M93 Residue of melamine and cyanuric acid in milk and tissues of dairy cows fed with different doses of melamine. J. S. Shen, J. Q. Wang*, H. Y. Wei, D. P. Bu, P. Sun, G. C. Luan, and Z. F. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*.

Melamine (MEL) may be degraded into cyanuric acid (CYA) and some other analogs by rumen microorganism. This study was conducted to investigate the residue of MEL and CYA in milk and tissues of dairy cows fed with different doses of MEL. Forty mid-lactation dairy cows (157 ± 43 DIM, 20.8 ± 1.4 kg of milk/d) were divided into 4 groups (n = 10/group) in a completely randomized design. The cows of the 4 groups were dosed with MEL (purity ≥99.5%) at 0 (Control), 300 (TRT 1), 500 (TRT 2) and 1000 (TRT 3) mg/d per cow, respectively. The whole trial lasted for 18 d (12 d feeding period, followed by 6 d clearance period). Milk samples were collected at d 1, 2, 3, 4, 8, 12, 13, 14, 15 and 18. On d 13, 3 cows of TRT 2 and TRT 3 were chosen randomly to slaughter, and tissue samples (kidney, liver, mammary, bladder, gluteus medius and longissimus dorsi) were collected. Milk and tissue samples were analyzed for MEL and CYA simultaneously by liquid chromatography tandem mass spectrometry (LS-MS/MS). Minor MEL was detected in concentrated feed background (6.23 ± 1.26 mg/kg), however, no CYA was detected. In MEL treated groups, milk MEL concentration increased quickly and reached a stable concentration at d 4, 8 and 12 after the first administration of MEL. Milk MEL concentration of treated groups in steady-state condition (0.18, 0.27 and 0.50 mg/L for TRT1, TRT2, TRT3, respectively) was significantly affected by MEL feeding dose ($P < 0.05$), with a linearly relationship between MEL intake and milk MEL concentration ($R^2 = 0.91$). No CYA was detected in milk of all groups. MEL residue in tissues of TRT 4 was about 2-fold higher than TRT 3, with the highest concentration in the kidney. The difference of CYA residue in tissues of TRT 4 and TRT 3 was not very obvious. Liver, kidney and bladder has higher CYA residue than other tissue. The CYA may come from the degradation of MEL in rumen.

Key Words: melamine, cyanuric acid, dairy cow

M94 Factors affecting microbiological and physicochemical characteristics of milk produced in dairies located in central Mexico (Altos de Jalisco). A. S. Aguilar, M. A. Lopez-Carlos, C. F. Arechiga*, J. I. Aguilera, F. Mendez-Llorente, H. Rodriguez, M. Rincon, and C. Diaz-Mora, *University of Zacatecas*, *Zacatecas, Mexico*.

Factors affecting physicochemical and microbiological characteristics of milk from 54 dairies located in 2 regions of central Mexico were evaluated. Effects of region, month, season, farm sanitary conditions; farm size and water hardness were evaluated. Variables measured were: protein, lactose, total solids (TS), non-fat solids (NFS), reductase, temperature, acidity, cryoscopy, and density; and the microbiological characteristics: CFU and SCC. Data was analyzed by SAS (proc- mixed). Fat, protein and lactose decreased in January and February. Northeast region scored higher values of milk quality compared with West region. Moreover, microbiological and physicochemical characteristics showed a quadratic trend with farm sanitary conditions. Whereas, physicochemical conditions showed a linear trend for volume, fat, protein, and lactose. A quadratic effect was observed for SCC in herds over 101 cows. Herd size showed a positive linear trend for milk volume, protein, TS, NFS and cryoscopy. Water hardness influenced microbiological characteristics, resulting in higher CFU and SCC by using medium-hard water compared with soft-water (196×10^6 vs. 126×10^6 for CFU and 748×10^6 vs. 631×10^6 SCC, respectively). Furthermore, soft-water hardness significantly affected fat, lactose TS, and NFS in milk. Finally, season of the year affected SCC (summer: 877×10^6 vs. autumn: 709×10^6; $P > 0.05$), and had no effect on CFU. In conclusion, factors evaluated confirmed the need to improved sanitary conditions and to monitor water hardness. It became evident the need to implement preventive medicine programs and stricter health controls for milking during periods with elevated incidence of subclinical mastitis especially in big herds.

Key Words: milk, microbiologic, physicochemical

M95 Determination of Cd and Pb content on tissues of beef cattle raised in a tropical pasture based system in Brazil. J. R. Lima*[1], M. B. M. Teixeira[2], J. L. B. Silva[2], E. F. Silva[2], R. G. Reis[2], L. R. D. A. Neto[1], H. M. Queiroz[2], and L. G. Nussio[1], [1]*University of São Paulo/ESALQ*, *Piracicaba, Brazil*, [2]*Ministry of Agriculture, Livestock and Food Supply*, *Campinas, São Paulo, Brazil*.

To satisfy the requirements of the international market it is necessary to monitor several quality traits in meat products such as heavy metals. The purpose of this study was to determine Cd and Pb in liver and Pb in kidney tissues of 27 beef steers pasture raised in a tropical grazing system to compare the accumulation of Cd and Pb at the same tissue and Pb in 2 different tissues. The animals were fed at *Brachiaria brizantha* (palisade grass) pastures stocked under a rotational grazing system for 8 mo receiving energy and mineral supplementation sources. They were allocated in 3 treatments with 3 replications up to the finishing period which was taken at feedlot in a random block design. The supplements supplied on pasture were: 1) mineral supplementation (MS) *ad libitum*; 2) MS + ground corn (0.3% of BW); 3) MS + ground corn (0.6% of BW). There was no intentional contamination with Cd and Pb of animal feed, soil or water to provide a positive control and the analyzed heavy metals were checked for all of them. Livers and kidneys samples were taken at slaughter and immediately frozen. The analysis method utilized was previously validated according to Commission Decision 2002/657/EC and Regulation 2007/333/EC and accredited, in accordance with ISO/IEC 17025/2005. Duplicates samples of about 10 g of each tissue were weighted and twice burned at 500°C in a muffle furnace and ashes were washed with HNO_3. The concentration was determined with a graphite furnace atomic absorption spectrometry (GF AAS) technique with quantification limits for Cd and Pb of 22µg/kg and 75µg/kg, respectively. Mean levels for Cd and Pb content in all samples of the liver and Pb in the kidney were bellow acceptable levels (Cd - 500µg/kg and Pb - 500µg/kg on wet basis) established by European Community (Regulation 2006/1881/EC) and bellow the quantification limits too. The lack of contamination of beef livers and kidneys with Cd and Pb suggests the meat from a typical grazing system in Brazil is considered safe based on the European Community regulations.

Support: FAPESP, CNPq

Key Words: heavy metals, beef cattle, graphite furnace atomic absorption spectrometry

M96 Effects of iodine intake and teat dipping practices on milk iodine concentrations. S. I. Borucki Castro*[1], R. Berthiaume[1], A. Fouquet[2], A. Robichaud[2], F. Beraldin[2], and P. Lacasse[1], [1]*Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada*, *Sherbrooke, Québec.*, [2]*Food Directorate, Health Products and Food Branch, Health Canada*, *Montréal, Québec.*

A study was conducted to determine the effects of different iodine levels in lactating cow diets and different post dip practices on the concentration of milk iodine. Sixty 3 cows in mid-lactation were assigned to a 3 × 3 factorial with: 0.25, 0.5 and 1.0 mg dietary iodine / kg DM and 3 different post dip managements: chlorhexidine with dip cup, 1% iodine dip cup and 1% iodine spray, for a total of 9 treatments. During the 13-d pre-experimental and the 17-d experimental period, non-iodized sanitizers were used in premilking management or flushing of the milking units. At the end of the pre-experimental period, where all cows were fed 0.5 mg iodine/ kg DM and chlorhexidine was used as post dip, the levels of milk iodine averaged 299.5 (±11.55) µg/kg and no relationship was found with lactation number, days of lactation or milk production. Dietary iodine and milking management both affected milk iodine concentrations ($P < 0.001$). Although teat dipping with 1% iodine had no effect on milk iodine concentration, the same solution applied by spraying greatly increased milk iodine levels ($P < 0.05$). The results from this study confirm that iodine should not be overfed to preserve the safety of milk. Spraying iodine-based teat dipping solutions result in large increases of milk iodine contents and should be avoided.

Table 1. Least square means (LSM) of milk iodine concentrations at the end of 17-d treatment (µg/kg)

	Post milking management		(SEM=42.6)	
Dietary iodine (mg/kg DM; SEM 42.5)	Chlorhexidine - dip	1% Iodine - dip	1% Iodine - spray	Diet (SEM = 24.3)[1]
Low (0.28)	143	201	593	312_a
Recommended (0.53)	261	289	700	417_b
High (0.99)	325	386	665	459_b
Post dip (SEM=24.3)[2]	243_a	292_a	653_b	

[1]LSM in the column with different subscripts differ ($P < 0.05$).
[2]LSM in the row with different subscripts differ ($P < 0.001$).

Key Words: iodine intake, milk iodine, teat dip

M97 Iodine concentrations in feeds in farms with contrasting levels of iodine in milk. S. I. Borucki Castro*[1], P. Lacasse[1], A. Fouquet[2], A. Robichaud[2], F. Beraldin[2], and R. Berthiaume[1], [1]*Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada*, *Sherbrooke, Québec, Canada,* [2]*Food Directorate. Health Products and Food Branch, Health Canada*, *Montréal, Québec, Canada.*

In a previous study, iodine concentration of bulk-tank milk in Canada (n = 501 farms) was found to vary considerably and appeared to be influenced by feeding practices. A subset of 200 participating farms was used to determine the relationship between milk iodine concentrations and concentration of this mineral in different feeds and diets of lactating dairy cows. The 30 farms with the lowest (Low) and the 30 farms with the highest (High) levels of iodine in milk were selected. Each of them completed a questionnaire providing information about the feeding management and samples of all feed ingredients, water and bulk tank milk were collected. The iodine offered on each of the farms was estimated using the amount recommended by the software Ration′L and the iodine concentration in the feed sampled and analyzed using inductively coupled plasma mass spectrometry. Milk iodine concentrations averaged 146 (±13.9) µg/kg (Low) and 487 (±44.6) µg/kg (High). Dietary concentrations of iodine offered daily were 33% lower ($P < 0.01$) for the group Low compared with the group High, 1.20 (±0.099) vs. 1.81 (±0.195) mg/ kg DM, respectively. A linear relationship ($P < 0.01$) was found between dietary iodine concentration and milk iodine levels: (y) Milk iodine (µg/kg) = 145 (±66.9) + 113 (±39.4) x (dietary iodine concentration, mg/kg DM). However, the low R^2 (0.15) indicates that other factors such as milking management and the presence of goitrogens, must have affected the concentrations of iodine in milk. Forages supplied approximately 17% of iodine requirements in the average lactating cow diet. Therefore, variation in the iodine content of forages are unlikely to cause iodine overfeeding. Conversely, 27% of mineral mix samples presented iodine concentrations above 100,000 µg/kg DM (and up to 322,000 µg/kg DM). More than 80% of the farms tested fed higher iodine levels than dietary iodine recommendations (0.50 mg iodine/kg DM; NRC, 2001). Our results suggest that iodine supplements should be used with caution in lactating cow diets.

Key Words: iodine, milk iodine, feed ingredients

M98 European Union principles for the risk assessment of feed additives. M. Anguita*, J. Galobart, and C. Roncancio-Peña, *European Food Safety Authority*, *Parma I43121, Italy*.

In the European Union (EU), all feed additives before being placed on the market undergo an authorization procedure as established in the Regulation (EC) No 1831/2003. In this procedure the European Food Safety Authority (EFSA), and in particular the Panel on additives and products or substances used in animal feed (FEEDAP), is the responsible of assessing the safety and the efficacy of the additive. Feed additives are substances, microorganisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water to perform one or more of the following functions: favorably affect the characteristics of feed or animal products, favorably affect the color of ornamental fish and birds, satisfy the nutritional needs of animals, favorably affect the environmental consequences of animal production, favorably affect animal production, performance or welfare or have a coccidiostatic or histomonostatic effect. To be authorized, feed additives should be safe, and therefore should not have (i) an adverse effect on the animal health, human health or the environment, and (ii) should not be presented in a manner which may mislead the user; the additives should also be efficacious. Any person seeking the authorization of an additive should submit an application to the European Commission and a technical dossier to EFSA. The dossier should be compiled following Commission Regulation (EC) No 429/2008 and the guidance documents that EFSA has prepared to help the applicants. The data provided in the technical dossier should allow a complete assessment of (i) the identity of the additive, (ii) the safety for the target animals, for the consumers of food products derived from animals fed diets containing the additive, for those persons handling the additive and for the environment and (iii) the efficacy of the additive, according to the claim made. The scientific assessment carried out by the FEEDAP Panel finishes with the adoption of a scientific opinion which will be the basis for the Commission to grant or deny the authorization of the product for its use in the EU market.

Key Words: additives, assessment, European Union

M99 Development an on-farm technique using lactic acid bacteria as a biomarker to detect of toxins in milk. M. H. Hathurusinghe*[1], A. AbuGhazaleh[2], M. R. Reddy[1], S. A. Ibrahim[1], M. Tajkarimi[1], and D. Song[1], [1]*North Carolina Agricultural and Technical State University*, *Greensboro, NC*, [2]*Southern Illinois University*, *Carbondale*.

Because many dairy farms lack adequate biosecurity there is a risk of intentional contamination of milk with harmful chemicals. Such contamination has potential health risk to the human consumers as well as major economic losses to the country. Most of the existing methods to detect toxins are expensive and time consuming. Thus, there is an urgent need for developing simple on-farm techniques that can detect toxins in raw milk. The objective of this study was to determine the effect of brodifacoum, bromadiolon, strychnine, and sodium cyanide on the growth of selected strains of lactic acid bacteria (LAB) and to test the potential of LAB as a biomarker for early detection of toxins in milk. Three strains of bifidobacteria, one strain of *Lactobacillus rhamnosus* GG and a commercially available yogurt culture were used to detect their sensitivity to selected toxins. Toxins at different concentrations were added into separate tubes containing different strains of bacteria. Samples were incubated at 37°C for 24 h. Milk samples without toxins were used as the control. The turbidity of the sample was recorded at 3, 9 and 24 h intervals. Results showed that *B. longum* was not sensitive to the tested toxins. *B. adolescentis* and *B. breve* were sensitive to the toxins and the inhibition was observed after 24 h. The yogurt culture and *L. rhamnosus* GG were the most sensitive to all the toxins at levels of 1µg/ml and 2µg/ml respectively, except for sodium cyanide which was 0.1µg/ml for both yogurt culture and *L. rhamnosus* GG after 3 h of incubation. These findings suggest that a highly sensitive, environmentally safe, fast and accurate test kit could be developed using selected LAB as a universal biomarker.

Key Words: toxin, lactic acid bacteria, biomarker

M100 Food safety in developing countries using no technology: The Wagashi study case. F. La Terra[1], G. Belvedere[1], M. Manenti[1], C. Pediliggieri[1], S. Mirabella[1], J. C. Codjia[2], S. Doko[3], and G. Licitra*[1,4], [1]*CoRFiLaC, Regione Siciliana*, *Ragusa, Italy*, [2]*University of Abomey-Calavi*, *Benin*, [3]*University of Parakou*, *Benin*, [4]*DACPA, Catania University*, *Catania, Italy*.

The Wagashi cheese, produced in Benin from Peuhl communities, is a cheese that has a homogeneous structure and can be found usually in round shaped 300 to 800 g pieces. To produce Wagashi cheese, milk coagulation occurs at temperatures higher than 70°C. This allows a first thermic treatment of milk extremely important when production hygiene conditions cannot be controlled. Fresh milk is first heated at 60°C for 5 min. After, a vegetal coagulant extracted from the latex of *Calotropis procera* leaves is added and temperature is raised and maintained at 95°C for 3 to 5 min until curd separates from whey. The latex of *Calotropis procera* is used to produce the Wagashi cheese with very low proteolytic activity that allows boiling the cheese over and over again, every 2 d, for about 20–30 d since it has been produced. This simple practice makes the cheese safe. Alternatively, cheese can be sun-dried to extend shelf life. The aim of this study was to assess variations of chemical, biochemical, and microbiological properties of Wagashi cheese, to evaluate the food security conditions. Production conditions and processes were reproduced and monitored in a laboratory trial. Eleven 500-g cheeses were produced using cow raw milk. Cheeses were sampled after cheese making process and, after 24h and 48h, before and after boiling for 10, 20, and 40 min. Cheese samples were analyzed for pH, soluble proteins (TCA 12%), soluble proteins pH 4.6, fat, total nitrogen, urea PAGE, total bacteria count (TBC), total and fecal coliform count, and *S. aureus* count. After 24h, 10-min boiling were sufficient to reduce TBC in the center of the cheese from 3.5×10^3 to 10^2 cfu/g. After 48h, 10-min boiling reduced TBC from 1.10×10^6 to 6.30×10^4 cfu/g, whereas 20 min were necessary to reduce TBC to 10^1 cfu/g. The proteolytic profiles of Wagashi cheese obtained before and after boiling treatment were in overlapping, confirming the low proteolytic activity. The results showed how the temperature during cheese making is an important factor for the microbiological food safety and in addition the re-boiling process for 20 min each 48h guarantee its shelf-life for several days.

Key Words: food safety, proteolysis, Wagashi cheese

M101 Stress-induced adaptive tolerance response influences virulence in *Campylobacter jejuni*. G. S. Kumar*, I. Hanning, Y. Ma, and M. Slavik, *University of Arkansas*, *Fayetteville*.

Campylobacter jejuni, the major cause of human gastroenteritis, is a fragile bacterium requiring special conditions in the laboratory for its growth. In nature, however, this organism is able to survive in very diverse and hostile environments and produce disease in humans. The mechanisms that the organism has evolved for its survival in stressful conditions are not fully understood. To determine the effect of acid stress on *C. jejuni*, 4 different strains of *C. jejuni* were exposed to an acid pH of 5.5 and then re-challenged with a pH of 4.5. Acid-adapted cells were found to have higher viability to survive further acid stress, but adaptation and survival were time-dependent. The effects of starvation stress also were studied. Expression of virulence gene *cadF* was upregulated by starvation stress, while virulence genes *cdtB* and *ciaB* were downregulated. Adhesion and invasion are thought to be important factors for the colonization of *C. jejuni* in the intestinal tract of host. In vitro studies with INT 407 tissue culture model (mammalian intestinal cells) were conducted with different times of exposure to acid (2h and 3h), to determine the effects of stress on adhesion and invasion of *C. jejuni*. All the tissue culture experiments were performed in replicates of 3. Results indicated that acid-adapted bacteria had increased adhesion and invasiveness, but varied with the strains and the time of exposure to the acid. Exposure of acid-adapted *C. jejuni* to further stress of starvation for 24 h did not have any significant difference in the adhesion and invasion abilities as compared with cells exposed to starvation stress only. These results indicate that *C.jejuni* surviving stress may be more resistant to further stress such as passage through the human gastrointestinal tract and that stress may be a significant factor in inducing some virulence genes.

Key Words: *C. jejuni*, stress, acid-adapted

M102 *Salmonella* Enteritidis challenge in chicks of different genotypes. P. E. N. Givisiez*[1], E. G. Santos[1], F. G. P. Costa[1], J. H. V. Silva[1], and A. Berchieri Jr.[2], [1]*Universidade Federal da Paraiba*, *Areia, PB, Brazil*, [2]*Universidade Estadual Paulista*, *Jaboticabal, SP, Brazil*.

Alternative poultry production is a promising business for small farmers, who should choose breeds based on robustness, consumer concerns and health issues. This study evaluated the resistance of Cobb and Naked Neck birds fed 2 threonine levels and challenged with *Salmonella* Enteritidis at 2 d of age. Twenty Cobb chicks (CC) and 20 naked neck chicks (NN) were distributed into a completely randomized design according to a 2 × 2 factorial (2 genotypes and 2 threonine levels) and 8 repetitions. Antibiotic-free corn-soybean diets with 0.821 and 0.917% digestible threonine were used. The birds were inoculated with *Salmonella* Enteritidis $^{Nal++}$ (1.2×10^8 CFU.mL^{-1}) and killed at 10 d of age for performance evaluation and bacterial counts (CFU.g^{-1}), which were

transformed into Log10. Data were subjected to ANOVA and means were compared using Tukey's multiple comparison test ($P < 0.05$). There was no interaction between genotype and threonine level ($P > 0.05$). Lower *Salmonella* counts ($P > 0.01$) were seen for NN compared with CC (1.37 vs 6.91). Furthermore, 94% of CC (15/16) and 25% (4/16) of NN were positive for salmonella counts. Body weight and weight gain were higher ($P < 0.01$) for CC (250.13 vs 120.19 g and 206 vs 77.75 g, respectively). Although threonine has been suggested to help diminishing enteric pathogen infection, higher threonine levels had no effect on salmonella counts or performance. The results corroborate the need of *Salmonella* control during the first week of rearing, since birds contaminated during this period may be infected until slaughter and there is potential risk for consumers. NN was best fitted for alternative production, considering the higher resistance to infection by *Salmonella* and slower growth rate. In conclusion, naked neck chicks were more resistant to *Salmonella* Enteritidis than Cobb independent of threonine dietary levels.

Key Words: alternative poultry, salmonellosis, nutrition