Occurrence of aflatoxin in dairy cow feed and raw milk in China. N. Zheng1,2, J. Q. Wang*1,2, Z. N. Yu1, X. M. Xu1,2, R. W. Han1,2, S. L. Li1,2, and X. Y. Qu1,2, State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. 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). 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 99.4% 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

Occurrence of organochlorine pesticide residues in raw milk in China by gas chromatography triple-quadrupole mass spectrometry, X. M. Xu1,2, N. Zheng1,2, J. Q. Wang*1,2, R. W. Han1,2, and S. L. Li1,2, State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. 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 μg·kg−1 to 6 μg·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%, 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 μg·kg−1 for Aldrin, 4.56 μg·kg−1 for Endosulfan I (α isomer), 4.33 μg·kg−1 for Endosulfan sulfate, 4.42 μg·kg−1 for DDT-p,p’, 5.47 μg·kg−1 for Dieldrin, 4.64 μg·kg−1 for BHC-α, 4.80 μg·kg−1 for DDD-p,p’, 4.56 μg·kg−1 for BHC-β, 4.56 μg·kg−1 for Lindane, 5.33 μg·kg−1 for DDE-p,p’. For all positive samples, only one sample contained Endosulfan (4.56 μg·kg−1) exceeded the legal limit of Japan (4 μg·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 is safe to consume.

Key Words: aflatoxin, feed, raw milk

Survey of 38 veterinary drug residues in raw milk in China. R. W. Han1,2, N. Zheng1,2, J. Q. Wang*1,2, Z. N. Yu1, X. M. Xu1,2, Y. P. Zhen1,2, X. Y. Qu1,2, and L. C. Huang1,2, State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. Ministry of Agriculture - Milk and Dairy Product Inspection Center (Beijing), Beijing, 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 99.4% 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
TH213  Occurrence of heavy metals in raw milk in China. X. Y. Qu, N. Zheng, J. Q. Wang, X. M. Xu, R. W. Han, Y. P. Zhen, and S. L. Li, State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. The heavy metals in milk are important to assess the quality of milk. The levels of heavy metals in milk in China were in the safe level. The concentrations of lead, chromium, mercury and arsenic in milk were 0.01 mg/kg, 0.002 mg/kg, 0.003 mg/kg, and 0.001 mg/kg, respectively. The data was statistically analyzed using the SPSS version 11.5 (SPSS, 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 0.28 mg/kg of aluminum, 0.08 mg/kg and 0.04 mg/kg of chromium, 0.005 mg/kg and 0.002 mg/kg of mercury, 0.106 mg/kg and 0.011 mg/kg of nickel, 0.033 mg/kg and 0.008 mg/kg of lead and 0.002 mg/kg and 0.001 mg/kg of arsenic. The levels of these heavy metals in milk were all within the 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, N. Zheng, J. Q. Wang, J. B. Cheng, R. W. Han, X. M. Xu, and S. L. Li, State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. The study was conducted to investigate the occurrence of mycotoxins in raw milk in China. A total of 178 raw milk samples were collected from 8 primary milk producing provinces of China, including Inner Mongolia, Heilongjiang, Sichuan, Shandong, and Tianjin. The concentrations of aflatoxin M1 (AFM1), ochratoxin A (OTA), α-zearalenol (α-ZEL) and zearalenone (ZON) in raw milk 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 less than 0.01 mg/kg. The levels of these mycotoxins in raw milk were all within the national legal limits. The concentrations of AFM1, OTA, α-ZEL and ZON in positive samples were 10.5 ng·kg\(^{-1}\), 42.3 ng·kg\(^{-1}\), 248 ng·kg\(^{-1}\), and 111.9 ng·kg\(^{-1}\), respectively. The samples contained AFM1, OTA, α-ZEL and ZON were found in all provinces. The lowest detection rates for 4 mycotoxins were 60% of AFM1, 35% of ZON in Inner Mongolia, 50% of OTA in Shandong, 23.8% of α-ZEL in Hebei, and the highest detection rates for 4 mycotoxins 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 concentrations of AFM1, OTA, α-ZEL and ZON were 95.5 ng·kg\(^{-1}\) (Inner Mongolia), 198.9 ng·kg\(^{-1}\) (Inner Mongolia), 648.3 ng·kg\(^{-1}\) (Anhui), and 111.9 ng·kg\(^{-1}\) (Inner Mongolia), respectively. The levels of AFM1, OTA, α-ZEL and ZON in milk were all within the national legal limits. The data was statistically analyzed using the SPSS version 11.5 (SPSS, Chicago, IL). 84% of the raw milk samples were collected from China’s 8 of the main milk-producing provinces. The data was statistically analyzed using the SPSS version 11.5 (SPSS, 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 0.28 mg/kg of aluminum, 0.08 mg/kg and 0.04 mg/kg of chromium, 0.005 mg/kg and 0.002 mg/kg of mercury, 0.106 mg/kg and 0.011 mg/kg of nickel, 0.033 mg/kg and 0.008 mg/kg of lead and 0.002 mg/kg and 0.001 mg/kg of arsenic. The levels of these 3 heavy metals in milk were all within the national legal limits, and the levels of these 3 heavy metals in milk were all within the national legal limits.

Key Words: occurrence, pesticide residue, raw milk

TH215  Effect of prophylactic use of antibiotics in intravaginal sponges on the response of inhibitor screening tests in goats milk. T. Romero, J. Balado, R. L. Althaus, M. P. Molina, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, Ministry of Agriculture Milk and Dairy Product Inspection Center (Beijing), Beijing, China. The objective was to determine whether the prophylactic use of antibiotics in 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 Framicos (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 days as well positive samples in microbiological tests were analyzed with receptor binding protein methods specific for tetracyclines and sulfonamides. 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 χ²-test was used to evaluate the degree of cleanliness of the samples. 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: mycotoxin, raw milk
The objective of this study was to qualitatively evaluate the occurrence of antibiotic residues in 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 contaminated fresh or frozen goat milk. This test kit had 100% sensitivity in detecting antibiotic residues in fortified goat milk with ampicillin, amoxicillin, cephalin 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 residues 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

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 antemicrobial and anthelmintic screening tests, respectively. The antimicrobial residues detection included quinolones, cefitofuro, thiapenocil, streptomycin, tylosin and tetracyclines; while anthelmintics detection included benzimidazoles, amino benzimidazoles, levamisole, avermectins, thiabendazole, moxidectin and tiabendazole. 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 (Anthelmints 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.

Key Words: pasteurization, antibiotic, Delvetest SP-NT
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

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**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

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**TH221 Detection of ceftiofur residues in milk of cows treated for mastitis using the BetaStar Plus assay.** K. Grooms*, 1 D. Grooms1, E. Jagodzinski2, B. Norby1, R. Erskine1, L. Halbert1, and J. Rice2, 1Michigan State University, College of Veterinary Medicine, East Lansing, 2Neogen 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

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**TH222 Macrocyclic lactones residues in milk from family farming properties in the state of Rio Grande do Sul, Brazil.** U. A. Souza1, J. Reck2, J. R. Martins2, A. Webster1, G. Klafeke2, G. Rubesam2, F. Barreto2, and L. Kindlein*1, 1Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, 2Institute of veterinary research Desiderio Finamor, Eldorado do Sul, Rio Grande do Sul, Brazil, 3Agriculture 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 (35) and South (17) of the state of Rio Grande do Sul. The residue of LM were extracted from bovine milk by addition of acetoniitrile 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 μg.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. Min1, B. R. Min*2, and J. H. Lee3, 1Tuskegee University, Department of Food and Nutritional Sciences, Tuskegee, AL, 2Tuskegee University, Department of Agricultural and Environmental Sciences, Tuskegee, AL, 3Fort 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 (BHI) soft agar was used for inoculation of L. monocytogenes and TSA 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 in hole in the plate to measure the inhibition effect. After incubation, the diameter of inhibition zone 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. Chai1, Y. Lu1, C. Man1, Y. Guo2, X. Dong1, Y. Lang2, M. Guo3, and Y. Jiang1,2, 1National Dairy Engineering and Technology Research Center, Northeast Agricultural University, Harbin, Heilongjiang, China, 2Department of Food Science, Northeast Agricultural University, Harbin, Heilongjiang, China, 3Department 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, septicaemia, 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 Xbal 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 to 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/cm². 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/cm². 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/cm². 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

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, Ile, 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 freundii (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

TH228 Variable antimicrobial effect of essential oils against different bacterial strains. A. D. B. Melo2, A. F. Amaral2, G. Schaefer2, G. M. Preis2, L. J. Lara2, L. V. C. Girao2, and M. H. Rostagno*1, 1USDA-ARS, West Lafayette, IN, 2Purdue 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: copper sulfate, zinc oxide, antibiotic susceptibility

TH227 Effect of exposure to copper sulfate or zinc oxide on bacterial antibiotic susceptibility profile. A. F. Amaral2, G. Schaefer2, L. J. Lara2, G. M. Preis2, A. D. B. Melo2, L. V. C. Girao2, and M. H. Rostagno*1, 1USDA-ARS, West Lafayette, IN, 2Purdue 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 after exposure to CuSO4 or ZnO under 2 ELISA test kits (Neogen and Ridascreen). Edible tissues with concentrations of CuSO4 or ZnO in the range of 0.012 – 0.06 ppm did not affect the antibiotic susceptibility profile of the strains tested, whereas exposure to CuSO4 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 CuSO4, 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

TH229 Clenbuterol hydrochloride residues in beef and beef liver tissues from different retailers points in Texcoco, Mexico. E. Olaya-Fernandez1, G. Aranda-Osorio*,1 E. Maldonado-Siman1, J. A. Cadena-Meneses1, M. Huerta-Bravo1, and O. Hernandez-Mendoza2, 1Universidad Autonoma Chapingo, Texcoco, Mexico, 2Colegio 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 (α = 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