

211 Influence of serum prolactin concentrations on fecal shedding of *E. coli* O157:H7 in cattle. R. L. Farrow*, T. S. Edrington, K. M. MacKinnon, R. C. Anderson, and D. J. Nisbet, *USDA - ARS, College Station, TX.*

Previous research in our laboratory demonstrated hormones known to respond to changing day length can influence fecal shedding of *E. coli* O157:H7 in cattle. Continuing with this research, we examined the effect of serum prolactin concentrations on fecal shedding of *E. coli* O157:H7 and cellular immune response. 2-Bromo- α -ergocryptine methanesulfonate salt (BROMO) a dopamine agonist and sulpiride a D-2 dopamine receptor blocker were administered to decrease and increase prolactin levels, respectively. Fifteen Holstein steers experimentally infected with *E. coli* O157:H7 were randomly assigned to receive BROMO (0.05 mg/kg BW), sulpiride (0.05 mg/kg BW) or control (ethanol) via s.c. injection, twice daily. Fecal samples were collected daily and shedding of *E. coli* O157:H7 was determined via an immunomagnetic separation technique. Blood samples were collected via jugular venipuncture for analysis of serum prolactin concentrations and circulating neutrophils were isolated from peripheral blood on d 7 and 14 and degranulation and oxidative burst (OB) assays conducted. When examined over the 14-d experimental period, BROMO decreased ($P = 0.0001$) the percentage of cattle shedding *E. coli* O157:H7 (56% vs. 25.33% for control and BROMO treatments, respectively) while sulpiride had no effect ($P > 0.10$). BROMO decreased ($P < 0.0001$) serum prolactin concentrations, while sulpiride injections had no effect ($P > 0.10$). Oxidative burst by neutrophils for BROMO vs. control showed no significant difference on d 7, however, on d 14 OB by neutrophils tended to be higher for BROMO vs. controls ($P = 0.08$). Serum prolactin concentrations tended to be negatively correlated with OB ($P = 0.09$). No significant differences were observed for degranulation in BROMO vs. control on either d 7 or 14. These results support our hypothesis that hormones influenced by day length are responsible for the seasonality of *E. coli* O157:H7.

Key Words: *E. coli* O157:H7, beef cattle, seasonal shedding

212 Oral delivery systems for encapsulating bacteriophage targeted at *E. coli* O157:H7. K. Stanford*, T. P. Stephens¹, T. A. McAllister², D. Niu^{1,3}, and R. P. Johnson⁴, ¹Alberta Agriculture and Rural Development, Lethbridge, AB, Canada, ²Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, ³Dalian University of Technology, Dalian, China, ⁴Public Health Agency of Canada, Guelph, ON, Canada.

Bacteriophages (PHAGE) are natural predators of *E. coli* O157:H7 in feedlot cattle and their environment. As PHAGE are inactivated at low pH, protection against gastric acidity may enhance efficacy of orally-administered PHAGE. *In vitro*, polymer encapsulation effectively protected 4 spray-dried PHAGE (wV8, rV5, wV7 and wV11) from acid digestion and a mean of 2.70×10^9 PFU was recovered across PHAGE types after 20 min exposure to pH 2.8. Twenty-four steers were administered 10^{10} CFU of naladixic acid-resistant *E. coli* O157:H7 (NalO157) on D0 and housed in 6 pens of 4 animals. Two pens served as CONTROL (NalO157 inoculation only) and remaining animals received 10^9 PFU of polymer-encapsulated PHAGE on d-1, 1, 3, 6 and 8. Two pens received PHAGE orally in a gelatin capsule using a starch carrier (BOLUS) while the remaining 2 pens received PHAGE top-dressed on barley silage in the feed bunk (FEED). A daily 150g sample of FEED was retained to verify PHAGE titre using a standard plaque assay. Fecal shedding of *E. coli* O157:H7 was monitored for 10 wk by collection of fecal grab and hide swab samples from individual animals and via collection of fecal pats, feed and water in environment. Acceptable viability of mixed PHAGE was found in BOLUS and FEED

treatments, averaging 1.82 and 1.50×10^9 PFU, respectively. However, treatment with PHAGE did not reduce numbers of animals shedding NalO157 compared to CONTROL, although duration of shedding was reduced by 14 d in BOLUS as compared to CONTROL animals. This study developed 2 successful delivery systems for PHAGE, but a better understanding of PHAGE-*E. coli* O157:H7 ecology is required to make PHAGE therapy a viable mitigation strategy in the feedlot.

Key Words: bacteriophage, *E. coli* O157:H7, cattle

213 Effects of Aviplus® on *E. coli* O157:H7 in pure culture and in mixed ruminal culture fermentations. T.R. Callaway*¹, E. Grilli², M. R. Messina², and A. Piva², ¹Food and Feed Safety Research Unit, Agricultural Research Service, USDA, College Station, TX, ²DIMORFIPA, University of Bologna, Bologna, Italy.

Foodborne pathogenic bacteria can be harbored in the gut of food animals and transmitted to humans through the food supply, through water supplies or animal contact. Populations of the pathogenic bacteria *E. coli* O157:H7 can be affected by changes in the native flora of the intestinal tract. Organic acid products have been suggested for use as non-antibiotic modifiers of the gastrointestinal fermentation of animals. However, the impact of these acids on the overall microbial ecology of the intestinal tract remains unknown. Therefore, this study was designed to examine the effects of these acids on populations of the foodborne pathogen, *Escherichia coli* O157:H7. Pure cultures 3×10^5 CFU/ml of *E. coli* O157:H7 were added to tubes that contained Aviplus® added at 0, 0.1, 1, 2, 5, and 10% (w/v; n = 3). Aviplus® did not affect ($P > 0.1$) the growth rate or final populations of *E. coli* O157:H7 in pure culture, indicating that Aviplus® does not directly kill this pathogen at levels similar to those found in the intestinal tract. Ruminal fluid was collected from cows fed concentrate and placed in *in vitro* buffers. *E. coli* O157:H7 was added to *in vitro* ruminal fermentation, respectively, that contained Aviplus® at concentrations of 0, 1, 2, 5, and 10% (w/v; n = 2) and were incubated for 24 h. Aviplus addition did not affect ($P > 0.1$) populations of *E. coli* O157:H7 in the ruminal fluid. The ruminal A:P ratios were reduced ($P < 0.07$) by Aviplus® treatment. Organic acid products, such as Aviplus®, can alter the intestinal microbial ecology and impact animal productivity and health, however *in vitro* it does not appear that Aviplus® has an impact on populations of tested foodborne pathogenic bacteria inoculated at relatively high initial populations.

Key Words: Aviplus, food safety, fermentation

214 Control of *E. coli* O157:H7 in corn silage with inoculants under anaerobic and aerobic conditions. A. F. Pedroso^{1,2}, A. T. Adesogan², O. C. M. Queiroz², and S. K. Williams², ¹Brazilian Agricultural Research Corporation, Embrapa Cattle-Southeast, Sao Carlos, Sao Paulo, Brazil, ²Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, Gainesville.

The aim was to determine if bacterial inoculants could eliminate *E. coli* O157:H7 (ECOL) in contaminated corn silages and if inoculants transferred antibacterial activity to silages. Chopped corn forage was ensiled in triplicate after treatment with: 1) distilled water (control); 2) 5×10^5 cfu/g of ECOL (EC); 3) EC and 1×10^6 cfu/g of *Pedococcus pentosaceus* and *Propionibacterium freudenreichii* (EC+BII); 4) EC and 1×10^6 cfu/g of *Lactobacillus buchneri* (LB; EC+LB); 5) EC and 1×10^6 cfu/g of LB and *P. pentosaceus* (EC+B500). Silos were opened after 3, 7, 31, and 82 d and analyzed for pH and ECOL counts as well



as VFA, lactate, and aerobic stability on d 82. By d 3, all silages had pH was <4 (SE=0.33; p=1) and pH did not increase subsequently; therefore ECOL was not detected in any silage. The Kirby-Bauer disc diffusion test showed that all pure cultures of inoculants had pH-independent antibacterial activity against ECOL but inoculated silages did not, suggesting that ECOL elimination was mediated by pH reduction. Inoculation with LB resulted in less lactate (SE=0.31; p<0.05), more acetate (SE=0.35; p<0.05), and greater aerobic stability (SE=7.1; p<0.05) versus control. Day-82 silages were reinoculated with EC at silo opening (immediate) or after 144 h of exposure (delay) and ECOL were enumerated 24 h later. All immediately reinoculated silages had low pH values (<4) and no ECOL 24 h later. Control, EC, and EC+BII silages reinoculated after the delay had relatively high pH values (4.71, 5.67, and 6.03) (SE=0.74; p<0.05) and ECOL counts (2.87, 6.73, and 6.87 log cfu/g) (SE=1.4; p<0.05), whereas those treated with LB had low pH values (<4) and undetectable (EC+B500) or low ECOL counts (1.96, cfu/g; EC+LB). Inoculants did not enhance elimination of ECOL during ensiling, but *L. buchneri* inoculants increased stability and eliminated or inhibited ECOL in aerobically exposed silages.

Key Words: *E. coli* O157:H7, inoculant, silage

215 Characterization of antimicrobial-resistant *Escherichia coli* from samples collected throughout processing of feedlot cattle at a commercial abattoir. T. W. Alexander¹, G. D. Inglis¹, L. J. Yanke¹, E. Topp², and T. A. McAllister¹, ¹*Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada*, ²*Agriculture and Agri-Food Canada, London, Ontario, Canada*.

This study investigated antimicrobial-resistant *Escherichia coli* from samples throughout abattoir processing of feedlot cattle fed diets containing chlortetracycline plus sulfamethazine (AS700) or no antimicrobials (control). For each treatment, samples analyzed were: 1) feces collected at the feedlot (N=30); 2) hides after euthinization (N=15); 3) carcasses after evisceration and after 24 h in the chiller (N=15 for both); 4) ground beef stored for 1 and 8 d (N=15 for both); 5) digesta and mucosa from nine sections of the lower digestive tract (N=5 for each); 6) environmental abattoir samples (N=10); 7) air samples during slaughter (N=15). Generic, ampicillin (AREC)-, and tetracycline (TREC)-resistant *E. coli* were isolated on MacConkey agar or MacConkey agar containing ampicillin or tetracycline. All animals harboured AREC and TREC. Compared to control animals, the number of AREC (26.5% vs. 7.9%) and TREC (50.9% vs. 12.6%) were greater in feces from AS700 treated animals (P < 0.05) but were similar between hide, digesta and mucosa samples. Generic *E. coli*, AREC and TREC were detected from carcasses after evisceration and after 24 h in the chiller and d1- and d8-ground beef samples. Generic *E. coli* were isolated from all air samples. Resistant *E. coli* were isolated from the abattoir environment after slaughter of both groups of animals. Susceptibilities to 11 antimicrobials and pulsed field gel electrophoresis (PFGE) analyses were conducted on 362 AREC and TREC isolates across all samples. Twenty-five antibiogram profiles were detected. Most (28.2%) AREC were multi-resistant to ampicillin, streptomycin, and tetracycline while TREC (53.5%) were mainly resistant to tetracycline. From PFGE, 11 and 26 genotypes were detected amongst AREC and TREC respectively. Generally, isolates from meat and environmental samples had genetic backgrounds similar to isolates from animal tissue, digesta, or hide samples. These data indicate that antimicrobial-resistant *E. coli* from feedlot cattle can contaminate meat products at the slaughter plant and enter the food chain.

Key Words: antimicrobial resistance, abattoir, *Escherichia coli*

216 Screening of class IIa bacteriocin-producing lactic acid bacteria from Chinese traditional fermented food by PCR based method. H. Yi, L. Zhang*, Y. Tuo, X. Han, and M. Du, *Harbin Institute of Technology, Harbin, Heilongjiang, China*.

Class IIa bacteriocins of lactic acid bacteria are by far the most investigated and have been considered as one of the most interesting and potential groups of antimicrobial peptides for use in food preservation. There are abundant and various traditional fermented foods in China, which are likely to be valuable database containing class IIa bacteriocin-producing LAB. However, how to screen the desirable LAB rapidly from the complex fermented food ecosystem presents challenge. Therefore, development of rapid and reliable method for screening of microbes producing class IIa bacteriocins from potential organisms holds the key to the discovery of new and applicable class IIa bacteriocins. A method based on colony-PCR was developed and applied to screen class IIa bacteriocin-producing bacteria from 43 traditional fermented products (18 raw milk or yoghurt samples, 15 koumiss and 10 fermented vegetables samples) collected from specific ecological localities (Qinghai, Gansu, Sinkiang, Tibet) throughout the northwestern China. Results showed that 6 of 275 isolates gave rise to PCR fragments. Fragments of 3 kb can be detected with strain SB31 and Q5, while 3.4 kb with strain J20, J23 and 3.8 kb with strain M18 and X20 were obtained. The discrepancy of the amplicons size suggests that the size of peptide between bacteriocin and histidine kinase exists strain-specific. Amplicons of 332 bp (with strain M18 and X20), 412 bp (with strain SB31 and Q5), 428 bp (with strain J20 and J23) indicate the presence of pediocin, enterocin, plantaricin, respectively. This assay showed agreement with the conventional well-diffusion method. It offers several advantages over the existing methods in terms of rapidity, simplicity and accuracy, which make it a promising alternative to the conventional protocols. In addition, isolates of LAB from natural niches with the plateau climate in the northwestern China could not only preserve the native microbes, but also eventually be an important resource for the development of novel starter cultures as well as food biopreservatives.

Key Words: class IIa bacteriocin, lactic acid bacteria, screen

217 *Salmonella* infection and immune response in finishing pigs. M. H. Rostagno*, S. D. Eicher, and D. C. Lay, *USDA, ARS, Livestock Behavior Research Unit, West Lafayette, IN*.

Finishing pigs infected with *Salmonella* pose food safety risks by carrying the pathogen into abattoirs. A study was conducted to determine the dynamic of *Salmonella* infection in finishing pigs, and immunological alterations that occur in *Salmonella*-carrier pigs, by longitudinally comparing infected to non-infected pigs. Pigs (n=24) were individually inoculated with *Salmonella* Typhimurium. Fecal and blood samples were collected from each pig, and 3 pigs were randomly selected and euthanized to collect additional samples (spleen, liver, mesenteric lymph node, ileum, and cecum) on days 1, 2, 7, 14, 21, 28, 35, and 42 post-inoculation (p.i.). A control group (n=15) of non-infected pigs was maintained for comparison by sampling at 1, 2, 7, 14, and 21 days. No inoculated animal showed any clinical sign of infection. Bacteriological data revealed that all inoculated pigs started shedding *Salmonella* within 24 h p.i., and persistently shed the bacteria up to the end of the study. Ileal and cecal content samples were all positive throughout the study. Mesenteric lymph nodes were also positive during the entire study and at the same level as intestinal content samples. All samples contained 3-4 logs (cfu/g) of *Salmonella* at 24 h p.i., and 4-5 logs (cfu/g) of *Salmonella* up to 4 wk p.i. Interestingly, levels of *Salmonella* dropped markedly (P<0.05) in all samples at 5 wk p.i., being detectable only by enrichment.