 operations during the months of March through August 2007. After adjusting for study design and incorporating weighting procedures, 76.9% of dairy operations had PCR evidence of *C. burnetii* in bulk tank milk. *C. burnetii* was detected in bulk tank milk on 69.8% of operations with less than 100 cows, 90.8% of operations with 100–499 cows, and 98.8% of operations with 500 or more cows. A higher percentage of bulk tank milk from operations in the West region (90.1%) were PCR positive for *C. burnetii* compared with those in the East region (75.7%). Weighted log-linear regression models for count data, which adjusted for herd size and region, were used to assess the effect of *C. burnetii* on multiple morbidity and mortality outcomes. Positive operations had a significantly higher percentage of calves born dead (6.0%) and a higher percentage of abortions (4.1%) than operations that tested negative (4.6 and 3.2%, respectively). In addition, positive operations removed a significantly higher percentage of cows due to reproductive problems. These findings provide further evidence that *C. burnetii* is more prevalent in the US dairy herd than previously thought. In addition, *C. burnetii* infection appears to be associated with abortions, calves born dead, and cows removed for reproductive problems. More research needs to be conducted to determine the source of *C. burnetii* on dairy operations, define the causal relationship between health outcomes, and to determine management practices that are likely to decrease transmission of the organism.

Key words: *Coxiella burnetii*, bulk tank milk, prevalence

364 Bulk milk somatic cell penalties in herds enrolled in dairy herd improvement programs. K. J. Hand*[1], A. Godkin[2], and D. F. Kelton[3], *[1]Strategic Solutions Group, Puslinch, ON, Canada, [2]Ontario Ministry of Agriculture, Food and Rural Affairs, Elora, ON, Canada, [3]University of Guelph, Guelph, ON, Canada.*

The objective was to assess the risk of bulk milk somatic cell count (BMSCC) penalties for herds enrolled in dairy herd improvement (DHI) programs compared with nonparticipatory herds. The data consisted of monthly average BMSCC for 2898 CanWest DHI herds and 1186 non-DHI herds in 2009, Ontario, Canada for a total of 48,250 records. The median average BMSCC (10^3 cells / ml) for CanWest DHI herds was found to be 228; whereas, the median in non-DHI herds was found to be 250. Two threshold BMSCC (10^3 cells / ml) penalty levels were considered, 399 and 499. For both penalty levels, the odds of a BMSCC exceeding either penalty threshold for DHI herds was modeled using a generalized mixed model with a binary link function. Random effects included herd; covariates included season of BMSCC (summer, May through September and winter, October through April), total milk shipped per month (l), fat paid per month (kg) and protein paid per month (kg). Furthermore, the odds of a DHI herd incurring a penalty under the Ontario Milk Act (where 3 out of 4 consecutive BMSCC exceeded penalty thresholds) was also examined and modeled with a similar generalized mixed model. The likelihood of a BMSCC exceeding a penalty threshold in a non-DHI herd compared with a DHI herd was found to be significantly greater than 1 at both penalty levels ($P < 0.004$). The likelihood of incurring a BMSCC penalty under the Ontario Milk Act was found to be not significant for a penalty threshold of 499 ($P = 0.18$), but significant for the penalty threshold of 399 ($P = 0.004$).

Key words: bulk tank, SCC, penalty

365 A novel analysis strategy of detection hydrolysate protein adulteration in milk. Z. Chen[1] and D. M. Barbano*[2], *[1]Analysis and Testing Center, Shandong University of Technology, Zibo, Shandong Province, PRC, [2]Department of Food Science, Cornell University, Ithaca, NY.*

Hydrolysate protein (HP) which has the analytical characteristics of protein molecules in the Kjeldahl total nitrogen test could pollute milk or be illegally added into milk to improve protein content would cause big food safety problem. The main component of HP is true protein, so it is difficult to distinguish HP true protein from milk. Hydroxyproline could be only used as identification tag for animal source protein (ISO 3496–1994). Therefore, how to detect exogenous true protein adulteration in milk is still a big challenge for milk quality control and food safety. Our objective was to develop an effective and quick analysis method to figure out HP adulteration in milk. Ultrafiltration (UF) and infrared (IR) analysis were considered to be appropriate techniques. Lactalbumin hydrolysate, casein hydrolysate (acid) and Vegetable Peptone No1 were used to simulate HP adulteration in milk. Every HP experiment (0, 1250, 2500, 3750 and 5000 mg/kg) was replicated 3 times. Stirred Cell 8400 with Micon Ultrafiltration Membrane and LactoScope FTIR were chosen as experiment instruments. 35 psi and 200 g was preferred to operation condition of UF. Excluding the cleaning procedure of UF, it takes 50 minutes for permeate collection and IR detection of one milk sample. All permeate protein is significantly correlated with the 3 HP added in milk ($P < 0.01$), the R square of 3 linear equation is 0.986, 0.897 and 0.953 respectively. The recovery percent of the 3 HP in permeate ranges from 81.32% to 107.25%. Compared with ISO 3496-1994, the proportion of true protein adulteration could be quantificationally and directly detected. The upper bound of 95% confidence interval for control milk permeate protein (n=9) is 0.0401%, it could be the lower limit reference critical value to distinguish whether HP adulteration in milk or not ($P < 0.05$). This paper developed a novel analysis strategy (UF, IR, critical value judgment), and it is verified to be an effective and quick analysis method to solve milk HP adulteration problem.

Key words: hydrolysate protein, milk adulteration, detection