**T98 Poultry offal meal traceability in meat quail tissues using the technique of stable carbon-13 and nitrogen-15 Isotopes.** C. Mori*[2], E. A. Garcia[1], C. Ducatti[1], J. C. Denadai[1], and K. Pelicia[1], *[1]São Paulo State University, Botucatu, São Paulo, Brazil, [2]São Paulo State University, Registro, São Paulo, Brazil.*

Studies on detection of animal byproducts in poultry meat are rare, and non-existent on quail meat. This study aimed at detecting increasing levels of poultry offal meal (POM) in quail meat, using carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotopes technique. Sixty 4 one-day-old male quails derived from commercial farm were fed experimental diets containing 0, 1.5, 3.0, 4.5, 6.0, 7.5, and 15% of POM. Diets were formulated to contain equal energy, protein, and amino acid levels. Four individuals per treatment were sacrificed at 42 d of age for breast muscle (pectoralis major), keel, and tibia, and subsequently prepared and submitted for isotopic analysis of C and N. Isotopic analyses of feed ingredients, feeds, and tissues were carried out at the Center of Stable Environmental Isotopes of the Biosciences Institute (CIE/IB), UNESP, Botucatu campus. Isotopic carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) ratios were determined in an isotopic ratio mass spectrometer (IRMS) type DELTA-S (Finnigan Mat) coupled to an Elementary Analyzer (EA 1108 CHN). The obtained isotopic results were submitted to multivariate analysis of variance (MANOVA) using GLM (General Linear Model) procedures of SAS statistical software. Data were generated by error matrices for each tissue, which were later graphically distributed in regions (ellipses) with 95% confidence of observing possible differences between experimental treatment means and control treatment means. The inclusion of animal byproducts in quail diets was detected by $^{13}C$ and $^{15}N$ analyses in the tissues of the birds, with the lowest detection level of 3% dietary inclusion of poultry offal meal. It was concluded that quail meat can be certified by the technique of stable isotopes.

**Key words:** quail, stable isotopes, traceability

**T99 Use of stable isotopes of carbon-13 and nitrogen-15 in quail eggs.** C. Mori*[2], C. Ducatti[1], C. C. Pizzolante[3], S. K. Kakimoto[3], and J. C. Denadai[1], *[1]São Paulo State University, Botucatu, São Paulo, Brazil, [2]São Paulo State University, Registro, São Paulo, Brazil, [3]São Paulo Agency of Agribusiness Tecnology, Brotas, São Paulo, Brazil.*

The objective was to trace the inclusion of bovine meat and bone meal (BMBM) in the diet of quails through the analysis of eggs and their fractions (yolk and albumen), using the stable isotope carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) technique. It was used 120 quails were used in 5 treatments and 4 replications of 5 birds each. After 42 d, 20 eggs were randomly selected per treatment over 3 consecutive days. From these collected eggs, 10 were used for yolk and albumen sampling and the other 10 for the total egg sampling. Treatments were: a control diet based on corn and soybean meal (T0) and other 5 diets containing inclusions of BMBM, namely: 1, 2, 3, 4 and 5%. Isotopic analyses of feed ingredients, feeds, and tissues were carried out at the Center of Stable Environmental Isotopes of the Biosciences Institute (CIE/IB), UNESP, Botucatu campus. Isotopic carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) ratios were determined in a isotopic ratio mass spectrometer (IRMS) type DELTA S (Finnigan Mat) coupled to an Elementary Analyzer (EA 1108 CHN). The obtained isotopic results were submitted to multivariate ANOVA (MANOVA) using GLM (General Linear Model) procedures of statistical software. Data were generated by error matrices for each tissue, which were later graphically distributed in regions (ellipses) with 95% confidence of observing possible differences between experimental treatment means and control treatment means. In the final product (eggs and their fractions) it was possible to detect the inclusion of 1% of BMBM in the diet. Thus, the technique of isotope carbon-13 and nitrogen-15 is able to track the inclusion of 1% of BMBM in diets of laying quails, through the analysis of eggs and their fractions, ensure safe information about the product be consumed, but also allows the certification of origin government agencies.

**Key words:** egg quail, stable isotopes, traceability

**T100 Adsorption capacity and efficacy assessment of bamboo charcoal an alternative adsorbent for aflatoxin B1 in a ruminal batch culture.** H. J. Yang* and Y. H. Jiang, *State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Fifty milligrams of bamboo charcoal (BC), activated charcoal (AC) and smectite power (SP) were weighed into tubes containing 4.5 mL McDougall′s buffer (pH 6.8) in the presence of 4 mg/L aflatoxin B1 (AFB1) and incubated at 39°C. The 5 replicate tubes were incubated for 1, 3, 6, 12, 24, 48 or 72 h, then removed to assess the AFB1 adsorption capacity (Q) and adsorption rate (Y). The mean value of Q did not differ between AC and BC (0.383 vs 0.381 mg/g), which Q means were greater than that of SP ($P < 0.05$). No differences of Y were observed between AC and BC (0.958 vs. 0.955), and a low Y (0.931) occurred in SP ($P < 0.01$). In a separate batch culture (75 mL, rumen fluid: McDougall′s buffer = 1: 2), a 2-factor randomized block design was applied in vitro with 2 binder blocks (SP and BC) at dose rates of 0, 0.1, 1, 10 g/L in the cultures to investigate effects of the binders on fermentation of a grain-rich substrate (*Leymus chinensis* hay: maize meal = 1: 4) in the presence of 1.0 mg/L AFB1. After a 72 h incubation, cumulative gas production was fitted to an exponential model: $Y = A \times [1\text{-}e^{-c \times (\text{time-lag})}]$, and the asymptotic gas production A was greater in BC than in SP ($P < 0.01$), and it increased linearly ($P < 0.01$) and quadratically ($P < 0.01$) against the binder dose rates. Both lag time (h) and the average gas production rate (AGPR, mL/h) did not differ between SP and BC. The residual AFB1 concentration in both binders decreased against the dose rates before a methanol washing procedure ($P < 0.05$), and it was greater in BC than SP after the washing ($P < 0.01$). No differences for pH and ammonia N were observed between the binders or their dose rates. Total VFA was lower in SP than BC ($P < 0.01$). The addition of SP numerically decreased total VFA production, but the addition of BC comparatively increased it. The addition of the binders quadratically increased molar propionate proportion ($P < 0.05$), and molar acetate proportion was greater in BC than SP ($P < 0.01$). These results suggested that the effectiveness of BC was comparable to SP, and that it could be an effective AFB1 sequestering agent.

**Key words:** bamboo charcoal, aflatoxin B1, rumen fermentation

**T101 Occurrence of mycotoxins in feedstuffs and feed samples from 2009-2010.** U. Hofstetter*, K. Naehrer, and I. Rodrigues, *Biomin Holding GmbH, Herzogenburg, Austria.*

A survey about the most important mycotoxins in feedstuffs from different countries/regions all over the world, specifically Asian-Pacific, Europe, Middle-East, Africa and Americas was initiated with the objective to identify major mycotoxin occurrence. From January 2009

until December 2010, a total of 3961 samples were analyzed for the presence of aflatoxins (Afla), zearalenone (ZON), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). Samples tested ranged from cereals, to processing by-products (e.g., DDGS) and other fodder like silage or finished feed. Analyses were performed by HPLC (high performance liquid chromatography), ELISA (enzyme-linked immunosorbent assay) or TLC (thin-layer chromatography) according to the standard procedures. For the purpose of data analysis, non-detect levels are based on the quantification limits (LOQ) of the test method for each toxin. The majority of the analyses were performed at Romerlabs (Austria, Singapore, USA) and SAMITEC (Brazil). From all survey samples 33%, 36%, 51%, 55% and 28% tested positive for Afla, ZON, DON, FUM and OTA, respectively. The most frequently detected mycotoxins were the *Fusarium* sp. toxins FUM and DON with an average contamination of all tested samples of 1280 µg/kg (median of all samples tested positive 1034 µg/kg, maximum 53700 µg/kg) and 434 µg/kg (median of all samples tested positive 460 µg/kg, maximum 29300 µg/kg), respectively. In the case of ZON a mean of 83 µg/kg (maximum 16712 µg/kg) was verified. The average contamination of Afla and OTA was 22 µg/kg and 4 µg/kg, respectively. Data were grouped according to occurrence of mycotoxins in different geographical regions, on the occurrence in diverse raw materials and on the co-occurrence of different mycotoxins. From all samples sourced worldwide, only 24% were below the respective detection limits of the analyzed mycotoxins. 76% of all analyzed samples were contaminated with one mycotoxin and 41% were contaminated with more than one mycotoxin.

**Key words:** mycotoxins, survey

**T102 Horizontal transfer of Stx2 gene from *E. coli* O157:H7 to non-pathogenic *E. coli* occurred under feedlot conditions.** W. F. Yue, M. Du, W. J. Means, and M. J. Zhu*, *Department of Animal Science, University of Wyoming, Laramie.*

Shiga toxins (Stx) are the key virulence factors of *Escherichia coli* O157:H7 which is responsible for hemorrhagic colitis and serious renal failure. It is commonly believed that *E. coli* O157:H7 picked up stx genes through bacteriophages. *E. coli* O157:H7 genome contains a pool of defective lambdoid prophages including prophages carrying stx genes. We hypothesized that strong UV radiation in combination with high temperature associated with global warming accelerates stx prophages activation, which facilitates the dissemination of stx genes into non-pathogenic *E. coli* in feedlots. Plaque analysis showed that UV radiation increased prophage activation in *E. coli* O157:H7 (EDL933), which was further enhanced by high temperature. Activated prophages were capable of converting non-pathogenic *E. coli* (MG1655) to Shiga toxigenic *E. coli* in culture media. To further confirm horizontal transfer of stxs, EDL933 and kanamycin resistant ($Kan^R$) MG1655 were mixed into fresh cattle feces and incubated at 37°C for 12 h. $Kan^R$-MG1655 was recovered by kanamycin antibiotic selection, which were further subjected to in situ hybridization to examine the possible presence of stx2. In situ hybridization results indicated that stx2 were horizontal transferred from EDL933 to MG1655. In summary, data implicated that high temperature combined with UV radiation accelerates the spread of stx genes through enhancing prophage activation. Cattle feedlot sludge is frequently exposed to UV radiation, in combination with elevated temperature associated with global warming, which may provide an environment promoting generation of new Shiga toxigenic *E. coli*. (USDA AFRI, 2010–65201–20599, Agricultural Experiment Station at University of Wyoming, NIH-INBRE P20RR016474).

**Key words:** *E. coli* O157:H7, prophage, Stx2

**T103 Antagonistic intestinal microflora produces antimicrobial substance inhibitory to *Pseudomonas* species and other spoilage organisms.** B. Hatew*[1,2], T. Delessa[1,3], V. Zakin[1], and N. Gollop[1], *[1]Agricultural Research Organization of Israel, Bet-Degan, Israel, [2]Wageningen University, Wageningen, the Netherlands, [3]Swiss Federal Institute of Technology, Zurich, Switzerland.*

Today an increase in consumers demand for fresh, natural and chemical preservative-free foods have enhanced attention to antimicrobial substances from Generally Recognized as Safe bacteria. Chicken intestines harbor a vast number of bacterial strains that play an important role in the health of chickens. Many of these bacterial strains produce antimicrobial substances which are active against aerobic spoilage bacteria of refrigerated poultry meat, especially *Pseudomonas* spp. In the present study, an antimicrobial substance produced by lactic acid bacteria isolated from the gastrointestinal tract of healthy chickens was isolated, characterized and purified. Based on 16S rRNA sequencing the bacteria strain was identified as *Lactobacillus plantarum*, and designated as *L. plantarum*-vN. The antimicrobial substance exhibited a broad-spectrum of activity against many important pathogenic and spoilage microorganisms including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella typhimurium* and *Erwinia amylovova*. The antimicrobial substance was found to be thermostable, insensitive to pH values ranging from 2.0 to 8.0, resistant to various organic solvents and to enzymatic inactivation. The inhibition kinetics displayed a bactericidal mode of action. This study revealed a new type of antimicrobial substance with low molecular mass of less than 1 kDa and having features not previously reported for lactic acid bacteria isolated from the chicken intestines. The novelty of the substance and its potential to inhibit gram-negative bacteria of spoilage and health significance, not normally inhibited by the majority of bacteriocins of lactic acid bacteria, may be of potential interest to food safety and preservation.

**Key words:** *Pseudomonas*, antimicrobial substance, *Lactobacillus plantarum*

**T104 Microencapsulated feed additives to reduce *Salmonella* shedding.** E. Grilli*[1], R. Bari[1], A. Piva[1], B. Tugnoli[1], and T. R. Callaway[2], *[1]University of Bologna, Ozzano Emilia, BO, Italy, [2]Food and Feed Safety Research Unit, ARS/USDA, College Station, TX.*

The reduction of *Salmonella* prevalence in food animals in Europe is regulated by EU Reg. 2160/2003, EU Reg. 1003/2005 and others. The purpose of these regulations is to detect and control *Salmonella* strains that represent a threat to public health and to ensure that preventive measures at each stage of production are taken. In this context, tailored nutritional strategies are now a priority, along with improved management and biosecurity. Aim of the study was to investigate the efficacy of an experimental microencapsulated blend of sorbic acid and naturally identical compounds (SAB) against *S. Typhimurium* in pigs. The active principles of SAB were dissolved in TSB and serial dilutions were prepared to reach final concentration of: 0, 200, 400, 600, 800, 1000, 2000, 3000, 4000, and 5000 mg/L. Each dilution tube was inoculated with *S. Typhimurium* at $10^6$ cfu/ml initial concentration. Compared with controls, after 24 h of incubation, SAB at 2000 and 3000 mg/L reduced ($P < 0.05$) *Salmonella* growth by 4–5 $Log_{10}$, respectively, and SAB at 4000 and 5000 mg/L completely inhibited ($P < 0.05$) its growth. Forty (n = 40) pigs housed in 20 pens were assigned to 4 dietary treatments: control group (challenged, not treated), and 3 treatment groups treated with 300, 3000, 30000 g/ton of SAB, respectively). After 1 week of adaptation pigs were challenged with $10^7$ cfu of *S. Typhimurium* mixed to the feed and a second challenge was

repeated via gavage after 7d. After 2d, and every 4d thereafter, fecal samples were collected from each pig and analyzed for *S. Typhimurium* qualitatively and quantitatively. Results demonstrated that 3000 and 30,000 g/ton SAB reduced ($P < 0.05$) *S. Typhimurium* prevalence by 40% and 50% after 2 wk, and at the end of the third week 100% of the animals in the same groups resulted negative for *S. Typhimurium*. This study demonstrated that intestinal delivery of microencapsulated sorbic acid and naturally identical compounds can result in a reduction of *S. Typhimurium* prevalence and fecal shedding in pigs. In-field trials are currently under exploitation to confirm our preliminary small-scale observations.

**Key words:** *Salmonella*, pig, microencapsulation

**T105 Improving voluntary oral interaction of dairy cattle with manila ropes to facilitate *E. coli* O157:H7 monitoring on dairies.**
A. F. Pedroso*[2,1], O. C. M. Queiroz[1], and A. T. Adesogan[1], *[1]Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, [2]Brazilian Agricultural Research Corporation, Embrapa Cattle-Southeast, 13560-970, São Carlos, SP, Brazil.*

This trial aimed to improve the efficacy of the manila rope method for *E. coli* O157:H7 detection on dairy herds. In an initial test, untreated and molasses-treated (3 min immersion in a autoclaved molasses solution - 8.5% in water) manila ropes (0.64 × 80 cm) were tied at alternate positions (30 cm apart) on the railing over feed bunks in 2 pens containing 22, 13.5 mo dairy heifers each. Seven ropes per treatment were placed immediately before feeding (0930 h) and observed from 0940 to 1130 h. Treatment with molasses resulted in a 16% increase in the total number of times the ropes were visited (chewed, licked or touched with the muzzle) in each pen, and in a 19% increase in an "efficacy index" estimated by multiplying the number of visits by the percentage of heifers visiting the ropes (85 and 83%, respectively, for ropes with and without molasses). Subsequently, the effect of rope to animal ratio was evaluated using 89, 11 to 22 mo heifers stratified by weight into 4 pens (19 to 26 animals/pen) and randomly assigned to 4 treatments. Ratios of 1, 3, 6 and 9 ropes/25 animals were tested in each pen. Molasses-treated manila ropes were placed in the pens as mentioned before and monitored from 0800 to 1100 h every 4 d. Increasing the rope to animal ratio linearly increased the percentage of heifers visiting the ropes, increased quadratically the number of visits per pen, and increased exponentially the efficacy index (Table 1). Molasses treatment and increasing rope to animal ratio in pens potentially makes the method more effective.

**Table 1.** Effect of rope to animal ratio on measures of oral interaction of heifers with manila ropes

| No. ropes/ 25 animals | % heifers visiting ropes | No. visits/ pen | Efficacy |
|---|---|---|---|
| 1 | $32.6 \pm 6.98^{b}$ | $11.0 \pm 9.73^{c}$ | $4.8 \pm 8.68^{c}$ |
| 3 | $45.6 \pm 6.98^{b}$ | $23.5 \pm 9.73^{c}$ | $12.0 \pm 8.68^{bc}$ |
| 6 | $71.8 \pm 6.98^{a}$ | $40.8 \pm 9.73^{b}$ | $32.3 \pm 8.68^{b}$ |
| 9 | $83.7 \pm 7.82^{a}$ | $72.6 \pm 10.79^{a}$ | $62.5 \pm 9.74^{a}$ |
| Equation | $y = 17.9x - 3.9$ | $y = 4.75x^2 - 12.95x + 18.45$ | $y = 0.9251e^{0.8582x}$ |
| $R^2$ | 0.978 | 0.997 | 0.995 |

[a,b,c]Means within a column with different letters differ ($P < 0.05$) by the Tukey test.

**Key words:** *E. coli* O157:H7, manila ropes

**T106 Effects of predipping practices on milk iodine concentrations.**
S. I. Borucki-Castro[1], R. Berthiaume[1], A. Robichaud[2], and P. Lacasse*[1], *[1]AAFC-Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada, [2]Food Directorate, Health Canada, Longueuil, QC, Canada.*

A study was conducted to determine the effects of udder preparation before milking on milk iodine concentrations. The study compared the use of pre-dip and post-dip solutions; and determined the effect of an incomplete removal of pre-dip solution on milk iodine. Thirty-two lactating cows were assigned to 4 treatments: no pre-dip (udder wash without iodine; control); pre-dip with a pre-dip solution containing 0.5% iodine (Theratec, GEA Farm Technologies) + complete cleaning with paper towel (complete); pre-dip with a post-dip solution 1% iodine (Teat-Kote, GEA Farm Technologies) + complete cleaning with paper towel (post); and pre-dip with a pre-dip solution 0.5% iodine (Theratec) + incomplete cleaning with paper towel (incomplete). Incomplete cleaning was achieved by cleaning only 3 of the 4 teats. All cows received a diet without goitrogenic feeds, and an iodine concentration of 0.50 mg /kg of feed offered. During the 14d pre-experimental and the 19d experimental periods, non-iodized sanitizers were used for post-milking dipping or flushing of the milking units. The first 2 weeks were used for adaptation to treatments and measurements were done on the final week to ensure a plateau in the milk iodine response. During the pre-experimental period, milk iodine concentrations were similar for all groups and averaged 160.4, 167.3, 157.0 and, 153.0 ± 17.3 µg/kg for control, complete, post and, incomplete; respectively. During the last week of treatment, milk iodine averaged 164, 189, 218 and, 252 ± 9.8 µg/kg for control, complete, post, and incomplete, respectively. Pre-planned orthogonal contrasts indicated that pre-dipping with a 0.5% iodine pre-dip solution (complete) tended to increase milk iodine content above that of control ($P = 0.08$) and, that iodine content of post ($P < 0.05$) and incomplete ($P < 0.001$) were higher than that of the complete treatment. These results indicate that pre-dipping is an acceptable practice but must be performed with the appropriate product and completely wiped out before milking.

**Key words:** iodophore, milking, milk safety

**T107 Effects of natural beta-acids extracted from hops on *Salmonella* and *Campylobacter* pure culture.**
N.A. Krueger*[1], R. C. Anderson[1], J. A. Byrd[1], M. D. Flythe[1], and D. J. Nisbet[1], *[1]Food and Feed Safety Research Unit, United States Department of Agriculture, Agriculture Research Service, College Station, TX, [2]Forage Animal Production Research Unit, United States Department of Agriculture, Agriculture Research Service, Lexington, KY.*

*Salmonella* and *Campylobacter* are important foodborne pathogens that may colonize the gut of food-producing animals. The objective of this experiment was to evaluate the effects of a hops β-acid solution on *S.typhimurium* and *C. jejuni* pure cultures. Nine-milliliter volumes with approximately $10^{-4}$ colony forming units (cfu) of overnight grown *S. typhimurium* (in tryptic soy broth; TSB) or *C. jejuni* (in Mueller Hinton; MH) added in triplicate to screw top tubes previously loaded with 1 mL MH, TSB, or hops β-acid extract solution to achieve 0, 62.5 (H1) or 125 ppm (H2) hops and were incubated anaerobically at 40°C. After 0, 3 and 6 h incubation, 1 mL sample from each culture was serially diluted and plated to XLT4 or Campy-Cefex agar for quantification of *S. typhimurium* and *C. jejuni*. $Log_{10}$ transformations of *S. typhimurium* colonies enumerated after 24 h incubation and *C. jejuni* colonies enumerated after 48 h microaerobic (85% $N_2$, 10% $CO_2$, 5% $O_2$) incubation were subjected to Mixed Model repeated

measures ANOVA to assess affects of treatment over time. Tubes containing *S. typhimurium* initially contained 3.35 ± 0.202 $\log_{10}$ cfu/mL. Concentrations of *S. typhimurium* in control and H1 cultures increased ($P < 0.05$) by 6 h incubation (5.80 ± 0.175 and 4.97 ± 0.227, respectively); however, S. Typhimurium concentrations in H2 cultures did not differ over time ($P > 0.05$). *Campylobacter jejuni* cultures initially contained 4.41 ± 0.135 log10 cfu/mL. Concentrations of *C. jejuni* in control cultures did not differ ($P > 0.05$) over time whereas *C. jejuni* concentrations in H1 and H2 treated cultures were reduced to below our limit of detection ($\log_{10}$ 1.5) by 3 h incubation and did not recover by 6 h incubation. Results of this study demonstrate that hops β-acids can effectively reduce *C. jejuni* but not *S. typhimurium* concentrations in vitro.

**Key words:** *Campylobacter*, *Salmonella*, natural beta-acids

**T108 *Staphylococcus aureus* virulence and metabolism are dramatically affected by *Lactococcus lactis* in cheese matrix.** M. Cretenet[1,2], S. Nouaille[3,4], J. Thouin[1,2], L. Rault[1,2], L. Stenz[5], P. François[5], J. A. Hennekinne[6], M. B. Maillard[1,2], J. Fauquant[1,2], P. Loubière[3,4], S. Lortal*[1,2], Y. Le Loir[1,2], and S. Even[1,2], *[1]INRA, STLO, Rennes, France, [2]Agrocampus Ouest, STLO, Rennes, France, [3]Université de Toulouse; INSA, Toulouse, France, [4]INRA, UMR792, Toulouse, France, [5]University of Geneva Hospitals, Geneva, Switzerland, [6]ANSES, LERQAP, Maisons-Alfort, France.*

In complex environments such as cheeses, the lack of relevant information on the physiology and virulence expression of pathogenic bacteria and the impact of endogenous microbiota has hindered progress in risk assessment and control. Here, we investigated the behavior of *Staphylococcus aureus*, a major foodborne pathogen, in a cheese matrix, either alone or in the presence of *Lactococcus lactis*, as a dominant species of cheese ecosystems. The dynamics of *S. aureus* was explored in situ by coupling a microbiological and, for the first time, a transcriptomic approach. *L. lactis* affected the carbohydrate and nitrogen metabolisms and the stress response of *S. aureus* by acidifying, proteolysing and decreasing the redox potential of the cheese matrix. Enterotoxin expression was positively or negatively modulated by both *L. lactis* and the cheese matrix itself, depending on the enterotoxin type. Among the main enterotoxins involved in staphylococcal food poisoning, sea expression was slightly favored in the presence of *L. lactis*, whereas a strong repression of sec4 was observed in cheese matrix, even in the absence of *L. lactis*, and correlated with a reduced saeRS expression. Remarkably, the agr system was downregulated by the presence of *L. lactis*, in part because of the decrease in pH. This study highlights the intimate link between environment, metabolism and virulence, as illustrated by the influence of the cheese matrix context, including the presence of *L. lactis*, on 2 major virulence regulators, the agr system and saeRS.

**Key words:** *Staphylococcus aureus*, cheese, bacterial interactions

**T109 Characterization of risk of food pathogens in Minas Frescal cheese.** R. Freitas[1], A. F. Carvalho*[1], L. A. Nero[1], G. G. Netto[1], and M. A. V. Brito[2], *[1]Federal University of Viçosa, Viçosa, MG, Brazil, [2]EMBRAPA CNPGL, Juiz de Fora, MG, Brazil.*

This work aimed to carry out a characterization of the risk found in the food pathogens *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* sp., in Minas Frescal cheese. The prevalence and level of contamination by microbial agents was studied as well as the presence of microorganisms indicators of hygienic conditions (total coliforms and *Escherichia coli*), in relation to cheese making methods (lactic acidification, acidification by lactic acid and curd) and inspection stamp (federal, state or municipal) to which they were submitted. The magnitude of ingesting this type of cheese by Viçosa consumers for the qualitative estimate of risk of contracting diseases after consuming this product was investigated. Of 99 cheese samples analyzed, 20 were contaminated with *S. aureus*, with microorganism enumeration in 65% being higher than $1.0 \times 10^3$ cfu/g. The pathogen *L. monocytogenes* was isolated from one cheese sample, while *Salmonella* sp. was not identified in any of the samples analyzed. High contamination of the product by *E. coli* was also verified in 30.3% of the cheese samples analyzed, in values above the limit allowed by the current legislation, rendering these samples improper for consumption. A high level of cheese ingestion by the population was verified for consumption frequency and amount consumed. Of the total of 400 persons interviewed, 15.5% were in the age range between 11 and 20 years old, 77.5% between 21 and 60 years old and 7% above 60. Among the individuals interviewed, 84 informed that they consumed Minas Frescal cheese, 177 stated they consumed between 2 and 7 d per week, with the 11 to 20 year old, the 21 to 60 and the over 60 groups corresponding, respectively, to 11.9%, 77.4% and 10.7% of high cheese intake. Based on the use of the product by the population surveyed and the presence of S. aureus, Minas Frescal cheese consumption has the potential to contribute to the occurrence of intoxication cases in the population exposed to it, mainly when risk groups are considered. However, for *L. monocytogenes* and *Salmonella* sp. the risk of infection from consuming the product is low.

**Key words:** Minas Frescal cheese, risk characterization, quality

**T110 Inhibition of *Listeria monocytogenes* growth in cheddar cheese by nanofiltration retentate of tryptic extract of whey proteins.** V. Demers-Mathieu[1,2], G. Robitaille[1], D. St-Gelais[1], S. Gauthier[2], and M. Britten*[1], *[1]Food Research and Development Centre, Agriculture and Agri-Food Canada, St Hyacinthe, QC, Canada, [2]Centre de recherche STELA & INAF, Département de Sciences des Aliments et de Nutrition, Québec, QC, Canada.*

The objective of the study was to investigate the efficiency of a nanofiltration retentate ($RT_{NF}$) of trypsin-hydrolyzed whey proteins to control the food-borne pathogen *Listeria monocytogenes* and the non-pathogen *Listeria innocua* in Cheddar cheese models. Reconstituted Cheddar cheeses (37% humidity) containing 0, 10 or 20 mg/g of $RT_{NF}$ and 3.5 or 1.75% salt/Humidity (S/H) were prepared from irradiated cheese powder, and were inoculated with $10^3$-$10^4$ cfu/g of *Listeria* and $10^7$ cfu/g of commercial lactic acid starter strains (*Lactococcus lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis* and *Leuconostoc cremoris*). Cheeses were stored 7 d at 30°C or 28 d at 4°C, and bacterial counts during the storage were carried out for *Listeria* species and for lactobacilli. The presence of $RT_{NF}$ in model cheese significantly decreased the survival of *Listeria* strains in a dose-dependent manner. The antimicrobial activity of $RT_{NF}$ in Cheddar cheese was greater against *L. monocytogenes* than against *L. innocua* and was higher at 30°C than at 4°C. Moreover, the combination of 20 mg/g $RT_{NF}$ and 1.75% S/H in cheeses incubated at either 30°C for 7 d or at 4°C for 28 d was the most efficient condition. For instance, *L. monocytogenes* bacterial counts significantly ($P < 0.001$) decreased by 1.1 and 1.5 log, respectively, when compared with model cheeses containing 1.75% or 3.5% S/H without $RT_{NF}$. Lactobacilli bacterial counts decreased during storage according to salt content, but was not affected by $RT_{NF}$ ($P > 0.05$). It is suggested that the presence of higher lactobacilli content in cheeses containing 1.75% S/H contributes to the decrease of *Listeria* bacterial

content in synergy with $RT_{NF}$. In conclusion, antimicrobial peptides from trypsin-hydrolyzed whey proteins could be useful as natural preservative to control *L. monocytogenes* growth in reduced-salt cheeses.

**Key words:** *L. monocytogenes*, antibacterial, cheese

**T111 Investigating contamination of bulk tank milk with *Listeria monocytogenes* on a dairy farm.** J. C. F. Pantoja*, A. C. O. Rodrigues, C. Hulland, D. J. Reinemann, and P. L. Ruegg, *University of Wisconsin, Madison.*

The objective of this study was to identify the source of bulk tank milk (BTM) contamination with *Listeria monocytogenes* on a dairy farm with a history of isolation of this pathogen from unpasteurized BTM. The herd was comprised of 711 cows that produced an average of 36 kg of milk per day, with BTM SCC of 250,000 cells/mL. Cows were milked in a parallel parlor and milk was stored in 2 bulk tanks after passing through a plate cooler. Free stalls were bedded with sand or manure solids. The farm was visited between September and November, 2010, in 2 study phases. Each phase consisted of 3 weekly visits performed to assess the presence of *L. monocytogenes* in the following samples: environmental (feces, bedding, silage, and water from troughs, hoses, and wells); milking machine (milk filters and swabs from the inner surface of liners, milk hoses, milk meters, milk line, gaskets, and receiver jar); in-line milk of each pen milked; mammary glands; and BTM. Daily BTM and milk filters were also collected. Of all samples collected during the 2 study phases (n = 299), *L. monocytogenes* was isolated from 66% of milk filters (19 of 29), 16% of BTM (7 of 44), 6% of water samples (2 of 33) and 1 of 18 in-line milk samples. No other samples were positive for *L. monocytogenes*. A subset of 27 pairs of BTM and milk filter samples collected on the same day was used to assess the agreement between the isolation of *L. monocytogenes* from these 2 sources. Of 18 *L. monocytogenes*-positive milk filter samples, only 4 (22%) were also BTM positive. Based on these results, the authors recommended that the milk filter be changed at mid-milking, after which daily milk filter and BTM samples were collected for 3 weeks as a follow-up assessment of the prevalence of *L. monocytogenes* in the BTM. No follow-up milk filter (n = 23) or BTM (n = 15) samples were positive. Although a specific on-farm source of BTM contamination could not be identified based on these preliminary data, results suggest that the milk filter is a point of concentration of this zoonotic pathogen.

**Key words:** listeria, milk quality, zoonosis

**T112 Prediction the growth of *Staphylococcus aureus* in raw milk using modified Gompertz and Logistic models.** B. Li[2], C. Man[1], M. Guo*[3], Y. Shan[1], F. Zhao[2], S. Yang[2], Y. Jiang[2], Y. Lang[2], and Y. Jiang[1,2], [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.*

*Staphylococcus aureus* in dairy foods could cause foodborne illness. Raw milk may be to be kept incorrect temperatures in developing countries where cold storage facilities are lacking. The objective of this study was to predict the growth of *S. aureus* in raw milk produced in different seasons. Nine raw milk samples were collected in winter, spring, and summer, confirmed *S. aureus* free using 3M Petrifilm Staph Express Count Plates and inoculated with *S. aureus* (ATCC 13565) at a final concentration of $10^2$ cfu/mL. The inoculated milk samples were incubated at 15°C, 25°C and 37°C with different incubation time and sampling interval according to the temperature. The enumerations of *S. aureus* were carried out in triplicates using the same method mentioned above. Growth data of *S. aureus* were analyzed with both a modified Gompertz model (mG model) and a modified Logistic model (mL model). The performance of each model were evaluated by calculating the root mean square error (RMSE), the Accuracy factor ($A_f$) and the Bias factor ($B_f$) between the observed and predicted values. Results showed that the $A_f$ values of the mG model ($1.0492_{Sum}$ and $1.0432_{Win}$) were closer to 1.0 than those of the mL model ($1.0851_{Sum}$ and $1.0663_{Win}$) during summer and winter. As for the $B_f$ values, the mG model also superior to the mL model ($0.9764_{Sum}$ and $1.0056_{Win}$ vs. $1.0293_{Sum}$ and $1.0198_{Win}$). The RMSE values of the mG model ($0.1032_{Sum}$ and $0.0936_{Win}$) were lower than those of the mL model ($0.1857_{Sum}$ and $0.1498_{Win}$). The results indicated a more accurate fitting between measured and predicted values by the mG model in both seasons. The $A_f$ values and RMSE values of the mG model were better than those of the mL model (1.0562 and 0.1057 vs. 1.0945 and 0.1926) for samples collected in spring, although the $B_f$ values of the mL model were closer to 1.0 than those of the mG model (0.9905 vs. 0.9890). The results showed that the mG model seems to be accurate for predicting the growth of *S. aureus* in raw milk of different seasons. This work was supported by the National Key Technology R&D Program of China (2009BADB9B06).

**Key words:** *Staphylococcus aureus*, prediction, raw milk

**T113 Rapid detection of viable *Listeria monocytogenes* in milk by loop-mediated isothermal amplification coupled with propidium monoazide treatment.** Y. Jiang[2], C. Man[1], M. Guo*[3], Y. Lu[1], F. Zhao[2], Y. Liu[2], B. Li[2], S. Yang[2], and Y. Jiang[1,2], [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.*

*Listeria monocytogenes* is a common foodborne pathogen in dairy foods. DNA-based loop-mediated isothermal amplification (LAMP) method cannot distinguish viable cells from dead ones, resulting in overestimate of *L. monocytogenes* contamination. Propidium monoazide (PMA) inhibits amplification of LAMP due to its ability of tightly binding to the DNA of dead cells. The objective of this study was to develop a procedure using LAMP method coupled with PMA treatment to detect viable *L. monocytogenes* in milk. *L. monocytogenes* (CMCC 54006) was cultured in tryptone soy broth containing 0.6% yeast extract at 37°C to logarithmic growth phase. Part of the cultured suspension was heated at 95°C for 3 min to obtain dead cells. The dead cell suspension was mixed with the viable cell suspension at a ratio of 9:1. The mix was inoculated in UHT sterilized milk with final concentrations of viable cells up to $10^7$ cfu/mL. The inoculated milk was treated with PMA (50 μmoL, final concentration) in the dark for 5 min, subsequently exposed to a 650W halogen lamp for 3 min. Then DNA extracted from the PMA treated samples as templates for LAMP. A set of 4 primers, including forward-inner, backward-inner, forward outer, and backward outer, were designed for LAMP to target 6 distinct regions on the hlyA gene of *L. monocytogenes*. The LAMP analysis was carried out in a reaction mixture containing the 4 primers and DNA templates at 63°C for 1 h and heated at 80°C for 2 min to terminate the reaction. The amplified samples were then analyzed by a 1.5% agarose gel electrophoresis and the gel was stained with ethidium bromide. The detection limit for viable *L. monocytogenes* in steril-

ized milk by the PMA-LAMP method was $6.37 \times 10^1$ cfu/ml in only 90 min. Results showed that the PMA-LAMP method is a rapid and sensitive technique to detect viable *L. monocytogenes* in milk. This work was supported by the National Key Technology R&D Program of China (2009BADB9B06).

**Key words:** *Listeria monocytogenes*, milk, detection

**T114 Simultaneous analysis of anions $Cl^-$, $NO_2^-$, $SO_4^{2-}$, $NO_3^-$ and $PO_4^{3-}$ in milk with ion chromatography.** D. Liu and Z. Chen*, *Analysis and Testing Center, Shandong University of Technology, Zibo, Shandong Province, China.*

The amount of anions is an important index of milk quality control. Some methods have been reported to determine the concentration of milk anions. However, some methods are time-consuming and susceptible to interference, and only one element can be determined at a time. Ion chromatography (IC) has been developed for the simultaneous analysis of cations and anions in the water, food, atmosphere, etc. Chromatography can yield the precise and reproducible data when the experimental condition is kept constant. In the present studies, the packaged milk was bought in the market, and the anions ($Cl^-$, $NO_2^-$, $SO_4^{2-}$, $NO_3^-$ and $PO_4^{3-}$) in milk were determined with the technique of IC. A Dionex ICS-2000 ion chromatograph with a Dionex gradient pump, eluent degassing module and conductivity detector was used. Anions were separated on an AS 11 HC ionexchange column, with an AS 11 HC guard column, and detected after suppression with an ASRS 300 anion electrical self-regenerating suppressor. The relative standard deviation (R.S.D.) for $Cl^-$, $NO_2^-$, $SO_4^{2-}$, $NO_3^-$ and $PO_4^{3-}$ was 3.44%, 9.52%, 2.38%, 1.67% and 3.48%, and the percent recovery was ranged from 80.21% to 120.24%. In addition, the concentration of 5 anions in the milk was detected, and the data were expressed as mean ± SD. Statistical analysis was performed by one-way ANOVA followed with comparison test. The concentration of $Cl^-$, $NO_2^-$, $SO_4^{2-}$, $NO_3^-$ and $PO_4^{3-}$ in the milk sample was 440.39 ± 0.15 mg/L, 8.33 ± 0.01 mg/L, 95.14 ± 0.02 mg/L, 2.14 ± 0.01 mg/L and 953.11 ± 0.33 mg/L (n = 6), respectively. There was significant difference among the concentration of $Cl^-$, $NO_2^-$, $SO_4^{2-}$, $NO_3^-$ and $PO_4^{3-}$ ($P < 0.05$). The results indicate that the IC technique is suitable for the rapid, precise and accurate determination of major anions in milk samples. Acceptable detection limits are obtained for the anions, and the time of anions analysis is significantly shortened with the technique of IC.

**Key words:** ion chromatography, anion, milk

**T115 Evaluation of a screening test for detecting antimicrobial residues in milk by visual reading and by reader equipment.** M. M. P. Araújo, M. A. Guerra, A. D. Lage, A. F. Cunha, L. M. Fonseca, M. O. Leite, M. R. Souza, C. F. A. M. Penna, and M. M. O. P. Cerqueira*, *Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.*

To evaluate the efficiency of Charm MRL BL/TET kit for detecting antimicrobials residues in milk, raw milk samples without antimicrobials were inoculated with 21 drugs in 4 different concentrations including the Brazilian legal limit (MRL) and the detection threshold of the kit. The 21 antimicrobials from 6 different groups and positive and negative controls were tested with 30 repetitions by level. The results obtained by the reader equipment and visual reading were compared by the MacNemar test at 95% of confidence. The temperature of the blocks was monitored and the milk quality was evaluated by total bacterial count; somatic cell count; and fat, protein, lactose, total solids, and solids non fat contents. The temperatures varied from 55 to 57°C in the Charm MRL BL/TET kit and were considered satisfactory. The titratable acidity of the milk samples varied from 14.35 to 16.56°D. Considering all the limit levels of antimicrobial detection (L3) defined by the kit's manufacturer, discrepancies were not observed. The kit detected the different antimicrobials at level 2 that correspond to MRL concentration (Brazilian legislation) and also some detected at level 1 (L1) that was half the L3 concentration. The detection at level 2 (L2) was 100% for the majority of the antimicrobials, exception for cloxacillin (93.3%). Ceftiofur, an important antimicrobial used in Brazil, was detected in 100% of all the milk samples. In relation to L3 level, the Charm MRL Beta/TET kit detected cefalexin in 23.3% and cefapirin in 20.0% of the samples; cefazolin was not detected. Even considered similar ($P > 0.05$), some discrepancies in results evaluated by visual reading and by the kit's reader indicating the presence of antimicrobial residues should be interpreted with caution due to public health hazards and economic problems. It can be concluded that the test kit is efficient for detecting antimicrobials in milk and it can be used as a screening test for monitoring these substances in milk. The kit's reader must be obligatory for screening antimicrobial residues in milk in Brazil.

**Key words:** milk, antimicrobials, detection