FOOD SAFETY

1053 (T093) Regulatory process for food additives used in animal foods. S. A. Benz[1], R. Christensen[*2], and M. G. Alewynse[2], *[1]Center for Veterinary Medicine, FDA, Woodbine, MD, [2]Nutrition & Labeling Team, Center for Veterinary Medicine, FDA, Rockville, MD.*

Animal food, both livestock feed and companion animal food, is composed of many different ingredients. With recent changes in ingredient availability, there is increasing interest in the use of novel ingredients in animal food. These ingredients may be intended to be a source of nutrients or, like enzymes, may affect the characteristics of the food itself. Under Federal law, ingredients that are not generally recognized as safe (GRAS) for an intended use are considered food additives that must be approved by the Food and Drug Administration (FDA) before they can be used in animal food. The food additive petition (FAP) process is the means to get approval of food additives and it is described in Title 21 of the Code of Federal Regulations Part 571 (21 CFR 571). Within FDA, the Center for Veterinary Medicine (CVM) approves animal FAPs when a firm demonstrates that the ingredient is safe and achieves its intended purpose. In a petition, 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 FAP, a regulation in 21 CFR 573 is established addressing the safe use of the substance in animal food. In September 2013, FDA published Guidance for Industry #221 on the Recommendations for the Preparation and Submission of Animal Food Additive Petitions. This draft guidance describes the types of information to be included in a petition, including: the name and all pertinent information concerning the food additive itself; chemical identity and composition of the additive; manufacturing methods and controls; intended use, use level and labeling; data establishing the intended effect (physical, nutritional, or other technical effect); a description of validated analytical methods to determine the amount of the food additive in the food; safety evaluations for the animal and humans consuming animal products; proposed tolerances for the food additive; proposed regulation; and environmental assessment. With the guidance, CVM seeks to provide the animal industry with knowledge of the types of information that are required to establish the safety of the use of an ingredient to help to ensure a safe animal food supply.

Key Words: FDA, food additive, guidance

1054 (T094) Persistence of *Escherichia coli* O157:H7 in feces from cattle fed diets with or without wet distillers grains with solubles. E. D. Berry[*], J. E. Wells, and V. H. Varel, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Feeding wet distillers grains with solubles (WDGS) to cattle can increase prevalence of *E. coli* O157:H7, but mechanisms for this increase are not fully understood. The objective of these experiments was to examine the persistence of *E. coli* O157:H7 in feces from cattle fed diets with or without WDGS. In the first study, fresh feces from steers fed 0, 20, 40 or 60% WDGS were collected and combined (6 to 8 animals/composite, $n = 8$ separate composites/diet treatment). Feces composites (600 g) were inoculated with a five-strain mixture of streptomycin-resistant *E. coli* O157:H7, incubated at room temperature, and sampled periodically up to 14 d. Feces samples were diluted and plated to determine *E. coli* O157:H7 levels. Compared to levels seen with diets containing WDGS, *E. coli* O157:H7 levels in feces from cattle fed 0% WDGS rapidly decreased ($P < 0.05$), from 6.28 $\log_{10}$ cfu/g on d 0 to 2.48 $\log_{10}$ cfu/g by d 14. From the same initial levels, *E. coli* O157:H7 in feces from cattle fed 20, 40, and 60% WDGS were 4.21, 5.59, and 6.13 $\log_{10}$ cfu/g of feces on d 14, respectively. A second study evaluated survival of *E. coli* O157:H7 in feces from cattle fed 0 and 40% WDGS. Steers were fed in eight pens (75 to 77 per pen; 4 pens/WDGS treatment). Feces were collected from 5 to 6 animals in each pen, both before and after the corn source was switched from high-moisture corn (HMC) to dry-rolled corn (DRC). Feces samples were combined within pen, inoculated, incubated, and analyzed for *E. coli* O157:H7 as described above, and examined in triplicate at 0, 1, 2, 4, and 7 d. Within corn source, *E. coli* O157:H7 persisted at higher levels ($P < 0.05$) in 40% WDGS feces at d 7. For 40% WDGS feces, *E. coli* O157:H7 persisted at higher levels ($P < 0.05$) at d 7 in feces when cattle were fed HMC compared to DRC. Greater persistence of *E. coli* O157:H7 in the feces and environment of cattle fed WDGS may play a role in the increased prevalence of *E. coli* O157:H7 seen in these animals, by increasing the risk for recolonization of animals. This work further suggests potential dietary approaches for reducing the occurrence and numbers of this pathogen in cattle fed WDGS. *USDA is an equal opportunity provider and employer.*

Key Words: *E. coli* O157:H7, distillers grains, cattle

1055 (T095) Characterization of Shiga toxin-producing *Escherichia coli* isolated from feces of cattle in commercial feedlots. T. W. Alexander[*1], T. A. McAllister[1], K. Stanford[2], T. Reuter[2], and E. Topp[3], *[1]Agriculture and Agri-Food Canada, Lethbridge, AB, [2]Alberta Agriculture and Rural Development, Lethbridge, Canada, [3]Agriculture and Agri-Food Canada, London, ON.*

Shiga toxin-producing *Escherichia coli* (STEC) are potential food and waterborne zoonotic pathogens. The objective of this study was to characterize the general population of *E. coli* (EC) in feedlot cattle and determine the proportion that is STEC. Four commercial feedlots with approximately 1,000 pens were sampled over a three-year period. Thirty percent of the pens were randomly selected for study enrollment. Pens were sampled when they had been filled with cattle and again after cattle had been on feed for > 60 days. Sampling consisted of collecting 1 to 2 g of material from 20 fresh fecal pats on pen floors and combining them into a single mixed sample per pen. In total, 291 pens were sampled and processed for EC isolation at both time points. A total of 3,578 EC were isolated and stored after plating pooled fecal pats onto MacConkey agar. Isolates of EC were then screened by multiplex real-time PCR for the virulence genes *stx*1, *stx*2, and *eae*. Isolates positive for *stx*1 or *stx*2 were further characterized for i) variants of *stx* using PCR, ii) susceptibility to 15 antimicrobials using broth dilution, and iii) genetic relatedness after pulsed-field gel electrophoresis (*P*FGE) of Xba1 restricted DNA. In total, 60 (1.7%) isolates tested positive for *stx*1 (N = 27), *stx*2 (N = 30) or a combination of *stx*1 + *stx*2 (N = 3) genes. The most prevalent *stx* variant was *stx*1d, followed by *stx*2a, *stx*2g, *stx*1a, *stx*1a + *stx*2a, and *stx*2b (N = 18, 15, 8, 7, 3, and 2, respectively). Undefined *stx*1 and *stx*2 variants were present in 2 and 5 isolates, respectively. Seasonality and time of isolation did not affect prevalence of total *stx*1 or *stx*2 ($P > 0.05$). However the majority of isolates with *stx*1d (15/18) and *stx*2g (7/8) were detected at > 60 days. Only 10 STEC (0.3%) also tested positive for *eae*. Sampling time point did not affect the prevalence of antimicrobial-resistant STEC ($P > 0.05$). Overall, 69.8% of STEC were resistant to at least one antimicrobial. The most prevalent resistance was to tetracycline which was common to all resistant STEC. From PFGE, 46 subtypes were observed. These data indicate STEC from feedlot cattle are diverse and their prevalence is low among the general population of EC. In addition, the majority of STEC were *eae*-negative, thus unlikely to be associated with outbreaks of hemolytic uremic syndrome.

Key Words: STEC, *Escherichia coli*, cattle

1056 (T096) Development of an ultrasensitive aptasensor for the detection of aflatoxin B1. X. Guo[1,2,3], F. Wen[1,4], N. Zheng[*1,3,4], Q. Luo[2], and J. Wang[1,4], *[1]Ministry of Agriculture—Laboratory of Quality and Safety Risk Assessment for Dairy Products, Beijing, China, [2]College of Animal Science and Technology, Xinjiang Agricultural University, Urumchi, China, [3]Ministry of Agriculture - Milk and Dairy Product Inspection Center, Beijing, China, [4]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Contamination of feed and food by aflatoxin B_1 (AFB_1), one of the most toxic of the mycotoxins, is a global concern. To prevent food safety scares, and avoid subsequent economic losses due to the recall of contaminated items, methods for the rapid, sensitive and specific detection of AFB_1 at trace levels are much in demand. In this work, a simple, ultrasensitive, and reliable aptasensor is described for the detection of AFB_1. An AFB_1 aptamer was used as a molecular recognition probe, while its complementary DNA played a role as a signal generator for amplification by real-time quantitative polymerase chain reaction (*P*CR). Under optimal conditions, a wide linear detection range (5.0×10^{-5} to 5.0 ng mL^{-1}) was achieved, with a high sensitivity (limit of detection (LOD) = 25 fg mL^{-1}). In addition, the proposed aptasensor exhibited excellent specificity for AFB_1 compared with eight other mycotoxins, with no obvious Ct value change. This aptasensor can also be used in quantifying AFB_1 levels in Chinese wildrye hay samples and infant rice cereal samples, demonstrating satisfactory recoveries in the range of 88–127% and 94–119%, respectively. This detection technique has a significant potential for high-throughput, quantitative determination of mycotoxin levels in a large range of feeds and foods.

Key Words: aflatoxin B_1, aptasensor, feed and food safety

1057 (T097) Cytotoxicity induced by ochratoxin A, zearalenone and α-zearalenol: Effects of individual and combined treatment. H. Wang[1,2,3,4], N. Zheng[1,2,3], S. Li[1,2,3], F. Li[4], and J. Wang[*1,2,3], *[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]Ministry of Agriculture - Laboratory of Quality and Safety Risk Assessment for Dairy Products, Beijing, China, [4]College of Animal Science and Technology, Gansu Agricultural University, Lanzhou, China.*

Mycotoxins, a series of secondary metabolites generated from moulds, mainly come from food and feed contaminated in the field, during drying and subsequent storage. Among all

the mycotoxins, ochratoxin A (OTA), zearalenone (ZEA) and α-zearalenol (α-ZOL) have evoked great concern owing to their high occurrence and serious harm to human health. The co-occurrence of OTA, ZEA and α-ZOL was found in animal feed and milk. The previous reports indicated that the co-occurrence of mycotoxins could increase their cytotoxicity. The aim of the present study was to investigate the cytotoxicity of combined mycotoxins of OTA, ZEA and α-ZOL on human Hep G2 cells by using the tetrazolium salt (MTT) assay and the isobologram analysis. Statistical analysis of data was carried out using SAS9.2, statistical software package. Our results demonstrated the significant ($P < 0.05$) cytotoxic effects of the two-toxin combination and the three-toxin combination on Hep G2 cells in a time- and concentration-dependent manner. The IC_{50} (inhibit concentration equal to 50%) values of Hep G2 treated with individual mycotoxin after 24 h, 48 h and 72 h of exposure were 1.86-8.89 μM, 29.48-55.79 μM, 20.91-52.30 μM for OTA, ZEA and α-ZOL, respectively. The combined indexes (CI) were 2.73–7.67 for OTA+ ZEA and 1.23–17.82 for OTA+ α-ZOL after 24 h, 48 h and 72 h of exposure at all inhibit concentration(IC) levels (IC_{10}-IC_{90}), indicated an antagonism. The CIs of ZEA+ α-ZOL were 1.29–2.55 after 24 h and 72 h of exposure (IC_{10}-IC_{90}), indicated an antagonism. The CIs of ZEA+ α-ZOL were 0.74-1.68 after 48 h of exposure, indicated an antagonism (IC_{10}-IC_{40}), additive effect (IC_{50}-IC_{70}) or synergism (IC_{80}-IC_{90}). The CIs were 1.41–14.65 for OTA+ ZEA+ α-ZOL after 24 h, 48 h and 72 h of exposure (IC_{10}-IC_{90}), indicated an antagonism. In conclusion, OTA was more toxic than ZEA and α-ZOL. The combined mycotoxins of OTA and ZEA, OTA and α-ZOL, OTA, ZEA and α-ZOL showed antagonism. And the combined mycotoxins of ZEA and α-ZOL showed antagonism, additive effect and synergism at different concentrations. But the result of combined mycotoxins affected by type of cell used endpoint of cytotoxicity, analysis assay of interaction, and other factors. So interaction of combined mycotoxins should be determined or re-validated in continuously toxicological data.

Key Words: ochratoxin A, zearalenone, α-zearalenol

1058 (T098) Efficacy of various levels of mycotoxin adsorbent to reduce aflatoxin M1 levels in milk of lactation cows fed aflatoxin B1. M. Dehghan banadaky[*1], R. Motameny[2], and S. Parhizkar[3], [1]*Dep. of Animal Science, Faculty of Agriculture, University of Tehran, Karaj, Iran,* [2]*Azad University, Tehran, Iran,* [3]*University of Tehran, Karaj, Iran*.

The aim of this study was to compare the ability of various levels of adsorbent Biotox (Biochem GmbH, Lohne, Germany) to reduce Aflatoxin in milk of Holstein cows. Twenty-four lactating Holstein cows in mid lactation were assigned to one of three treatments ($n = 8$) for 35 d. Diet formulated according to the nutrient requirements of dairy cattle (NRC, 2001). The following treatments were investigated 1- Aflatoxin diet plus 60 g/d/cow of Biotox (Biochem GmbH, Lohne,. Germany), 2- Aflatoxin diet plus 20 g/d/cow of Biotox and 3- Aflatoxin diet without mycotoxin adsorbent (Control). Aflatoxin diet provided 350 μg/d/cow of AFB. Individual dry matter intake and milk yield were recorded daily. Milk samples were collected at each milking time weekly. Blood samples (10 ml) were collected weekly from the coccygeal vein and centrifuged to separate plasma. Quantification of aflatoxin (B1 or M1) in TMR samples, milk and plasma done using microtitre plate enzyme linked immunosorbent assay (ELISA) method. Weekly data were analyzed using the PROC MIXED of SAS as repeated measurement data. Aflatoxin M1 concentrations for the Biotox60, Biotox20 and control (no adsorbent) treatments averaged 338.0, 439.0 and 490.1 ng/kg, respectively. Compared to the control group, AFM1 concentrations in milk were reduced ($P = 0.015$) by the addition of 20 and 60 g/d/cow of Biotox. Aflatoxin M1 excretion via milk, as calculated from milk AFM1 concentration and total milk volume produced, was 9.87, 12.87 and 14.66μg/d/cow in the Biotox60, biotox20 and control treatments respectively. Biotox significantly decreased AFMI excretion in milk ($P = 0.033$). Transfer rate of AF from feed to milk (TR), as calculated from [(excretion of AFM1/AFB1 consumption) × 100] averaged 2.90, 3.85 and 4.22% for the Biotox60, biotox20 and control treatments respectively. Biotox in 60 g/day dose significantly decreased Aflatoxin TR ($P = 0.029$). The results of the analysis of variance on plasma AFM1 showed that treatment did not affect plasma AFM1, but numerical increase in plasma AFM1 of control group showed. Results of the current study indicate that the Biotox was effective in reducing milk AFM1 concentrations, AF excretion, and AF transfer from feed to milk, but this efficacy was improved in higher dosage (60g/day/cow) at least in high polluted diets.

Key Words: aflatoxin, transfer rate, milk

1059 (T099) Inhibitory activity of *Staphylococcus aureus* against *Lactococcus* spp. isolated from artisanal Minas cheese. F. F. Ângelo[1], L. M. Fonseca[*2,3], and M. A. V. P. Brito[4], [1]*Universidade Federal da Paraíba/CTDR, João Pessoa, Brazil,* [2]*Universidade Federal de Minas Gerais* (*School of Veterinary Medicine), Belo Horizonte, Brazil,* [3]*University of Wisconsin-Madison/CAPES Est. Senior 18183-12-3, Madison,* [4]*EMBRAPA Gado de Leite* (*CNPGL), Juiz de Fora, Brazil.*

Production of antimicrobial substances by *Staphylococcus aureus* isolated from food has been reported. Since it is a highly prevalent etiologic agent for mastitis in dairy herds, inhibition of starter culture due to *S. aureus* inhibitory activity is possible during the processing of fermented dairy products, such as cheeses. The objective of the current work was to evaluate the antimicrobial substances produced by samples of *S. aureus* isolated from cow's milk during mastitis occurrence and their effect against strains of *Lactococcus* spp. Individual milk samples,

obtained from 54 herds, were analyzed for *S. aureus* presence and isolated strains were tested for inhibitory activity using the deferred-antagonism assay, with *Corynebacterium fimi* (NCTC 7547) as indicator. Proteic nature of the antimicrobial substances was investigated using protease type XIV from *Streptomyces griseus* (Sigma P-5147). Inhibitory spectrum was tested against nine *Lactococcus* spp. strains, previously isolated from artisanal Minas cheese. Descriptive statistics was used. Antimicrobial activity using the deferred-antagonism assay was detected in 262 (40%) of the 655 *S. aureus* strains tested. All 262 strains were inactivated by a proteolytic enzyme tested, indicating their proteic nature, a characteristic of bacteriocins. From 262 positive strains, 55 were selected based on the diameter of inhibition zone (> 10mm) for inhibitory activity against *Lactococcus* spp. Noteworthy, 42 strains (76%) presented some inhibitory activity against *Lactococcus* spp., and one strain of *S. aureus* presented inhibitory activity against five *Lactococcus* spp. strains. The results indicate that some *S. aureus* strains inhibit samples of *Lactococcus* spp. isolated from artisanal Minas cheese. Additional work is recommended to investigate further implications of this finding.

Key Words: *Staphylococcus aureus*, bacteriocins, mastitis

1060 (T100) Microbiological quality and safety of commercial local yogurt products in Giza Governorate, Egypt.

M. M. Motawee[*1], and S. A. Ibrahim[2], *[1]National Organization for Drug Control and Research, Giza- Egypt, Egypt, [2]North Carolina A&T State University, Greensboro.*

Yogurt is the most popular dairy product in Egypt. The popularity of yogurt can be attributed to its sensory characteristics and nutritional value. The microbiological characteristics of yogurt also contribute greatly to in the quality and shelf life of the final product. Thus, the objective of this study was to examine the microbiological quality and safety of yogurt products available in the local market in Giza Governorate, Egypt. One hundred yogurt samples were collected from local stores and stored in refrigerators prior to microbiological examination which was done within 24 h. Samples were stored under chilled conditions for 14 d at 7ºC ±1 and examined for yogurt culture viability, psychrophic bacteria (*Pseudomonas*), *Salmonella, Staphylococcus, E. coli, Aspergillus, and Bacillus*. The identification of each isolate was molecularly conformed using 16s rRNA. Our results showed that the yogurt culture maintained 6-7 log CFU/ml during the chilled storage. The psychrophic bacteria ranged from 5-6 log CFU/ml, whereas the other tested groups ranged from 2-4 log CFU/ml. The population of the tested bacteria did not change during chill storage. Our findings demonstrated that pathogenic bacteria could survive in commercial yogurt products. However, the presence of yogurt culture could help prevent foodborne illness in consumers. Our research demonstrates the importance of having standardized hygienic quality control practices in place to ensure the highest yogurt quality. Therefore, the implementation of HACCP or an equivalent safety protocol is paramount in preventing future outbreaks of foodborne illness in such popular dairy products in Egypt.

Key Words: yogurt, foodborne, quality.

1061 (T101) Stability of 10 β-lactam antibiotics in raw milk under different storage conditions.

H. Wang[1,2,3], N. Zheng[1,3], F. Wen[1,3], H. Wang[2], and J. Wang[*1,3], *[1]Ministry of Agriculture - Laboratory of Quality & Safety Risk Assessment for Dairy Products, Beijing, China, [2]College of Animal Science and Technology, Yangzhou University, China, [3]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

B-lactam antibiotics are used to cure mastitis which inflicts severe economic losses in dairy farming. However, improper use of antibiotics may lead to residues in milk, which could be toxic and dangerous for human health, and may cause allergic reactions and antimicrobial resistance. At present, several methods for detecting the residue of β-lactam require milk samples to be sent back to a testing laboratory and stored until further analysis. In this study, we attempted to evaluate the stability of 10 β-lactam antibiotics, include amoxicillin (AMOX), cloxacillin (CLOX), oxacillin (OXAC), penicillin G (*P*ENG), nafcillin (NAFC), cefoperazone (*P*ER), cephapirin (*P*IR), ceftiofur (TIO), cefazolin (ZOL) and cefalonium (LON) in raw milk under different storage conditions, such as storage temperature and time, thawing temperature, freeze-thaw cycle times, and the addition of preservatives. Raw milk samples were collected from a local farm in Beijing and transported to our lab as soon as possible. Milk samples were spiked with the Maximum Residue Limits (MRL) levels in China and the antibiotic residues were determined by UPLC-MS/MS. Results showed that most of these antibiotics were quite stable (recovery = 90 ~ 120%) under different storage conditions, and their degradation rate increased with the increasing storage temperature and time, thawing temperature and freeze-thaw cycle times. Among the variables, preservatives played a critical role in the stability of β-lactam. The storage of PIR at room temperature for 1 day in raw milk containing preservatives resulted in a degradation rate up to 41.7%. Above all, the milk samples should be stored at -80ºC less than 7 days so that the β-lactam residues can be analyzed accurately.

Key Words: raw milk; β-lactam; storage conditions

1062 (T102) Risk warning of veterinary drug residues in raw milk based on shewhart control chart.

R. Han[1,2,3], N. Zheng[3,4], Z. Yu[2], X. Qu[1,3,4], S. Li[1,3,4], Y. Zhang[1,3,4], X. Zhou[1,3], and J. Wang[*1,3,4], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Food Science and Engineering, Qingdao Agricultural University, China, [3]Ministry of Agriculture - Laboratory of Quality & Safety Risk Assessment for Dairy Products, Beijing, China, [4]Ministry of Agriculture - Milk and Dairy Product Inspection Center, Beijing, China*.

The aim of this study was to develop a dynamic risk warning method of veterinary drug residues in raw milk. Risk warning methods of veterinary drug concentration above MRLs (C1 risk warning), abnormal detection rates (J-Pn risk warning) and average-standard deviation (X-δ risk warning) were developed based on theory of Shewhart Control Chart. Flumequine and danofloxacin residues data of raw milk from a large dairy company were collected. Fifty raw milk samples were detected in each week and total of 1,000 continuous data of 20 weeks were analyzed. The data were divided into 20 groups according to time series. C1 risk warning was not triggered, because none of samples exceeded MRLs. J-Pn risk warning was used for danofloxacin, because most of samples were not detected. Central line (CL) was calculated with the value of 5.95 and upper control limit (UCL) was 12.82. Control charts indicated that numbers of samples detected in each week were stable and all less than 12.82, so J-Pn risk warning was not necessary in 1-20 weeks. For flumequine, X-δ risk warning was used, because most of samples were detected. The value of CL was 1.5 and UCL was 2.82. Results analyzed by control charts showed average of flumequine in each week were stable and less than 2.82, so X-δ risk warning was not necessary in 1-20 weeks. In this study, abnormality of detection rate and average-standard deviation was also assumed and analyzed.

Key Words: risk warning, veterinary drug residues, Shewhart Control Chart

1063 (T103) Stability of flavonoids in grape seed and grape marc meal extract (GSGME).

M. Würzbach, E. Holl, and B. Eckel[*], *Dr. Eckel GmbH, Niederzissen, Germany.*

A secure food supply is crucial to all consumers. The use of a plant derived component poses a challenge for quality control: environmental factors lead to variations of plant composition, regarding the desired active substances, during the growth period. After harvest the active component should stay stable. The following study investigates the processing and storage stability of plant derived components, using the example of plant derived flavonoids in GSGME (AntaOxE, Dr. Eckel GmbH, Niederzissen, Germany). Flavonoids have shown anti-inflammatory properties and are effective for animal nutrition (Gessner et al., 2013; Fiesel et al., 2013). The stability of flavonoids in GSGME was studied under common processing conditions with different stressors: time of storage (9 mo), standard packaging material and temperature stress to simulate manufacturing processes. Flavonoid content was analysed using the Folin-Ciocalteu-method (Waterhouse, 2003). It was decided to compare storage characteristics in bags which were shut and sewn or open, stored under cool to room temperature (22°C) conditions. Each measurement was taken in triplicate. The samples, with an initial flavonoid concentration of about 86g/kg ± 0.9g/kg, were analysed every 3 mo. To rule out light as a potential influence, some samples were stored in daylight and some in darkness. Heat stability of flavonoids was tested in a cabinet dryer (Heraeus) at different temperatures, ranging from room temperature to 140°C for 4 hr. In the present study, the analysis of the flavonoid content after 3 mo of storage showed a higher concentration (up to 156g/kg ± 1.6g/kg) than before. This increase in flavonoid concentration may be caused by isomerisation. We found no influence of sealing and packaging on flavonoid activity. For the samples stored under daylight conditions, we analysed an increase in flavonoid content of maximum 25% compared to the other groups after 9 mo. For temperatures of 50°C, 75°C and 100°C, the samples showed flavonoid contents above the initial concentration of 86g/kg ± 0.9g/kg. At 120°C and 140°C, the flavonoid content decreased to 70 to 82g/kg. The study showed that both storage up to nine months or temperatures up to 120°C did not lead to a reduction of the initial concentration of flavonoids in GSGME. It even had a positive influence on concentrations. The results indicate that GSGME can be used without any concern with regard to flavonoids. Further studies should be conducted to explain the increase of flavonoids during storage and which molecules are modified during that time.

Key Words: flavonoids, stability, AntaOxE

1064 (T104) Effect of lysozyme or antibiotics on fecal zoonotic pathogens in nursery pigs.

J. E. Wells[*], E. D. Berry, N. Kalchayanand, L. A. Rempel, and W. T. Oliver, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Lysozyme is a 1,4-β-N-acetylmuramidase that has antimicrobial properties. The objective of this study was to determine the effect of lysozyme and antibiotics on zoonotic pathogen shedding in feces in nursery pigs housed without and with an indirect disease challenge. Two replicates of 600 pigs each were weaned from the sow at 26 d of age (d 0), blocked by litter and gender, and then randomly assigned to one of 24 pens in either a nursery room that had been fully disinfected or a nursery room left unclean after the previous group of pigs. Within a room, pigs were randomly assigned to control diets (C; 2-phase nursery regime), control diets + antibiotics (C + A; chlortetracycline and Denegard), or control diets +

lysozyme (C + Lyso; 100 mg/kg diet). Rectal swab samples were collected on d 0 and 28 of treatment, and enriched and cultured for *Campylobacter* spp. and shigatoxigenic *Escherichia coli* (STEC) O26, O45, O103, O111, O121, O145 and O157. Enrichments from rectal swab samples were also analyzed for presence of enterohemorrhagic *E. coli* (EHEC) virulence genes (*hly*A, *eae*, *stx*1, and *stx*2). Overall, the percentage of samples positive for *Campylobacter* spp., *hly*A, *eae*, and *stx*1/*stx*2 on d 0 were 43.8, 27.4, 25.3, and 14.3%, respectively, and all were different on d 28 (70.6, 17.4, 78.7, and 3.0%, respectively; $P < 0.05$). Room hygiene on d 0 had little effect on d 28 results, except the percentage positives for *hly*A was greater (21.6 vs 13.4%; $P < 0.02$) and for *eae* was less (74.7 vs 82.6%; $P < 0.02$) in unclean compared to clean rooms, respectively. Percentage of samples culture positive for *Campylobacter* spp. was lowest for C + Lyso diet, but similar for C and C + A diets (43.2, 83.7, and 84.8.respectively; $P < 0.01$). Diet had little effect on the EHEC virulence genes *hly*A or *eae* ($P > 0.1$), but there was a tendency for lower percentage of samples positive for *stx*1/*stx*2 in C + A or C + Lyso diet groups compared to C diet (5.8, 1.2, and 2.1%, respectively; $P < 0.07$). The STEC types tested were rarely detected and not affected by time, hygiene or treatment ($P > 0.1$). Thus, lysozyme in the diet can reduce fecal shedding of *Campylobacter* spp. from nursery swine. *USDA is an equal opportunity provider and employer*.

Key Words: antibiotics, lysozyme, swine

1065 (T105) Thermophilic spore forming bacilli: attachment and biofilm formation on stainless steel. M. C. Enes Ribeiro[1], G. Theodore Walsh[2], M. Lucia Gigante[1], and R. Jimenez-Flores[*2], *[1]Faculty of Food Engineering, University of Campinas, Campinas, SP, Brazil, [2]Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

Studies suggest that the spores of thermophilic bacilli possibly bind to the stainless steel surface in greater numbers than vegetative cells. The aim of this study was to evaluate the attachment and biofilm formation by spore-forming bacteria on stainless steel. The microorganisms (CM12, SL12, SL9, CM3, CH7, and SH6) were isolated from milk powder plants in the USA and belong to the collection of cultures at DPTC/Cal Poly. To evaluate the spores attachment and biofilm formation, cleaned stainless steel coupons were used. The experiments were carried out using either whole or fat free UHT milk in order to form a fouling film on the coupons surface. The system was kept at 55°C for 20 hours at 200 rpm. After film formation, the coupons were removed from the matrices and immersed into an aqueous spore solution held at the same conditions to verify the ability of attachment and biofilm formation. Spores were enumerated after heat treatment (80°C/12 minutes) for either 5 minutes or for 20 hours. In addition, a SDS-PAGE gel was performed in order to verify the proteins present in the spores. The effect of the matrices (fat free or whole milk), microorganisms (six different strains), time of exposure (5 minutes or 20 hours), and their interactions on the spores attachment and biofilm formation were evaluated by ANOVA and Tukey's test for comparison between means ($P < 0.05$). Although, the spore strain and the time of exposure significantly affected the attachment and biofilm formation, none of the interactions among the factors were significant. Spores attachment ranged from 3 to 4 log cfu/cm^2. The highest average attachment was observed by SL12 and SH6 (4.01 and 4.08 log cfu/cm^2, respectively), while CM12 and SL9 showed the lowest average attachment (3.42 and 3.52 log cfu/cm^2, respectively). The strains that showed the lowest average attachment (CM12 and SL9) also presented less protein in band density around 25 and 50kDa when compared to the other four strains in the SDS-PAGE. Despite the attachment after 5 minutes was significantly higher than 20 hours, the difference observed was only 0.12 log cfu/cm^2. The results suggest that these matrices did not interfere with the spores attachment and biofilm formation. This could be a strain-dependent characteristic since spores protein may play an important role on attachment. *Acknowledgments: CNPq, CAPES.*

Key Words: dairy, spores, biofilm

1066 (T106) The consumer profile of certified beef in the XXI century. M. E. A. Canozzi[1], J. Magero[1], R. C. T. Mesquita[1], J. O. Barcellos[2], D. Streit Júnior[1], and L. Kindlein[*1], *[1]Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [2]Universidade Federal Do Rio Grande Do Sul, Porto Alegre, Brazil*.

The beef production chain has been reinvented as a consequence of changes in perceptions and importance of its various players. The emergence of the current model, which combines quality and food safety, was in the late twentieth century. It was due to the expansion of the international trade in meats and an awareness of the connection between health crises and animal derived products. These changes in the supply chain and the difficulty of the consumer to judge quality and food safety favored the emergence of the certification processes in the beef market. The aim of this study was to characterize the publications and evaluate the consumer profile of certified beef. A systematic search of descriptive and/or sensory research published between 2002 and 2012 was performed. Three hundred twenty-seven indexed papers were found in the literature on the proposed subject. Only 34 papers were selected for (10.4%) in depth evaluation, based on its importance and meta-analysis methodology. Over these ten years there was a gradual increase in the number of publications. Concerning the spatial distribution of publications, the highest proportion was from the European continent (46.2%), followed by North (23.1%) and South America (15.4%). More than half of the analyzed articles (60.5%) studied traceability, and less repre-

sentative was animal welfare and geographical origin (11.6%/each). There was a predominance of studies on accreditation type most sought or demanded by consumers (35.8%), followed by willingness of the consumer to pay a premium price (33.9%) and the relationship between socio-demographic characteristics and certification type (30.7%). The consumer demand for certified beef in the 21st century increased after the health crises and the quality differential became a relevant point in the buying decision. This process was influenced by social and demographic characteristics.

Key Words: food certification, meta-analysis, traceability

1067 (T107) Identification of horsemeat presence in beef commercial butcheries using the polymerase chain reaction (PCR) technique. G. Aranda-Osorio*[1], B. Alarcón-Zúñiga[1], M. Huerta-Bravo[1], O. Hernández-Mendo[2], and G. Reséndiz-González[1], *Departamento de Zootecnia, Universidad Autónoma Chapingo, México, [2] Colegio de Postgraduados, Montecillo, México.*

The objective of the present study was to determine the presence of horsemeat in beef commercial butcheries through the use of the polymerase chain reaction (PCR) technique. Statistically, horsemeat is not consumed in México, however, the country occupied important places in the world as producer (4º with 64,695 ton) and exporter (4º with 14,026 ton), remaining an important amount (a little more than 50,000 ton) that should be commercialized into the domestic market, thus the question is if some portion of this amount is commercialized as beef in one of the biggest markets in the country, México City. To give answer to this question, there were sampled 22.5% of the Delegational markets of México City (the City is divided in 16 Delegations, each one have different numbers of markets, depending on their size and population). From a completely random design there were selected two butcheries per market, in each butchery, there was asked (bought) for a portion of specific cut of beef, approximately 250 g of "aguayón" (*Biceps femoris*), although it was not always the case. Once the sample was obtained, it was immediately identified and kept on ice for transportation to the meat lab, once arrived to it, the samples were prepared: from the center of the meat cut (to avoid contamination) a subsample of 1 g was deposited in a vial and freeze (-80ºC) until lab analysis. The PCR technique used was based in the methodology reported by Matsunaga *et al.* (1999). Fortunately, the results showed that only 5.5% of the samples were positive to equine. Horsemeat is as nutritious a beef, and has the advantage of being a lean meat, thus less saturated fatty acids, less probability of cardiovascular problems, although, the key point is the fraud to the consumer.

Key Words: DNA identification, meat substitution