

## Food Safety: Advances in Food Safety

**648 Antimicrobial use in preweaned calves: Effects on fecal *E. coli* resistance.** D. A. Moore,\* D. Barone, A. C. B. Berge, T. E. Besser, and W. M. Sischo, *Washington State University*.

Antimicrobial use in food animals can potentially lead to resistant bacteria and future inability to use some antimicrobials for humans. The objective of this study was to determine the effect of fed antimicrobials and antimicrobial treatment on resistance in calf fecal *E. coli*. Newborn calves (n = 118) were allocated to 1 of 4 groups and monitored for 28 d. Calves in the conventional therapy group were treated as per dairy protocol with trimethoprim-sulfa, spectinomycin, penicillin, and bismuth-pectin for diarrhea. The targeted therapy group included a physical examination and treatment with bismuth-pectin for diarrhea and ceftiofur and trimethoprim-sulfa in cases of fever. Within treatment groups, calves were equally assigned to receive neomycin and tetracycline in their milk for the first 2 weeks of life or not. Daily health evaluations and treatments were recorded. Fecal swabs, taken on d 1 and at 2 and 4 weeks were cultured for *E. coli*, 3 isolates of which were tested for susceptibility to 12 antimicrobials. Resistance patterns were designated susceptible; resistant to at least streptomycin, sulfa and tetracycline (SSuT); or resistant to all  $\beta$ -lactams and SSuT. On the first sampling, 86% of isolates were mostly susceptible but by 14 d only 7 isolates were susceptible. Isolates were 3.1 times more likely to have  $\beta$ -lactam/SSuT resistance at 2 weeks compared with 4 weeks ( $P < 0.001$ ). Using ordinal logistic regression, feeding antibiotics in milk did not result in higher levels of antibiotic resistance ( $P = 0.52$ ). However, at the 14-d sampling, an isolate was 10 times more likely to have a higher level of resistance (either SST or  $\beta$ -lactam/SSuT) if the calf had been treated compared with no treatment (95% CI 3.7, 30.7). At 4 weeks, neither antibiotics in the milk for the first 14 d nor treatment were associated with resistance patterns seen ( $P = 0.12$ ). In conclusion, antimicrobial treatment has a significant effect on recovery of multidrug resistant fecal *E. coli*, but the effect is not completely sustained over time.

**Key Words:** antimicrobial, resistance, calf

**649 Effect of pre-slaughter stressors on intestinal microbial populations of pigs.** M. H. Rostagno\*<sup>1</sup>, B. T. Richert<sup>2</sup>, and D. C. Lay Jr.<sup>1</sup>, <sup>1</sup>USDA-ARS, Livestock Behavior Research Unit, West Lafayette, IN, <sup>2</sup>Purdue University, Department of Animal Sciences, West Lafayette, IN.

The swine intestinal microbiota is a complex ecosystem, which may be disturbed by many factors. Studies have focused on the relation between antimicrobial use and resistance in intestinal microbial populations, whereas the effect of non-antimicrobial factors, such as stress, remains unknown. During the process of being transported from production farms to abattoirs, market pigs are exposed to several stressors. The occurrence of antimicrobial resistance in market pigs is critical, since the most likely transmission route from animals to humans is through contamination of carcasses. Therefore, 2 experiments were conducted to determine the effect of common pre-slaughter stressors (feed withdrawal, transportation, and lairage) on levels of antimicrobial resistance in commensal coliforms and lactobacilli in market pigs. In the first study, no effect of transportation and lairage on total fecal coliforms or its subpopulations resistant to antibiotics was observed ( $P > 0.10$ ). Also, no effect was observed on total fecal lactobacilli populations, as well as on subpopulations resistant to tetracycline and erythromycin ( $P >$

0.10). However, a significant increase of the fecal levels of ampicillin-resistant lactobacilli was caused by transportation and lairage ( $P < 0.05$ ). In the second study, while total ileal coliforms were not affected by feed withdrawal and/or transportation ( $P > 0.10$ ), populations of lactobacilli resistant to tetracycline and erythromycin decreased ( $P < 0.05$ ) in response to the stress of feed withdrawal and transportation. Interestingly, no effects of the investigated stressors were observed in any of the cecal microbial populations analyzed. These studies show that stress does affect intestinal microbial populations in market pigs with lactobacilli being more susceptible to the effects of stressors compared with coliforms. Moreover, it was revealed that interactions between type of stress, region of the intestinal tract, and microbial population analyzed may influence the pattern of antimicrobial resistance observed. There is a critical need for further investigation of the quantitative effect of stressors on intestinal microbial populations of pigs, and its food safety implications.

**Key Words:** swine, stress, microbial ecology

**650 Screening of antimicrobials and salt substitutes for use in reduced sodium dairy products.** T. Taylor,\* A. Lathrop, N. Farkye, and A. Lammert, *California Polytechnic State University, San Luis Obispo*.

Pressure has been put on the food industry to reduced sodium levels. Since reduction or elimination of salt from dairy products could potentially change the microbial stability an alternative antimicrobial agent may be needed. In addition to the use of antimicrobials salt replacers could be used to maintain acceptable flavor. To determine which antimicrobials have potential for success in reduced sodium dairy products, this study evaluated 8 commercially available antimicrobials (lauric arginate, natural enzyme systems and various fermentates). Antimicrobials were also tested in combination with 6 commercial sodium reduction agents to determine if their use would interfere with antimicrobial activity. Sodium reduction agents contained one or more of the following components calcium salt, potassium salt, sodium chloride, cultured corn sugar and trehalose. Milk and low-sodium cheese agar systems were used as the growth medium for screening. Antimicrobials with and without sodium reduction agents were added to the agar systems, then a 5-strain cocktail of *Listeria monocytogenes*, *Salmonella* or *Escherichia coli* O157:H7 was spread plated at 3 concentrations:  $10^1$ ,  $10^2$  and  $10^4$  cfu/plate. Samples were then incubated at 30°C and observed for growth after 24 and 48 h. A nisin-containing antimicrobial blend, lauric arginate, a natural enzyme system and a fermentate inhibited growth of all pathogens on milk agar after 48 h. In the cheese agar, pathogen growth was observed after 48h with the nisin-containing antimicrobial blend, and the fermentate, whereas while lauric arginate showed pathogen growth in 48 h or less. No pathogen growth was observed in the cheese agar containing the enzyme system. When the nisin-containing antimicrobial blend, lauric arginate and fermentate were tested in combination with sodium reduction agents containing potassium chloride, growth of *Salmonella* and *E. coli* O157:H7 was observed within 48h. Regardless of the salt substitute, there was no pathogen growth when the natural enzyme system was tested. Results from this study can aid in the selection of antimicrobials and sodium reduction agents for use in dairy products. However, testing in the dairy product itself is needed to confirm screen results and ensure a safe product.

**651 Control of native microbiota in skim milk by pulsed electric fields and tangential-flow microfiltration versus high-temperature short-time pasteurization.** D. Khanal,\* A. Chugh, M. Walkling-Ribeiro, L. Duizer, and M. W. Griffiths, *University of Guelph, Guelph, Ontario, Canada.*

Conventional high-temperature short-time pasteurization (HTST) is the established food preservation technology for commercial processing of milk. However, during HTST treatment degradation of valuable nutrients and loss in sensory quality in milk occurs due to substantial heat exposure. Pulsed electric fields (PEF) and tangential-flow microfiltration (TFMF) are emerging food processing technologies that could represent a non-thermal alternative to heat pasteurization for skim milk processing. This study investigated the effect of non-thermal technologies on the reduction of native microbiota in skim milk and compared PEF and TFMF efficacies to that of HTST. Fresh raw skim milk (0.05% fat) was incubated at 8°C for 96–120h to promote the native microbial growth up to  $10^8$  cfu/mL. Incubated milk was treated with PEF for electric field strength, product flow rate and maximum processing temperature combinations using 28 kV/cm, 20 mL/min and 40°C (PEFL), 40 kV/cm, 35 mL/min, and 55°C (PEFM), and 40 kV/cm, 25 mL/min, and 65°C

(PEFH) that led to respective treatment times of 2805, 1122, and 1571  $\mu$ s, while TFMF-treated milk was processed at a flow rate of 300 mL/min and 35°C with membranes of different pore sizes (0.2, 0.45, and 0.65  $\mu$ m). Thermal control treatments were carried out with HTST at 75 and 95°C for respective holding times of 20 and 45 s. ANOVA was used for all treatments ( $n = 3$ ). Microbial loads in stand-alone PEFL- and PEFM-treated milk were reduced by respective 1.3 and 2.3  $\log_{10}$  ( $P < 0.05$ ), in contrast to higher inactivation ( $P < 0.05$ ) of 4.5 and 3.7  $\log_{10}$  achieved by similarly effective PEFH and 0.65  $\mu$ m pore-sized TFMF ( $P \geq 0.05$ ), respectively. However, these treatments were all less effective for microbial decontamination than microfiltration at 0.2 and 0.45  $\mu$ m ( $P < 0.05$ ) inactivating respective 6.0 and 5.9  $\log_{10}$ , which was comparable to the low and high HTST treatments ( $P \geq 0.05$ ) that led to 5.7 and 5.8  $\log_{10}$  reductions of native microbiota in skim milk, respectively. Results obtained in this study highlight the potential of TFMF and heat-assisted PEF for skim milk processing and suggest their combination in a hurdle technology as non-thermal processing alternative to HTST.

**Key Words:** pulsed electric field, tangential-flow microfiltration, high-temperature short-time