**19 ADSA Pioneer: Milk quality - Developments in testing and grading of raw milk.** W. S. LaGrange*, *Iowa State University*, *Ames*.

Milk testing was initiated due to consumer pressure to have wholesome uniform products. Initially, the buyer smelled and sometime tasted the milk. When the Babcock test came along milk richness or fat content could be standardized. Older milk could be determined from titration and alcohol tests. Then keeping quality tests such as Methylene blue and Resazurin became important to manufacturers. A lot of time was spent in identifying spoilage and pathogenic organism in raw milk. As technology became available rapid and sophisticated tests became the standard of the milk industry. In the developed world many tests are determined prior to the milk being unloaded at the manufacturing plant.

**20 Transglutaminase polymerization of a modified whey protein ingredient.** D. A. Clare* and C. R. Daubert, *North Carolina State University*, *Raleigh*.

Transglutaminase promotes protein crosslinking reactions through an acyl transferase mechanism involving protein-bound glutaminyl residues and primary amines including the ε-amino group of lysine residues in soy, myosin, gluten, oat globulin, peanuts, casein and whey. In this study, a modified whey protein concentrate (mWPC) was prepared to varying protein concentrations, 5.6% - 10% or 8% - 14.3% solids (w/w), in deionized water, pH 8.0, and crosslinked with microbial transglutaminase (TGase) at an enzyme/substrate ratio of ~5 units of activity/g mWPC. Test mWPC dispersions, prepared in the presence and absence of 10 mM dithiothreitol (DTT), were then incubated with the enzyme for various time intervals at 40°C. Representative samples were analyzed with respect to their (a) SDS-PAGE banding profile, (b) degree of crosslinking as determined by OPA assays, (c) apparent viscosity measurements, (d) gelation parameters, and (e) emulsion properties.
SDS-PAGE results showed that polymer formation occurred in all experimental mWPC dispersions treated with TGase, even those devoid of dithiothreitol (DTT); however, the rate of the reaction was slower. For example, quantitative OPA analyses revealed ~30% cross-linking in whey protein samples containing 10mM DTT after a 3h treatment period compared to 15% polymerization in equivalent solutions prepared under non-reducing conditions. These finding were attributed, in part, to maintenance of the redox status of the active cysteine residue found in the enzymatic catalytic site.
Pseudoplasticity was observed in all shear rheological experiments. Furthermore, the apparent viscosity and gel strength of TGase-mWPC dispersions was slightly lower than non-treated controls while the gelling temperature was raised. Notably, both the viscosity and stability of emulsions, prepared with these dispersions, were significantly improved. Scanning electron microscopy revealed a larger pore size in mTGase crosslinked mWPC samples, attributed to polymer formation. Ultimately, these approaches may provide novel whey-based food ingredients with unique functional characteristics for expanded application within the world marketplace.

**Key Words:** Whey Proteins, Transglutaminase, Functionality

**21 Production of conjugated linoleic acid in milk by lactic acid bacteria.** A. J. Pandit*, S. K. Anand, K. F. Kalscheur, and A. N. Hassan, *South Dakota State University*, *Brookings*.

Geometrical and positional isomers of conjugated linoleic acid (CLA) have several reported health benefits. Dairy products like cheese are increasingly being viewed as a possible dietary source of CLA. Increasing CLA level in these products could help bridge the gap between daily CLA requirement and effective dietary intake. This study was undertaken to evaluate CLA production and their isomeric distribution in milk during fermentation by lactic acid bacteria (LAB). A total of 155 cultures of LAB, including 46 cultures from the Dairy Science Department's culture collection, 68 isolates from retail cheeses, and 41 raw milk isolates were screened for CLA production. Cultures were individually inoculated in milk at 2% levels and incubated for 4 h at 37°C. Fat was extracted from milks at the end of incubation and the butyl esters were analyzed by gas chromatography. Control milks had an average CLA content of 0.41 ± 0.10 gm/100 gm of fatty acids (FA). The 13 cultures (7 *Lactococcus* and 6 *Lactobacillus*) from the Department collection produced CLA values ranging from 0.43 ± 0.03 to 0.63 ± 0.14 gm CLA/100 gm FA in milk. A higher CLA level was produced by five isolates from retail cheeses (4 *Lactococcus* and 1 *Lactobacillus*). The highest CLA production by one of the *Lactococcus* strains was 1.12 ± 0.35 gm/100 gm FA. The remaining 4 isolates (3 *Lactococcus* and 1 *Lactobacillus*) produced 0.87 ± 0.22, 0.63 ± 0.16, 0.56 ± 0.15, 0.62 ± 0.10 gm CLA/100 gm FA, respectively. These cultures increased mainly the cis-9, trans-11 isomer and also produced 3 to 5 unidentified CLA isomers. On an average, cis-9, trans-11 represented 62.62%, and trans-10, cis-12 represented 17.77% of the total CLA. This study shows that lactic acid bacteria can increase levels of CLA in fermented milk and possibly be used for making dairy products enriched with CLA.

**Key Words:** Conjugated Linoleic Acid, Lactic Acid Bacteria, Milk

**22 Immuno-stimulatory AT oligodeoxynucleotide from *Lactobacillus gasseri* requires a specific self-stabilized structure.** T. Shimosato*[1], M. Tohno[2], T. Sato[3], Y. Kawai[2], T. Saito[2], and H. Kitazawa[2], [1]*Shinshu University*, *Minamiminowa, Nagano, Japan*, [2]*Tohoku University*, *Sendai, Miyagi, Japan*, [3]*Yokohama City University*, *Yokohama, Kanagawa, Japan*.

Some probiotics (*live microorganisms which when administered in adequate amounts confer a health benefit on the host*) enhance the secretion of anti-inflammatory cytokines by innate immune cells. We have been studying immuno-stimulatory DNA from probiotics as an immuno-active factor in probiotics. Recently, we found an immuno-stimulatory AT oligodeoxynucleotide (ODN) containing a unique core sequence (5′-ATTTTTA-3′) in *L. gasseri* genomic DNA. Interestingly, although the AT-ODN does not contain any CpG sequences, it exerts mitogenic activity and augments Th1 type immune responses via Toll-like receptor (TLR) 9. We then identified 280 different AT-ODNs in *L. gasseri* genomic DNA, and mitogenicity and NF-kappaB reporting assays showed that 13 of these 280 AT-ODNs were strongly immuno-stimulatory in the TLR9 transfectant. Of these, AT-ODNs LGAT-145

and LGAT-243 were the most potent. LGAT-243 had the greatest activity and was more potent in the induction of Th1 type cytokines than the swine prototype, ODN D25. We further found that a six-base secondary loop structure containing a self-stabilized 5′-C...G-3′ stem sequence is important for induction of the potent immuno-stimulatory activity. The present study identified novel strong immuno-stimulatory AT-ODNs in the genomic DNA of probiotics. Moreover, these results are the first to demonstrate that AT-ODNs with a specific loop and stem structure are important for immuno-stimulatory activity. Finally, the data shows that TLR9 is a receptor for not only CpG but also for AT ODNs. These findings may help our understanding of intestinal immuno-regulation mediated by probiotic DNA through TLR9, for the development of new probiotic foods.

**Key Words:** AT-ODN, *Lactobacillus gasseri*, TLR9

**23 Complete genome sequence and comparative genome microarray of *Lactobacillus casei* provides evidence for genome expansion and reveals significant intraspecies differences.** H Cai*[1], J. R. Broadbent[2], and J. L. Steele[1], [1]*University of Wisconsin*, *Madison*, [2]*Utah State University*, *Logan*.

*Lactobacillus casei* are industrially important lactic acid bacteria (LAB) that have been primarily used as probiotics and specialty cultures for cheese flavor development. They have remarkable ecological adaptability to diverse habitats. The genome sequence of *L. casei* ATCC334 had been analyzed. It contained a 2,895,264-bp chromosome and a 29,061-bp plasmid with a GC% of 46.6 and 42.2, respectively. A total of 2,951 ORFs was predicted. Several unique features of the genome supported the hypothesis that the genome had been expanded for versatile adaptation to diverse ecological niches: (i) a correlation was observed between the relatively high GC% of the genome and versatile adaptability of LAB, (ii) relatively low numbers of rRNA and tRNA genes relative to genome size likely indicated presence of large amount of unessential genes; and (iii) significantly higher number (~3.25% of total ORFs) and larger diversity of transposases (11 families) suggested the key role of transposases in genome expansion and horizontal gene transfer of the species. Additionally, the genome contained relatively high number of transcriptional regulators (>4% of total ORFs) and transporters. About 361 secreted proteins, 19 glycosyltransferases and several mucus- and fibronectin-binding proteins were identified. About 28 peptidases and homologs for PrtP and PrtM were predicted. Comparative genome microarray between ATCC334 (a cheese isolate) and a silage isolate 12A revealed ~14.4% of the genes that are present in ATCC334 are missing in 12A, most of which are transposase- and bacteriophage- associated genes or genomic regions. Loci involved in sugar transport, metabolic enzymes and transcriptional regulators also differ. Collectively, the genomic features of *L. casei* provided evidence for genome expansion that may have facilitated its diverse ecological adaptability. Significant gene content differences were identified between the cheese and silage isolates, which may underlie the ecological adaptation of these strains to their specific ecological niches.

**Key Words:** *Lactobacillus casei*, Genome Sequence, Adaptation

**24 Effect of feeding pasture and long chain omega-3 fatty acid (LCn-3FA) supplements on the composition and oxidative stability of milk & butter.** M. C. Rose*[1], H. P. V. Rupasinghe[1], S. M. Budge[2], K. Glover[1], and A. H. Fredeen[1], [1]*Nova Scotia Agricultural College*, *Truro, NS, Canada*, [2]*Dalhousie University*, *Halifax, NS, Canada*.

Enriching milk with LCn-3FA, namely docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), to improve its nutritional profile may also increase rate of lipid oxidization and the production of off-flavors. Antioxidant content of forage may ameliorate the effect, but antioxidant content of forages is reduced by ensiling. The objective of this experiment was to determine the effect of feeding LCn-3FA and forage type with different anti-oxidant potential on the oxidative stability of milk. Eight mid-lactation Holstein cows (BW 612.5 ± 9.49kg; BCS 2.71 ± 0.03 out of 5; milk yield 16.83 ± 0.56kg) were used to evaluate four treatments: pasture (with concentrate fed twice daily at milking according to milk yield 1:4 w/w) or total mixed ration (TMR containing ensiled forage and 11.06 kg concentrate DM per cow daily) with or without a supplement of rumen- protected LCn-3FA. Cows were assigned randomly to treatment in two 4x4 Latin squares with 28 d periods. Milk was collected on days 27 and 28. No significant differences ($P>0.05$) in gross milk composition among the treatments were observed, which averaged 4.11 ± 0.23% fat; 3.10 ± 0.09% protein; 4.69 ± 0.03% lactose. Butter was made by centrifugation at 15,000 x g for 15 min at 4°C. Oxidation of raw milk and butter was determined using the Thiobarbituric Acid Reactive Substances (TBARS) method. TBAR absorbance at 530nm was significantly higher ($P=0.02$) in milk and butter of cows fed TMR supplemented with LCn-3FA compared with those fed pasture and LCn-3FA. These results indicate that a pasture - based diet may improve the shelf life of milk and butter enriched with LCn-3FA.

**Key Words:** Long Chain Omega-3 Fatty Acids, Lipid Oxidation, Forage

**25 The protective effect of proceesed cheese against hyperlipidemia in rats.** M. H. Abd El-Salam* and D. A. Mohamed, *National Research Centre*, *Cairo, Giza, Egypt*.

The effects of ingestion of processed cheeses containing different types of fats on plasma lipid profile and lipid peroxidation of hypercholestrolemic rats were studied. The study included three types of processed cheeses; the first two contained vegetable oils and the third contained milk fat only. Five groups of rats (8 animals each) were fed balanced diet (normal control), hydercholesterolemic diet (control) and hypercholesterolemic diet containing one of the tested processed cheeses respectively for 8 wks. The body weight and food intake were recorded and gain in body weight and food efficiency ratio were calculated. Blood analysis was carried out at the end of the experiment for total lipids and cholesterol, LDL, HDL, triglycerides (Tg) and plasma malondialdehyde (MDA). Feeding the different processed cheeses with hypercholesterolemic diet showed variable reductions in the plasma lipids except HDL which was increased as compared to control. The highest reduction was observed in group that received processed cheese containing milk fat only. Also, this group showed significant reduction in lipid peroxidation as measured by MDA. These results reflect the potential beneficial effects of consumption of processed cheese containing milk fat only towards cardiovascular diseases.

**Key Words:** Processed Cheese, Hypercholesterolemia, CLA

**26 Antimicrobial activity of dried spearmint and its extracts for use as soft cheese preservatives.** M. Foda[1], M. El-Sayed*[1], M. E-Mogazy[1], A. Hassan[2], and N. Rasmy[2], [1]*National Research Center*, *Cairo, Egypt*, [2]*Faculty of Agriulture*, *Cairo, Egypt*.

Spearmint which is common herb in the Mediterranean region was evaluated (dried or its extracts) as inhibitor factor against the growth of four strains of bacteria, four strains of yeast and four strains of moulds, using filter paper disc agar diffusion technique. Obtained results showed that water and ethanolic extracts had no inhibitory effect on all strains under test except the moderate activity of ethanolic extract against Gram positive bacteria (Bacillus cereus and Staphylococcus aureus). Essential oil was the strongest antimicrobial inhibitor, so, its antifungal activity and minimum inhibitory concentration (MIC) were examined. To evaluate the antimicrobial activity of spearmint, two types of UF-soft cheese were prepared using different concentrations of dried spearmint and its essential oil. Spearmint essential oil showed significant reduction on counts of total bacteria, proteolytic bacteria and psychrotrophic bacteria compared with control samples. Moulds and yeasts were totally inhibited by using the essential oil.

**Key Words:** Soft Cheese, Antimicrobial Activity, Spearmint

**27 Is Levowitz–Weber the appropriate confirmatory stain for direct microscopic somatic cell counting of ovine milk?** K. H. Petersson*[1], L. Connor[1], C. S. Petersson-Wolfe[2], and K. A. Rego[1], [1]*University of Rhode Island*, *Kingston*, [2]*Virginia Polytechnic Institute and State University*, *Blacksburg*.

The stain approved for regulatory use in bovine and ovine milk is the non–specific methylene blue based Levowitz–Weber (L–W) stain in contrast to the approved stain for goat milk, the DNA specific Pyronin–Y–Methyl Green (PMG) stain. Due to the presence of cytoplasmic particles in ovine milk the use of the L–W stain has the potential to artificially elevate the MSCC, particularly in late lactation. The objective of the current study was to compare the MSCC of sheep (n=42) over an entire lactation using 3 different counting methods: 1.) Direct Microscopic Somatic Cell Count (DMSCC) with L–W stain, 2.) DMSCC with PMG stain, and 3.) MSCC using the automated Fossamatic cell counter (FOSS). All staining procedures were conducted in accordance with U.S. regulatory procedures. Composite milk samples were collected from Fresian ewes (1–7 yr of age) within 2 wk of lambing and then monthly thereafter throughout the entire lactation. Each milk sample was divided into 2 aliquots. The DMSCC staining procedures (L–W and PMG) were conducted on one aliquot within 48 h of sample collection. The second aliquot was bronopol–preserved and Fossamatic cell counting began within 72 h of sample collection. The same bronopol–preserved sample was tested for fat, protein, milk urea nitrogen and total solids content. Sample collection began in October 2007 and will continue until May 2008. Descriptive statistics on the preliminary data have been summarized. To date, all study animals have been sampled from lambing through 90 days in milk. The average MSCC for L–W, PMG and FOSS were $98\pm9 \times 10^3$ (Mean±SEM), $72\pm7 \times 10^3$ and $345\pm40 \times 10^3$ cells/ml, respectively. Preliminary statistical analyses suggest significant interactions between the counting method and milk components fat, protein and total solids, as well as month of lactation. Additionally, milk urea nitrogen level appears to have a significant negative association with MSCC. A full statistical analysis will be examined following the completion of data collection in May 2008.

**Key Words:** Ovine Milk, Somatic Cell Count, Staining Procedure