

Food Safety: Food Safety Advances

T85 Occurrence of several antibiotic residues in raw milk in ten provinces of China. R. W. Han^{1,2}, J. Q. Wang^{*1}, N. Zheng¹, X. M. Xu¹, Y. P. Zhen¹, X. Y. Qu¹, P. Sun¹, and Z. N. Yu³, ¹State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, ²College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, Shandong, China, ³Haidu College, Qingdao Agricultural University, Laiyang, Shandong, China.

The objective of this study was to investigate the occurrence of antibiotic residues in raw milk in China. A total of 199 raw milk samples from 10 provinces of China were examined for β -lactams, tetracyclines, sulfonamides and quinolones. SNAP Beta-Lactam Test kit (Idexx) was used for qualitative detection of β -lactam antibiotics, and sample was positive with the ratio 1.06 or higher. The limits of detection (LOD) of R-Biopharm test kits (Ridascreen) which used for quantitative detection of tetracyclines, sulfonamides and quinolones were 1.5 μ g/kg, 5.0 μ g/kg, and 0.25 μ g/kg, respectively, which were far below the maximum residue limits (MRL, regulated by EU, CAC, and China). Only one positive sample from Beijing for β -lactams was found with a ratio of 1.39. No sample was positive for tetracyclines, while 47.23% samples for quinolones and 20.1% samples for sulfonamides were positive. Sulfonamides and quinolones were positive in all provinces. Shanghai (detection rate 5%) was lowest for quinolones and Hebei (detection rate 80%) was highest. Beijing (detection rate 10%) was lowest for sulfonamides and Shanghai (detection rate 80%) was highest. The maximums detected for sulfonamides and quinolones were 16.28 μ g/kg (Guangdong) and 23.25 μ g/kg (Tianjin), respectively. All samples for tetracyclines, sulfonamides and quinolones were under their MRL. In general, the residue levels of the 4 major antibiotics in raw milk in China are safe for people. But stringent measures still needed to control antibiotic residues in raw milk because of the high detection rate of some antibiotics.

Key Words: antibiotic residues, raw milk, China

T86 Occurrence of aflatoxin M1 in raw milk and UHT milk in China. N. Zheng, J. Q. Wang,* R. W. Han, X. M. Xu, Y. P. Zhen, X. Y. Qu, and P. Sun, State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

Aflatoxin M1 (AFM1) can cause teratogenic and mutagenic effects, and has been classified as a secondary group of carcinogenic compounds by the International Agency for Research on Cancer of World Health Organization. AFM1 is the only mycotoxin that has a legal limit in milk worldwide. The limits of 50 ng/L by the European Union and 500 ng/L by the USA have been adopted by many countries and international organization. The objective of the present study was to investigate the occurrence of AFM1 in raw milk and UHT milk in China. 360 raw milk samples and 153 UHT milk samples were collected from primary milk producing regions in China, including Beijing, Hebei, Shanxi, Shanghai, Guangdong provinces. The AFM1 was examined using a competitive enzyme immunoassay kit (R1111, R-Biopharm AG, Darmstadt, Germany), with the limits of detection of 5 ng/L. The data was statistically analyzed using the SPSS version 11.5 (SPSS, Inc., Chicago, IL). 78.1%

of raw milk samples and 54.9% of UHT milk samples contained AFM1. It showing that the occurrence of AFM1 in raw milk were higher than that in UHT milk. The concentration of AFM1 in positive raw milk samples and UHT milk samples were in the range of 5 to 123 ng/L and 6 to 160 ng/L, respectively. However, the content of AFM1 between positive raw milk samples and positive UHT milk samples had no significant ($P > 0.05$) difference. AFM1 contents in all positive samples were far below the China and the US legal limit of 500 ng/L, but 10% of the raw milk samples and 20.3% of the UHT milk samples exceeded the EU legal limit of 50 ng/L. Therefore, the potential for contamination of AFM1 in milk in China should be considered, and effective measures should be applied to decrease the contaminant of milk with AFM1.

Key Words: aflatoxin M1, milk, China

T87 Purple prairie clover condensed tannins inhibit *Escherichia coli* through disruption of outer and inner membranes. X. L. Liu^{*1,2}, L. Jin¹, Z. Xu¹, Y. Q. Hao², T. A. McAllister¹, and Y. Wang¹, ¹AAFC, Lethbridge, AB, Canada, ²Inner Mongolia Agricultural University, China.

It has been suggested that feeding condensed tannin (CT)-containing forage could be a strategy to mitigate pathogens such as *Escherichia coli* O157:H7 in ruminants. Although antimicrobial activity of CT against G⁺ bacteria is well documented, few of them possess activity against G⁻ bacteria, including *E. coli*. Our previous research revealed that CT isolated from purple prairie clover (PPC; *Dalea purpurea* Vent.) possesses a broad-spectrum of anti- *E. coli* and *E. coli* O157:H7 activity, which was bacterostatic up to the concentration of 400 μ g/mL, through an unknown mechanism. The objective of this study was to evaluate the effect of PPC CT on cell aggregation, cell membrane permeability and integrity. *E. coli* (ATCC25922) was aerobically cultured in M9 media containing 0 or a sub-lethal concentration (MIC = 12 μ g/mL) of 10 μ g/mL of CT at 37°C for 10 h. The bacteria were separated from the media and re-suspended in phosphate buffer (adjusted to OD₄₂₀ = 1.0). Inner membrane (IM) permeabilization was determined by measuring cytoplasmic β -galactosidase activity and outer membrane (OM) disruption by fluorescence measurement of the uptake of 1-N-phenyl naphthylamine (NPN) by the cell membrane. Cell aggregation was assessed using fluorescence spectrophotometry and fluorescence microscopy and cell wall integrity was examined by transmission electron microscopy (TEM). All determinations except for TEM were done 3 times over 2-wk period with 4 replicates for each time. *E. coli* cultured with 10 μ g/ml CT had higher ($P < 0.01$) extracellular β -galactosidase activity than those cultured without CT, indicating an increase in the IM permeability by CT. The NPN uptake by OM was increased ($P < 0.001$) by culturing *E. coli* with 10 μ g/ml of CT. Fluorescence microscopy showed that *E. coli* cells aggregated immediately after CT was added to the cell suspension and this was accompanied by a reduction ($P < 0.01$) in fluorescence. Examination by TEM revealed that the OM structure was also disrupted. These observations suggest that PPC CT inhibit *E. coli* growth through disruption of cell membrane structure and function. Whether inhibition of *E. coli* O157:H7 by PPC CT involves the similar mechanism needs to be further studied.

Key Words: condensed tannin, *E. coli*, cell membrane permeability

T88 Antimicrobial resistance of *Salmonella enterica* isolated from bulk tank milk and milk filters in the United States. J. S. Van Kessel*¹, J. Sonnier¹, S. Zhao², and J. S. Karns¹, ¹*Environmental Microbial and Food Safety Laboratory, USDA-ARS, Beltsville, MD*, ²*Center for Veterinary Medicine, US FDA, Laurel, MD*.

Non-typhoid *Salmonella* is frequently associated with dairy cattle and their environment. Despite well-developed milking hygiene protocols, fecal contamination of the bulk milk can still occur; *Salmonella* has often been isolated from bulk milk. The prevalence of *Salmonella* in US bulk tank milk was determined as part of the NAHMS Dairy Surveys in 2002 and 2007. In-line milk filters were also tested in the 2007 survey. The objective of this study was to determine the level of antimicrobial susceptibilities in *Salmonella* isolated from bulk milk and milk filters in the NAHMS surveys. Susceptibilities to 15 antibiotics were determined for 176 *Salmonella* isolates, representing 26 serotypes, using a Sensititer automated antimicrobial susceptibility system. Resistant isolates were screened by PCR for the presence of the bla_{CMY} gene and class I integrons, and further characterized by pulsed-field gel electrophoresis (PFGE). Thirty isolates (17.0%) including 6 serotypes [Newport (14/14), Dublin (7/7), Typhimurium (3/5), Kentucky (4/22), Anatum (1/13), and Infantis (1/2)] exhibited resistance to at least one antimicrobial agent. Twenty isolates (11.4%) including all 14 *Salmonella* Newport, 2 *Salmonella* Dublin, 2 *Salmonella* Typhimurium and 1 *Salmonella* Infantis displayed the typical MDR-AmpC phenotype, which showed resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline (ACSSuT), plus resistance to amoxicillin/clavulanic acid, cefoxitin, ceftiofur and ceftriaxone. All MDR-AmpC isolates carried the bla_{CMY} gene and 5 resistant isolates contained class I integrons (2.8%). Two-enzyme (*Xba*I and *Bln*I) PFGE discerned clades within serotypes, although it did not discriminate isolates based on year, antibiotic resistance profile, or geographic location. Testing raw milk and milk filters may be a useful means of surveying dairy farms for the presence of antimicrobial resistant *Salmonella*. These data suggest that there is a low but notable presence of antimicrobial resistance in salmonellae from raw milk.

Key Words: antimicrobial resistance, raw milk, *Salmonella*

T89 The effects of tetracycline analogue on prevalence of resistance genes encoded by *Escherichia coli* isolated from feedlot cattle. X. Jin^{1,2}, T. A. McAllister¹, Q. Li², and T. W. Alexander*¹, ¹*Agriculture and Agri-Food Canada, Lethbridge, AB, Canada*, ²*Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China*.

The effects of administering feedlot cattle subtherapeutic levels of chlortetracycline (CT) or CT and therapeutic levels of oxytetracycline (CT-OX) on resistance genotype in *Escherichia coli* were investigated. Fecal samples were collected from cattle that had been housed in commercial feedlots for greater than 60 d and had a documented history of antimicrobial use. Isolates were tested for susceptibility to tetracycline, chlortetracycline, and oxytetracycline using disk diffusion or broth microdilution. Detection of *tet*(A), *tet*(B), and *tet*(C) genes encoded by tetracycline-resistant isolates (n = 176) was performed by multiplex PCR. All isolates encoded at least one or a combination of 2 resistance genes. Prevalence of *tet*(A) was similar between groups of *E. coli*, however prevalence of *tet*(B) was lower (18% versus 34%; $P < 0.05$) and *tet*(C) was greater (46% versus 28%; $P < 0.05$) in CT isolates. The nature of the *tet* determinants was further assessed in a group of intermediately tetracycline-resistant isolates (n = 52). The *tet*(C) gene was present in 92% of these isolates. Minimum inhibitory concentrations showed that susceptibility was dependent on tetracycline analog and the type of resistance determinant. Copies of *tet*(C) transcripts,

analyzed by real-time PCR, indicated that upregulation did not occur in tetracycline-resistant isolates when compared with intermediately-resistant isolates. However, sequence analysis of the *tet*(C) gene revealed a T→G substitution at position 1063 in intermediately-resistant isolates that may have affected phenotype. These data provide insight into the relationship between the type of tetracycline analog administered to cattle and the prevalence of resistance genes in *E. coli*.

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

T90 Cranberry juice and cranberry fiber are accepted by newly weaned pigs. S. D. Eicher*¹, B. T. Richert², and M. H. Rostagno¹, ¹*USDA-ARS, West Lafayette, IN*, ²*Purdue University, West Lafayette, IN*.

Cranberry products offer a novel mechanism to control the ability of pathogens to colonize their host. However, it is not known how well pigs will consume juice or feed with cranberry fiber incorporated. The objective of this experiment was to determine if cranberry liquid or fiber products were palatable to newly weaned pigs. Pigs were weaned onto a pelleted diet for 3 d before starting this experiment. In a randomized complete block design, 60 4 recently weaned pigs (22 d of age) were blocked by weight into 8 pens with 4 pens/preference test (pen = experimental unit). Pigs were assigned to treatments to test for preferences of cranberry juice (0, 1, or 10% of 7.5 Brix) or a control dry feed versus dry feed supplemented with 4% cranberry fiber (both cranberry products were from Marshall Ingredients, NY). Control dry feed was provided to pigs on the liquid preference test, and water was provided for those on the dry feed preference test. Liquid choices were added as needed and weighed back every 3-d period. Dry feeds were also added as needed and weighed back every 3 d. Occurrences of eating or drinking of the test liquids and feeds were taken for 2 h from 0900 to 1100 and 1400 to 1600 for the first 3-d period by 1-min duration scan samples every 10 min. Cranberry juice (10%) was consumed more readily than water or than the 1% cranberry juice ($P = 0.01$) during the last 2 periods. Behavior tended to support the consumption of 10% cranberry juice, particularly in the morning observations, but overall was not different among liquid treatments ($P = 0.15$). Although the cranberry supplemented dry feed was eaten more on the first observation period, the treatments were preferred similarly over the remainder of the observations; and overall, the dry feeds were consumed relatively equally, 163 vs. 135 g/d for control and cranberry feeds respectively ($P > 0.10$). Results of this study support the use of cranberry products as a liquid (10%) or as fiber (4%) added to dry feed. Consequently, cranberry products will be used in follow-up research to determine its efficiency as microbial and immune modulators in young pigs.

Key Words: nursery pigs, cranberry

T91 Evaluation of hygienic and sanitary quality of jerked beef commercialized in Salvador city, Bahia, Brazil. L. Pereira, M. Silva, W. Costa, and R. Matoso,* *UFBA, Salvador, Bahia, Brazil*.

The objective of this study was to assess the sanitary quality of jerked beef sold in the city of Salvador, Bahia. We collected 30 samples of dried beef in retail markets located in different neighborhoods of the city. These samples were collected in the form of presentation available to consumers, observing their physical integrity. Each sample was subjected to processing and then plated in liquid and specific solid media to investigate aerobic mesophilic heterotrophic bacteria, coagulase-positive *Staphylococcus*, yeasts and molds. The plates for detection of aerobic heterotrophic bacterial mesophilic and *Staphylococcus*

coagulase-positive were incubated at 35°C for 48 h while the plates for the detection of yeasts and molds were incubated at 28°C for 5 d. Afterward colonies were read and counted, in addition to the complementary tests (smear stained by Gram's method, the catalase and coagulase test). It was observed that 100% of samples had counts of aerobic mesophilic microorganisms ranging from 1.2×10^6 to 9.3×10^6 cfu/g, higher than acceptable levels for other meat products contained in Resolution RDC No. 12 of Ministry of Health. Although the counts of molds and yeasts were lower, ranging from 2×10^2 to 8.3×10^4 cfu/g. When correlating the results with those previously described in literature, it is possible

to verify that the samples were in low hygiene conditions. Finally, the presence of *Staphylococcus* coagulase positive in 100% of the samples indicates that there was poor handling of the food product. It is extremely necessary to establish more rigorous standards for the microbiological quality of jerked beef to determine the quality of these products intended for human consumption. These standards should guarantee foods with good hygiene conditions what would increase product lifetime, avoid economic losses and prevent risks to public health.

Key Words: meat products, public health, microbiological quality