TH210 Occurrence of aflatoxin in dairy cow feed and raw milk in China. N. Zheng[1,2], J. Q. Wang*[1,2], Y. P. Zhen[1,2], X. M. Xu[1,2], R. W. Han[1,2], S. L. Li[1,2], and X. Y. Qu[1,2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences,, Beijing, China, [2]Ministry of Agriculture - Milk and Dairy Product Inspection Center (Beijing), Beijing, China.*

Aflatoxin (AF) M1 is carcinogenic and exists in milk due to dairy cows consuming AFB1 contaminated feeds. The legal limit of AFM1 in milk is set as 50 ng/L by European Union (EU) et al. and 500 ng/L by China, US, Japan et al. Meanwhile, the legal limit of AFB1 in dairy cow feeds also is established to control the level of AFM1 in milk. A limit of 10 μg/kg is set by China, Japan et al. and 5 μg/kg is set by EU et al. Furthermore, countries such as US and Canada set the legal limit of total AF (AFB1+AFB2+AFG1+AFG2) in dairy cow feed as 20 μg/kg. In the present study, the occurrence of AFs in dairy cow feed and raw milk in China was investigated. Two hundred dairy cows feed samples and 2 hundred raw milk samples were collected from 10 of the main milk-producing provinces in China. AFB1, B2, G1 and G2 in the feed samples were analyzed using the HPLC method. AFM1 in the raw milk samples was determined using the ELISA method. The data was statistically analyzed using the SPSS version 11.5 (SPSS, Inc., Chicago, IL). AFB1 and AFB2 were found in the feed samples, but not AFG1 and AFG2. In the feeds, 17.5% of feed samples contained only AFB1, 11.5% of samples contained only AFB2, and 24.5% of samples contained both AFB1 and AFB2. Totally 42% of the samples contained AFB1 within the range of 0.05–3.53 μg/kg, and 36% of the samples were positive for AFB2, with the content ranging from 0.03 μg/kg to 0.84 μg/kg. The content of AFB1 was significantly ($P < 0.05$) higher than that of AFB2 in the feeds. The AFB1 content in the positive feed samples was below the legal limit in China of 10 μg/kg and even below the EU legal limit of 5 μg/kg. The total content of AFs was below the U.S legal limit of 20 μg/kg. For the raw milk samples, 32.5% were positive for AFM1, containing 5.2–59.6 ng/L, a level far below the legal limit in China and the US of 500 ng/L, though 3 samples contained AFM1 exceeding the EU legal limit of 50 ng/L. It suggested that the AFM1 in raw milk in China is in the safe level due to the low concentration of AFB1 in dairy cow feeds.

Key Words: aflatoxin, feed, raw milk

TH211 Survey of 38 veterinary drug residues in raw milk in China. R. W. Han[1,3], N. Zheng[1,2], J. Q. Wang*[1,2], Z. N. Yu[3], X. M. Xu[1,2], Y. P. Zhen[1,2], X. Y. Qu[1,2], and L. C. Huang[1,2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture - Milk and Dairy Product Inspection Center (Beijing), Beijing, China, [3]College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, Shandong, China.*

The aim of the study was to investigate the occurrence of veterinary drug residues in raw milk of China. A total of 178 raw milk samples were collected from 8 provinces of China in July, 2012. Thirty 8 veterinary drugs including 14 β-lactams, 8 quinolones, 8 sulfonamides, 5 tetracyclines and 3 macrolides were determined with a developed multiclass method by ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The limit range of quantification was 0.03–10 ng/mL and the recovery range was 67.9–117.7%. The data was statistically analyzed using the SPSS version 11.5 (SPSS Inc., Chicago, IL). A total of 21 veterinary drugs including 5 β-lactams, 6 quinolones, 6 sulfonamides, 2 tetracyclines and 2 macrolides were detected and the detection percentage were in the range of 1.7–37.1% for β-lactams, 0.6–47.8% for quinolones, 1.7–24.7% for sulfonamides, 3.4–14.6% for tetracyclines and 1.7–34.8% for macrolides. The maximum concentrations for detected veterinary drug residues were 7.68 ng/mL for β-lactams (cefoperazone), 11.2 ng/mL for quinolones (ciprofloxacin), 1.93 ng/mL for sulfonamides (trimethoprim), 5.78 ng/mL for tetracyclines (doxycycline) and 76.25 ng/mL for macrolides (lincomycin). The percentage of 5.1% samples was found no veterinary drug residues, while 94.9% samples contained veterinary drug residues with the maximum type of 7. Chi-squared statistics analysis showed there were no differences ($P > 0.05$) on the veterinary drugs' detection percentages among 8 provinces. No samples exceeded the maximum residue levels of the veterinary drugs regulated by China, European Union and Codex Alimentarius Commission. It showed the veterinary drug level in raw milk was safe for consumption in China.

Key Words: veterinary drug, raw milk, China

TH212 Occurrence of organochlorine pesticide residues in raw milk in China by gas chromatography triple-quadrupole mass spectrometry. X. M. Xu[1,2], N. Zheng[1,2], J. Q. Wang*[1,2], R. W. Han[1,2], and S. L. Li[1,2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture - Milk and Dairy Product Inspection Center (Beijing), Beijing, China.*

Most of the countries and international organizations have issued standards and regulations to limit pesticide residues in milk. China government set maximum residue limits (MRLs) of 18 organochlorine pesticides in milk. In the present study, a total of 178 raw milk samples from 8 primary milk producing provinces of China, including Inner Mongolia, Heilongjiang, Sichuan, Shandong et al., were determined for 18 organochlorine pesticides using Gas chromatography triple-quadrupole mass spectrometry (GC-MS/MS; 7000B, Agilent, USA) to assess the contamination of pesticide residues in raw milk in China. The limits of quantification (LOQ) were from 0.5 $\mu g.kg^{-1}$ to 6 $\mu g.kg^{-1}$ for 18 organochlorine pesticides respectively. Pesticides in milk samples were extracted by a solid phase system with acetone. An extract aliquot of acetone was injected into the GC-MS/MS. Recoveries of pesticides spiked in raw milk samples were 78–122%. Among 18 organochlorine pesticides, 10 pesticides of Aldrin, Endosulfan I, Endosulfan sulfate, DDT-p,p', Dieldrin,BHC-α, DDD-p,p', BHC-β, Lindane, DDE-p,p', were found in milk samples. The frequency of total samples containing detectable levels of 18 organochlorine pesticides residues was 1.2%, 1.2%, 1.2%, 2.4%, 2.4%, 2.9%, 4.1%, 14.1%, 14.7%, 22.4% in total milk respectively. There were no differences ($P > 0.05$) on the pesticides' detection among 8 provinces after Chi-squared statistics analysis. The maximum concentrations were 5.27 $\mu g\ kg^{-1}$ for Aldrin, 4.56 $\mu g\ kg^{-1}$ for Endosulfan I (α isomer), 4.33 $\mu g\ kg^{-1}$ for Endosulfan sulfate, 4.42 $\mu g\ kg^{-1}$ for DDT-p,p', 5.47 $\mu g\ kg^{-1}$ for Dieldrin, 4.64 $\mu g\ kg^{-1}$ for BHC-α, 4.80 $\mu g\ kg^{-1}$ for DDD-p,p', 4.56 $\mu g\ kg^{-1}$ for BHC-β, 4.56 $\mu g\ kg^{-1}$ for Lindane, 5.33 $\mu g\ kg^{-1}$ for DDE-p,p'. For all positive samples, only one sample contained Endosulfan (4.56 $\mu g\ kg^{-1}$) exceeded the legal limit of Japan (4 $\mu g\ kg^{-1}$), while the others were within the legal limit set by China, European Commission, New Zealand. This survey show the level of pesticides in milk in China is safe to consume.

Key Words: occurrence, pesticide residue, raw milk

TH213 Occurrence of heavy metals in raw milk in China. X. Y. Qu[1,2], N. Zheng[1,2], J. Q. Wang*[1,2], X. M. Xu[1,2], R. W. Han[1,2], Y. P. Zhen[1,2], and S. L. Li[1,2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture - Milk and Dairy Product Inspection Center (Beijing), Beijing, China.*

The levels of heavy metals are an important component to assess the quality of milk. The nations set the legal limit to control the levels of heavy metals in milk. China government set legal limit of 4 heavy metals, including lead of 0.05 mg/kg, chromium of 0.3 mg/kg, mercury of 0.01 mg/kg and arsenic of 0.05 mg/kg. The object of this study was to assess the levels of aluminum, lead, chromium, nickel, cadmium, mercury and arsenic residues in raw milk in China. One hundred and 70 8 raw milk samples were collected from China's 8 of the main milk-producing provinces. The heavy metals were determined using a validation simultaneous analysis method of inductively coupled plasma-mass spectrometry (ICP-MS). The limits of detection (LOD) of aluminum, lead, chromium, nickel, cadmium, mercury and arsenic were 0.01 mg/kg, 0.002 mg/kg, 0.01 mg/kg, 0.003 mg/kg, 0.001 mg/kg, 0.001 mg/kg and 0.001 mg/kg, respectively. The data was statistically analyzed using the SPSS version 11.5 (SPSS, Inc., Chicago, IL). A total of 48.9% of the milk samples contained aluminum, and 12.9% samples contained chromium, 23.6% samples contained nickel, 9.0% samples contained arsenic, 27.5% samples contained mercury, 28.1% samples contained lead, and no sample was positive to cadmium. For the positive samples, the maximums and means were 1.52 mg/kg and 0.28mg/kg of aluminum, 0.08 mg/kg and 0.04mg/kg of chromium, 0.005 mg/kg and 0.002mg/kg of mercury, 0.106 mg/kg and 0.011 of nickel, 0.033 mg/kg and 0.008mg/kg of lead and 0.002 mg/kg and 0.001 of arsenic. Aluminum, chromium and mercury were existed in all provinces, and the levels of these 3 heavy metals had no significant ($P > 0.05$) difference in 8 provinces. Nickel, lead and arsenic were not found in Tianjin and Inner Mongolia. The concentrations of lead, chromium, mercury and arsenic in all positive samples were below China's national legal limits. It showed that the heavy metals in milk in China were in the safe level.

Key Words: heavy metal, raw milk, China

TH214 Occurrence of four mycotoxin residues in raw milk in China. L. C. Huang[1,3], N. Zheng[1,2], J. Q. Wang*[1,2], J. B. Cheng[1,3], R. W. Han[1,2], X. M. Xu[1,2], and S. L. Li[1,2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture - Milk and Dairy Product Inspection Center (Beijing), Beijing, China, [3]College of Animal Science and Technology, Anhui Agricultural University, Hefei, China.*

A study was conducted to investigate the occurrence of mycotoxins in raw milk in China. A total of 178 raw milk samples from 8 primary milk producing provinces of China, including Inner Mongolia, Heilongjiang, Sichuan, Shandong, et al., were examined for aflatoxin M_1 (AFM_1), ochratoxin A (OTA), α-zearalenol (α-ZEL) and zearalenone (ZON). Mycotoxins were simultaneously determined by UPLC-MS/MS (TQ-S, Waters, USA), with the limits of quantification (LOQ) of 3, 12, 9 and 3 $ng \cdot kg^{-1}$ for AFM_1, OTA, α-ZEL and ZON, respectively. LOQs were sensitive enough to meet the requirement of the maximum residue limits (MRLs) regulated by European Union, Codex Alimentarius Commission and China. The data was statistically analyzed using the SPSS version 11.5 (SPSS, Inc., Chicago, IL). 84% of the raw milk samples were contaminated with AFM_1, 38% with OTA, 74% with α-ZEL and 87% with ZON. The average concentration of AFM_1, OTA, α-ZEL and ZON in positive samples were 14.7, 20.4, 115.2 and 22.1 $ng \cdot kg^{-1}$, respectively. The samples contained AFM_1, OTA, α-ZEL and ZON were found in all provinces. The lowest detection rates for 4 mytcotonxins were 60% of AFM_1 and 35% of ZON in Inner Mongolia, 50% of OTA in Shandong, 23.8% of α-ZEL in Hubei, while the highest detection rates for 4 mytcotonxins were 100% of AFM1 in Shandong and Tianjin, 96.3% of OTA in Anhui, 100% of α-ZEL in Shandong, 100% of ZON in Shandong, Shanxi and Tianjin. The maximums of AFM_1, OTA, α-ZEL and ZON were 95.5 $ng \cdot kg^{-1}$ (Inner Mongolia), 198.9 $ng \cdot kg^{-1}$ (Inner Mongolia), 648.3 $ng \cdot kg^{-1}$ (Anhui) and 111.9 $ng \cdot kg^{-1}$ (Inner Mongolia), respectively. Among the 4 mycotoxins, only AFM_1 is set MRL in milk all over the world, and 50 $ng \cdot kg^{-1}$ represented by European Union and 500 $ng \cdot kg^{-1}$ represented by US and China are 2 peak limits. In the present study, the AFM_1 concentrations of 4 samples were between 50 $ng \cdot kg^{-1}$ and 500 $ng \cdot kg^{-1}$. However, high percentages of positive samples for OTA, α-ZEL and ZON were found in milk in China. So the contaminant of mycotoxins in raw milk in China should be considered, and the effective measure should be applied to decrease the contaminant of mycotoxins.

Key Words: mycotoxin, raw milk, China

TH215 Effect of prophylactic use of antibiotics in intravaginal sponges on the response of inhibitor screening tests in goats milk. T. Romero[1], J. Balado[2], R. L. Althaus[3], M. C. Beltrán[1], and M. P. Molina*[1], *[1]Instituto de Ciencia y TecnologAnimal. Universitat Politecnica de Valencia, Valencia, Spain, [2]Diputacion Provincial de Castellón, Ares del Maestrat, Castellón, Spain, [3]Cátedra de Biofisica, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Argentina.*

The objective was to determine whether the prophylactic use of antibiotics in the placement of intravaginal sponges in estrus synchronization treatments in caprines may be the cause of the presence of inhibitors in milk and, therefore of positive results in screening tests. Ninety-eight Murciano-Granadina goats were used, divided into groups of 14 animals each. Intravaginal sponges were placed in 6 groups using commercial antibiotics: Terramycin Oral (oxytetracycline), Hipradoxi (doxycycline), and Framicas (sulfathiazole 96% and framycetin 4%) with 2 different concentrations of each. In a control group goat sponges were placed without any antibiotic. Milk samples were collected daily along the 7-d-post-treatment period. SCC and pH were determined in all samples and analyzed by means of 3 microbiological screening methods (BRT MLR, Delvotest MCS and Eclipse 100). Moreover, milk of the first 3 d as well as the positive samples in microbiological tests were analyzed with receptor binding protein methods specific for tetracyclines and sulfonomides. When sponges were removed, the degree of cleanliness was visually assessed to evaluate antibiotic efficacy. The detection limits of the methods were calculated using logistic regression, and the χ^2-test was used to evaluate the degree of cleanliness of the sponges. Microbiological methods presented positive results, the BRT MLR method showed the highest number of them for all antibiotics, even in the control group, indicating a lower selectivity of the method for goat milk. By contrast, positive results were lower in Delvotest MCS and Eclipse 100 for all treatments. Antibiotic sponges presented superior odor, color and minor adhesions than the control group; treatments with tetracyclines (oxytetracycline and doxycycline) having a higher degree of cleanliness with the higher dose. It can be concluded that the prophylactic use of oxytetracycline, doxycycline and sulfathiazole in intravaginal sponges used in estrus synchronization treatments in goats does not seem to constitute a risk of antibiotic residues in milk.

Key Words: goat milk, intravaginal sponge, antibiotic screening method

TH216 Validation of new SNAP Beta-Lactam antibiotic residue test kit for goat milk screening. S. S. Zeng*, K. Tesfai, E. Vasquez, I. Portugal, and C. Watson III, *Langston University, Langston, OK.*

Experiments were conducted to validate the SNAP Beta-Lactam Test Kit (SNAP NBL) for screening antibiotic residues in goat milk for human consumption. Raw goat milk collection, preparation of drug-fortified goat milk samples, drug-incurred study, sample testing, data collection and analysis, and result interpretation were performed according to the FDA-DVM validation protocol (i.e., SNAP-Beta Lactam Test Form, FDA 2400n). Results indicate that the SNAP NBL Test Kit did not show any positive readings (i.e., 100% specificity) in unfortified and uncontaminated fresh or frozen goat milk. This test kit had 100% sensitivity in detecting antibiotic residues in fortified goat milk with ampicillin, amoxicillin, cephapirin and penicillin G at their respective tolerance and/or safe levels set forth by FDA for cow milk. It must be noted that this test might be more sensitive for goat milk than for cow milk with detection below the tolerance and/or safe levels of the drugs, leading to possible sub-violative positive results. The SNAP NBL Test Kit was also effective in screening for antibiotic resides in milk throughout lactation after goats were treated with antibiotic drugs. The clearance time of antibiotic residues from the mammary glands to tolerance levels and detection levels of the SNAP test depended on the drugs used and the amounts applied. In conclusion, the SNAP NBL Test Kit was effective in screening antibiotic residues in goat milk and is recommended for use in dairy goats

Key Words: goat milk, antibiotics, SNAP test

TH217 Antimicrobial residues in pasteurized milk assessed by the inhibition test of microbial growth and HPLC-DAD. A. P. A. Magnavita[1], S. A. A. Fernandes[1], S. P. B. Ferrão[1], S. A. Gualberto[1], and S. V. Matarazzo*[2], *[1]Universidade Estadual do Sudoeste da Bahia, Itapetinga, Bahia, Brasil, [2]Universidade Estadual de Santa Cruz, Ilheus, Bahia, Brasil.*

The objective of this study was to qualitatively evaluate the occurrence of antibiotic residues in pasteurized milk, under State Inspection, marketed in the state of Bahia, Brazil. Milk samples were collected monthly between November/2010 and October/2011 from commercial establishments. The Delvotest SP-NT was applied to 252 pasteurized milk samples, in duplicate, of 21 different brands. In the positive and/or suspect samples both oxytetracycline (OTC) and tetracycline (TC) were quantified by HPLC-DAD. Of all the samples, it was observed that 207 were negative (82.0%), 19 positive (7.6%) and 24 suspect (9.6%). The positive and/or suspect samples were concentrated in the first semester of the year (January to July). Of the 19 positive samples in the screening test, OTC was detected in all and TC in 6. In the 24 suspected samples, in only one was the presence of OTC not detected and in 8 no TC was found. Of the milk brands evaluated, the presence of antibiotic residues was not detected in 4; in the other brands both positive and suspect samples were verified in some period of the year. The screening test used was effective for identifying the presence of antibiotic residues (OTC and TC), confirmed by liquid chromatography. Results indicate the presence of antibiotic residues above legal limits, however, in low proportion. In 10 of the 16 dairy facilities with antibiotic residues in milk, the EDI was less than the maximum recommended by the European Union. In general, pasteurized milk sold in cities of Bahia presented low occurrence of antibiotic residues, of which OTC was predominant. This requires that the presence of antibiotic residues is controlled in milk from Bahia.

Key Words: pasteurization, antibiotic, Delvotest SP-NT

TH218 Regulatory processes for substances used in animal food. M. G. Alewynse* and S. A. Benz, *Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD.*

There is increasing interest in the marketing of novel substances for use in animal food. Animal food includes both livestock feed and companion animal food. These substances may be intended to be a source of nutrients or, like enzymes, may affect the characteristics of the food itself. Food falls under the regulatory authority of the US Food and Drug Administration (FDA). Within FDA, the Center for Veterinary Medicine (CVM) regulates both food and drugs intended for animals. The Division of Animal Feeds is responsible for regulating substances used in, or as, animal food. Substances added to a food must be safe and achieve their intended purpose. Two regulatory pathways are available for new substances. The food additive petition process is described in regulation 571 in Title 21 of the Code of Federal Regulations (21 CFR 571). The safety of the substance at the intended use rate must be addressed for both the animal and the environment. For food producing species, the safety of human food obtained from the animals must also be addressed. When FDA approves a food additive petition, a regulation in 21 CFR 573 is established addressing the proposed use of the substance in animal food. The second pathway is for qualified experts to determine that a particular use of a substance in animal food is exempt from the premarket requirements of the Federal Food, Drug, and Cosmetic Act because this use is generally recognized as safe (GRAS). A GRAS determination generally demands the same quantity and quality of data/ information needed for a food additive approval with the added requirement that this information be in the public domain. Sponsors can notify CVM about a GRAS determination through the animal food GRAS notification program. CVM maintains an internet list of animal food GRAS notices and CVM's conclusions about each notice. More information about these processes is available at http://www.fda.gov/AnimalVeterinary/Products/default.htm. Another pathway for substances that raise no safety concerns is provided by the Association of American Feed Control Officials to establish an ingredient definition in its Official Publication.

Key Words: animal food regulation, food additive/GRAS, ingredient definition

TH219 Detection of antimicrobial and anthelmintic residues in bulk tank milk from Minas Gerais State, Brazil. F. N. Souza[1], A. F. Cunha[1], L. C. A. Picinin[2], M. O. Leite[1], C. F. A. Penna[1], M. R. Souza[1], L. M. Fonseca[1], and M. M. O. P. Cerqueira*[1], *[1]Department of Food Technology and Inspection, Belo Horizonte, Minas Gerais, Brazil, [2]Department of Food Science and Technology, Florianopolis, Santa Catarina, Brazil.*

The present study aimed to detect residues of drugs in bulk tank milk samples from Minas Gerais State, Brazil. A total of 70 and 83 milk samples were submitted to antimicrobial and anthelmintic screening tests, respectively. The antimicrobial residues detection included quinolones, ceftiofur, thiamphenicol, streptomycin, tylosin and tetracyclines; while antithelmintics detection included benzimidazoles, amino benzimidazoles, levamisole, avermectins, thiabendazole, moxidectin and triclabendazole. The preparation of the milk samples and the detection of the antimicrobials (Anti Microbial Array II, cat no. EV3524A; Randox Laboratories Ltd., UK) and of the anthelmintics (Anthelmintics Array, cat no. EV3770; Randox Laboratories Ltd., UK) residues were performed by biochip array platform, using competitive antibody-capture immunoassay as recommended by the manufacturer's protocol. Here, 2.86%, 2.86% and 11.3% of the bulk tank milk samples were positive for the antimicrobials quinolones, streptomycin and tetracyclines

residues, respectively. Regarding the anthelmintics, 55.42%, 53.57%, 60.24%, 67.47%, 73.49%, 45.78% and 6.02% were positive for amino benzimidazoles, levamisole, avermectins, thiabendazole, moxidectin, triclabendazole and benzimidazoles residues, respectively. No milk sample had value above the Brazilian maximum residue levels (BMRLs) for antimicrobials, although 6.02% (n = 5) of the milk samples showed values above the BMRLs for avermectins (≥10 μg/L). The findings of the present report indicate the need of a stricter monitoring of the veterinary drugs residues in milk produced in Minas Gerais State. To reach this objective, continuous monitoring programs should be applied to offer a safer product to consumers.

Key Words: veterinary drug, milk, dairy cattle

TH220 Development of phage-based technologies to reduce *E. coli* O157:H7 contamination of beef products and produce. Y. Pan*, Y. Hong, J. Zhang, and P. D. Ebner, *Purdue University, Department of Animal Sciences, West Lafayette, IN.*

E. coli O157:H7 has developed into a ubiquitous pathogen with infections associated with products ranging from ground beef to produce to processed foods. We had previously demonstrated that phage-based technologies could reduce foodborne pathogen transmission and colonization in live animals. Here we examined whether a phage cocktail consisting of 3 phages could reduce *E. coli* O157:H7 in experimentally contaminated ground beef and produce. The 3 phages were isolated from human wastewater samples and belonged to the Myoviridae and Siphoviridae families. The phages were chosen from a growing library based on their rapid growth (40 to 50 min life cycle) and in vitro killing efficiencies. Phages were added to ground beef (20g) contaminated with *E. coli* O157:H7 (10^7 cfu) at an MOI = 1.0. Phage treatment significantly reduced ($P < 0.05$) the concentration of viable *E. coli* O157:H7 in contaminated ground beef by 2.0 $\log_{10}$ compared with untreated samples when stored at room temperature for 24h and 0.5 $\log_{10}$ when stored at refrigerated condition (4C). Likewise, the phage cocktail reduced *E. coli* O157:H7 by 0.5 $\log_{10}$ in undercooked ground beef (internal temperature of 46C; $P < 0.05$) compared with untreated samples. Similarly, spinach samples (3 pooled leaves) were inoculated with *E. coli* O157:H7 (10^7 cfu) and treated with the phage cocktail at an MOI = 1.0. Concentrations of *E. coli* O157:H7 in phage treated spinach samples were reduced 3.3 $\log_{10}$, 2.9 $\log_{10}$ and 2.8 $\log_{10}$ at 24, 48, 72 h, respectively, compared with untreated samples when stored at room temperature. Similar experiments were conducted with Swiss cheese, but no significant differences were found. Taken together, these results provide additional support for the development of phage-based approaches to control *E. coli* O157:H7 contamination in food products.

Key Words: *E. coli* O157:H7, phage, ground beef

TH221 Detection of ceftiofur residues in milk of cows treated for mastitis using the BetaStar Plus assay. K. Grooms*[1], D. Grooms[1], E. Jagodzinski[1], B. Norby[1], R. Erskine[1], L. Halbert[1], and J. Rice[2], *[1]Michigan State University, College of Veterinary Medicine, East Lansing, [2]Neogen Corporation, Lansing, MI.*

Development of a cow-side assay to detect antibiotic residues in milk would be advantageous to reduce risks of violative residues in marketed milk. The objective of this project was to evaluate the effectiveness of a rapid immunomigration assay (BetaStar Plus, Neogen Corporation, Lansing, MI) in detecting cephalosporin residues in milk from individual cows treated for mastitis. This assay is currently FDA approved for detecting β-lactam and cephalosporin residues in commingled milk. 38 dairy cows with clinical mastitis from 4 dairy farms were enrolled and treated with intramammary ceftiofur hydrochloride (Spectramast-LC, Pfizer Animal Health) according to the manufacturers label recommendation. Composite milk samples were collected a) before first antibiotic treatment, b) before the last antibiotic treatment, c) the last milking of the product-labeled milk withhold, d) the first milking after the product-labeled milk withhold had been met, and e) 72 h after the product-labeled milk withhold had been met. Samples were tested using the BetaStar Plus assay within 48 h of collection. Parallel samples were submitted to the Iowa State University Cyclone Analyte Detection Service for liquid chromatography mass spectrometry (LC MS). There were 2.6%, 57.9%, 42.1%, 15.8%, and 0.0% BetaStar Plus assay positive samples at each of the respective time points. The assay had a sensitivity and specificity of 93.3% and 78.3% respectively when compared with LC MS analysis using FDA published residue tolerance levels for ceftiofur (or ceftiofur metabolites) as a threshold. If the detection of any ceftiofur residue (or ceftiofur metabolites) by LC MS was considered as "positive," the sensitivity and specificity of the BetaStar Plus assay was 87.0% and 94.4% respectively. The BetaStar Plus assay could be useful to detect ceftiofur residues in milk from individual cows treated for mastitis before being sold for human consumption.

Key Words: antibiotic, residue, mastitis

TH222 Macrocyclic lactones residues in milk from family farming properties in the state of Rio Grande do Sul, Brazil. U. A. Souza[1], J. Reck[2], J. R. Martins[2], A. Webster[1], G. Klafke[2], G. Rubesam[3], F. Barreto[3], and L. Kindlein*[1], *[1]Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, [2]Institute of veterinary research Desidério Finamor, Eldorado do Sul, Rio Grande do Sul, Brazil, [3]Agriculture Ministry, Livestock and Supply, National Agricultural Laboratory, Porto Alegre, Rio Grande do Sul, Brazil.*

The objective of this study was to determine the presence of macrocyclic lactones (LM) residues in milk on cattle and Northwest or southern regions of the state of Rio Grande do Sul, Brazil. The control of chemical residues in animal products in Brazil is regulated by regulatory agencies through programs such as the National Residue Control (PNCR) and the Program for Residue Analysis of Veterinary Drugs (PAMVet), aimed at ensuring food security the consumer. The practices adopted in agricultural production, however, involve the use of drugs to control infectious and parasitic diseases in farm animals. The LM veterinary drugs are effective in controlling endo and ectoparasites, being widely used in dairy cattle. For this experiment, we collected 72 samples of milk cooling tanks properties smallholder farming regions Northwestern (55) and South (17) of the state of Rio Grande do Sul. The residue of LM were extracted from bovine milk by addition of acetonitrile and purified by freezing of the matrix co-extracts with a temperature of about −20°C. The purified extract was analyzed by LC-MS/MS. Among the 72 samples, 18 (25%) had LM residues whose active ingredients identified were: ivermectin (n = 15), moxidectin and ivermectin (n = 1), moxidectin (n = 1) and abamectin (n = 1). The southern region of the state showed higher occurrence of LM residues in milk (35.3%, 6/17) compared with the Northwest (21.8%, 12/55). The residues found in milk samples were below the limit set by Codex Alimentarius (<10 $\mu g.mL^{-1}$), but these drugs cannot be used in lactating animals whose milk is intended for human consumption. The results indicate the improper use of antiparasitic drugs in dairy herds studied. It is concluded that milk from dairy farms of the Northwest and Southern regions of the state of Rio Grande do Sul have LM residues.

Key Words: antiparasitic drug, food security, residue

TH223 Inhibitory effects of mint oils alone or combining with tannin extract against foodborne pathogens. B. J. Min[1], B. R. Min*[2], and J. H. Lee[3], *[1]Tuskegee University, Department of Food and Nutritional Sciences, Tuskegee, AL, [2]Tuskegee University, Department of Agricultural and Environmental Sciences, Tuskegee, AL, [3]Fort Valley State University, Department of Agricultural Sciences, Fort Valley, GA.*

Antimicrobial study was carried out to investigate inhibition effect of mint oil alone or combining with tannin extracts against foodborne pathogenic bacteria using agar diffusion assay. Commercial chestnut tannins (CNT; containing 80% hydrolysable tannins) and 2 mint oils; 2% peppermint (PP) and 2% spearmint (SP) were tested. Experimental solutions (n = 3/treatment) were prepared with 70% ethanol (EtOH) only (control), T1 (PP+EtOH), T2 (SP+EtOH), T3 (CNT+PP+EtOH), T4 (CNT+SP+EtOH) or T5 (CNT+PP+SP+EtOH) were investigated. To determine concentration of CNT extract before using in combination with mint oils, CNT was diluted with 70% ethanol solution at different ratio (1:10, 1:100, 1:500, 1:1000 dilutions) and tested inhibition activities against foodborne pathogens. A brain heart infusion broth (BHIB) soft agar was used for inoculation of *L. monocytogenes* and TSB soft agar was used for *E. coli* O157:H7 and bacteria was grown to 6 to 7 Log cfu/mL. Each 50 µL of solutions from the prepared solutions was dispensed into hole in the plate to measure the inhibition effect. After incubation, the diameter of inhibition zones was measured at least 3 cross-section points, and mean value was used for inhibition zone. From the screening test, CNT ethanol solution with 1:100 dilutions was selected for further study. All data were analyzed as a completely randomized design. Antilisterial effect of T1 (12 mm) was higher ($P < 0.05$) than effect of T2 (11.3 mm). For the combined treatment of T3 and T4 exhibited larger ($P < 0.05$) inhibition zone against *L. monocytogenes* compared with inhibition zone of T1 and T2. However, there was no significant effect of T5 to increase ($P > 0.05$) inhibition activity compared with T3 and T4. Among the prepared solutions, inhibition zone of T3 against both pathogenic bacteria was significantly larger ($P < 0.05$) than inhibition zone of other prepared solutions. The results indicate that the incorporation of mint oil with tannins might be applicable to the livestock feeding system for improving the quality of feeding to livestock and of their products.

Key Words: mint oil, tannin extract, food safety

TH224 A genotyping tool for *Enterobacter sakazakii* isolates from powdered infant formula and environment. Y. Chai[2], Y. Lu[1], C. Man[1], Y. Guo[2], X. Dong[2], Y. Lang[2], M. Guo*[3], and Y. Jiang1,2, *[1]National Dairy Engineering and Technology Research Center, Northeast Agricultural University, Harbin, Heilongjiang, China, [2]Department of Food Science, Northeast Agricultural University, Harbin, Heilongjiang, China, [3]Department of Nutrition and Food Sciences, The University of Vermont, Burlington.*

Enterobacter sakazakii is a foodborne pathogen. It has emerged as a cause of neonatal meningitis, septicemia, enterocolitis and associated with neonatal high mortality rate. In many cases, powdered infant formula (PIF) has been identified as the source of infection. The objective of this study is to discriminate 70 *E. sakazakii* isolates, separated from PIF in different areas and from processing environment, with pulsed-field gel electrophoresis (PFGE) to find the relation between sample and environment. All isolates were identified as *E. sakazakii* by biochemical profiles based on API 20E method and esculin test. In addition, PFGE was carried out with *Xba*I restriction enzyme. To obtain the best reaction conditions of PFGE, the bacteria liquid concentration was optimized. The OD value of bacteria liquid exhibited between 1.0 and 2.0 at 610 nm. The 70 isolates and 5 *E. sakazakii* type strains from ATCC or other laboratory displayed different banding patterns with PFGE analysis. There were 45 distinct pulsotypes among the 75 *E. sakazakii* strains. The result indicated that PFGE subtyping technique had very strong discriminatory power. Combining information on sample origin and pulse type, the No. 41 and the No. 44 isolated from PIF in the same region showed the same pattern. It demonstrated that both of them were from the same contamination source. Similarly, the No. 51 and the No. 53 isolates from PIF in the same area showed the same PFGE pattern. Furthermore, there were 3 strains, the No. 60 was from processing environment but the No. 71 and the No. 72 were from PIF, showed the same PFGE pattern. This revealed that the contamination of PIF was caused by processing environment. The results of this study showed that PFGE could be applied as an effective and reliable tool for distinction and tracing of *E. sakazakii* from PIF and environment. This work was supported by National Science and Technology Project (2013BAD18B11, 2012BAK17B04 and 2012BAD28B02).

Key Words: *Enterobacter sakazakii*, pulsed-field gel electrophoresis, powdered infant formula

TH225 The effectiveness of hurdle strategies consisting of pulsed light treatment and antimicrobials on the inactivation of pathogenic bacteria on cheese. L. Hsu*, B. M. Miller, and C. I. Moraru, *Cornell University, Ithaca, NY.*

Cheese products have been involved in outbreaks or recalls involving contamination with *Listeria monocytogenes* or *Escherichia coli* O157:H7. As one possible source of pathogens is post-process contamination, inclusion of a terminal decontamination step will help ensure cheese safety. Pulsed light (PL) treatment, consisting of short, high-energy light pulses, can effectively inactivate microorganisms on surfaces. This study examined the effectiveness of PL on inactivation of *E. coli* ATCC 25922 and *L. innocua* FSL C2–008 on cheese. PL was used alone or in combination with the antimicrobials nisin and natamycin, and applied directly on the cheese or through clear packaging. White Cheddar and Kraft singles were cut into 2.5 cm × 5 cm slices. *E. coli* and *L. innocua* were grown to stationary phase in tryptic soy broth and brain heart infusion at 37°C, respectively. Ten droplets of 10 µL were spot inoculated on each cheese slice, to yield initial inoculums of 5 or 7 log cfu/cm^2. Inoculated samples were dried overnight at 4°C. For treatments through packaging, low density polyethylene or commercial packaging was placed on top of the cheese sample before PL. For the combination treatments, cheese slices were dipped into 2.5% Nisaplin or 50 ppm natamycin for 2 min, air-dried for 2 min, spot-inoculated, and air-dried for 15 min. Cheese samples were exposed to PL doses of 1.1–13.2 J/cm^2. Treated samples were stomached for 2 min in Butterfield phosphate buffer, plated on selective media, and survivors enumerated by standard plate counting. Experiments were triplicated and data statistically analyzed. PL achieved 2.5–2.8 log reductions of *L. innocua* on Kraft singles and Cheddar, respectively, and 2.0–3.0 log reductions of *E. coli* on cheddar, after 3.3 and 6.6 J/cm^2. Packaging did not reduce PL effectiveness. Nisin enhanced inactivation compared with just PL. Natamycin reduced PL effectiveness by up to 1 log and thus should not be used with PL. This data suggests that PL, applied directly or through packaging, could be a realistic approach for decontamination of cheese surfaces and that nisin may further enhance its effectiveness.

Key Words: pulsed light, cheese, pathogen

TH226 Microbial assay and proximate composition of suya meat (an intermediate moisture meat) in Osun State, Southwest Nigeria. A. O. Akinwumi*, A. A. Odunsi, G. O. Adebayo, and T. O. Akande, *Ladoke Akintola University of Technology, Ogbomoso, Oyo, Nigeria.*

In an attempt to assess the influence of environment on meat quality, 72 ready-to-eat suya (a popular intermediate moisture meat) samples were collected at various major suya selling points within the 6 agricultural zones of Osun State, southwest Nigeria for microbial assay and proximate analysis. The agricultural zones are Ede, Ife, Ilesha, Iwo, Osogbo and Ikirun. Twelve samples each were collected at 2 selected local governments in each zone. The swabs were taken to the laboratory and serial dilution, inoculation of diluents into a sterile nutrient agar for incubation and catalase test, and Gram staining for characterization and identification were conducted. Findings identified the following isolates and their frequencies of occurrence as, *Staphylococcus aureus* (22.2%), *Staphylococcus saprophyticus* (11.1%), *Bacillus subtilis* (22.2%), *Klebsiella oxytoca* (5.6%), *Escherichia coli* (16.7%), *Salmonella* species (11.1%), and *Citrobacter freundi* (11.1%). *Bacillus subtilis* and *Staphylococcus aureus* were observed in all the zones of the state. The proximate composition of suya samples with the presence of the isolates compared with the control (without the presence of the microbes) showed a significant higher ($P < 0.05$) protein content (23.78 – 27.27%) and fat (34.23 – 42.79%). This has shown that there is need to improve on the hygienic processing and handling of suya to ensure safety of the consumers.

Key Words: suya, intermediate moisture meat (IMM), microbial assay

TH227 Effect of exposure to copper sulfate or zinc oxide on bacterial antibiotic susceptibility profile. A. F. Amaral[2], G. Schaefer[2], L. J. Lara[2], G. M. Preis[2], A. D. B. Melo[2], L. V. C. Girao[2], and M. H. Rostagno*[1], *[1]USDA-ARS, West Lafayette, IN, [2]Purdue University, West Lafayette, IN.*

Copper sulfate and zinc oxide have been extensively used as alternatives to antibiotics for health and growth promotion in animal production. However, there are emerging questions regarding potential cross-resistance between these compounds and antibiotics in gastrointestinal microbial populations. Therefore, an in vitro study was conducted to determine if exposure to high or low concentrations of copper sulfate (CuSO4) or zinc oxide (ZnO) affects bacterial susceptibility to antibiotics. Strains of *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* were exposed to 500, 10 and 0 ppm of CuSO4 or 2000, 10 and 0 ppm of ZnO in Mueller Hinton broth for approximately 24 h. A total of 3 independent repetitions of the study were conducted. After exposure, the minimum inhibitory concentration (MIC) for each strain was determined against a panel of antibiotics using a commercially available microdilution assay (Sensititer, Trek Diagnostic Systems). No effect on the MICs was observed when the strains were exposed to different concentrations of ZnO. However, when exposed to 500 ppm of CuSO4, the MICs for *Staphylococcus aureus* and *Enterococcus faecalis* (gram-positive bacteria) increased ($P < 0.05$), whereas when exposed to 10 ppm, the MICs did not change. Curiously, the opposite was observed with *Salmonella* Typhimurium, which had its MICs decreased ($P < 0.05$) after exposure to 500 ppm of CuSO4, whereas the MICs did not change after exposure to 10 ppm. In the case of *Salmonella* Enteritidis and *Escherichia coli*, no changes in MICs were observed after exposure to CuSO4. In conclusion, this study demonstrates that exposure to ZnO does not affect the antibiotic susceptibility profile of the strains tested, whereas exposure to $CuSO_4$ causes qualitative changes (i.e., bacterial response to antibiotics). However, it was shown that these changes are variable, not only according to the concentration of $CuSO_4$, but also according to the strain tested. Further studies are ongoing using intestinal microbial mixed populations to determine potential quantitative effects.

Key Words: copper sulfate, zinc oxide, antibiotic susceptibility

TH228 Variable antimicrobial effect of essential oils against different bacterial strains. A. D. B. Melo[2], A. F. Amaral[2], G. Schaefer[2], G. M. Preis[2], L. J. Lara[2], L. V. C. Girao[2], and M. H. Rostagno*[1], *[1]USDA-ARS, West Lafayette, IN, [2]Purdue University, West Lafayette, IN.*

Essential oils are increasingly being used as feed additives to promote health and growth in swine and poultry production. The antimicrobial activity of essential oils is widely accepted, but scarcely understood. Moreover, very little attention has been given to their use in bacterial sub-lethal concentrations. Therefore, a study was conducted to investigate the antimicrobial activity and to determine the minimum inhibitory concentration (MIC) of essential oils from *Origanum vulgare* (oregano), *Melaleuca alternifolia* (tea tree), *Cinnamomum cassia* (cassia) and *Thymus vulgaris* (white thyme) against *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis*. The MIC of the essential oils studied was determined by disk diffusion and broth dilution methods (3 independent replicates for each strain, oil and method). All essential oils tested showed antimicrobial effect against all bacterial strains, suggesting a broad spectrum against gram-positive and gram-negative bacteria. However, MICs for gram-negative bacteria were the same, or in most of the cases, lower in comparison to MICs for gram-positive bacteria, suggesting increased sensitivity to the oils tested. Overall, the white thyme oil had the strongest antimicrobial effect (0.015 - 0.06%), followed by the oregano (0.03 – 0.06%), cassia (0.03 – 0.25%) and tea tree (0.12 – 0.25%) oils. The MICs determined by broth dilution were lower than MICs determined by disk diffusion ($P < 0.05$). This study demonstrates the powerful antimicrobial effect of essential oils. However, it also shows that results need to be cautiously interpreted, taking in consideration the antimicrobial susceptibility methods applied. Even though essential oils are assumed to be safe additives, further studies are needed to better understand their effects on microbial populations. Therefore, studies on the potential development of resistance to essential oils, as well as cross-resistance to antibiotics through exposure to sub-lethal concentrations (i.e., sub-MIC) are currently in progress.

Key Words: essential oil, antimicrobial, feed additive

TH229 Clenbuterol hydrochloride residues in beef and beef liver tissues from different retailers points in Texcoco, Mexico. E. Olaya-Fernandez[1], G. Aranda-Osorio*[1], E. Maldonado-Siman[1], J. A. Cadena-Meneses[1], M. Huerta-Bravo[1], and O. Hernandez-Mendo[2], *[1]Universidad Autonoma Chapingo, Texcoco, Mexico, [2]Colegio de Postgraduados, Montecillo, Mexico.*

The objective of this study was to evaluate the risk likelihood of acquiring beef and bovine liver with concentrations above 2 µg/kg of clenbuterol (limit level from NOM-004-ZOO-1994) from different meat retailers points (i.e., street markets, butcher shops and supermarkets). There were sampled 6 commercial retailers' points for each category (36 in total). Samples were taken in November–December and April, high and low beef production seasons, respectively, and analyzed under 2 ELISA test kits (Neogen and Ridascreen). Edible tissues with concentrations above 2 µg/kg of clenbuterol (Neogen) were confirmed

by Ridascreen. The concentrations of clenbuterol were evaluated by the GLM procedure of SAS (2004) for sampling, distribution center and tissue type effects. Means were compared using the Tukey test ($\alpha = 0.05$). To determine the risk of probability, the CATMOD procedure was utilized. The evaluation of the kits was carried out with Student's *t*-test. There were differences ($P < 0.05$) due to sampling season, retailers points and tissue type. In average, the concentrations of clenbuterol in tissues collected during the high season were 1.40 µg/kg of clenbuterol, while for those collected during the low one were only 0.75 µg. Edible tissues from street markets and butcher shops had concentrations of 1.81 and 1.71 µg/kg of clenbuterol, respectively, whereas tissues from supermarkets had only 0.05 µg. Beef liver showed concentrations of 1.79 µg/kg of clenbuterol, while muscle presented only 0.58 µg. In general, the risk likelihood of obtained edible tissues with high concentrations of clenbuterol per kg of product was higher during the high than for the low beef production season, for street markets and butcher shops than for supermarkets (concentrations considered negligible), and for beef liver than for beef. The Neogen kit appears to be an economic and practical alternative to analyze clenbuterol concentrations in beef edible tissues.

Key Words: clenbuterol residue, edible tissue, risk likelihood