60 Observational study of factors associated with seasonal variation in milk urea nitrogen observed on intensively and extensively managed pastures, during the summer 2000 grazing season, in Prince Edward Island, Canada. E Leger, I Dohoo, G Keefe, J Wichtel, P Arunvipas, and J VanLeeuwen, *Atlantic Veterinary College*.

The effects of pasture management and pasture supplementation on milk urea nitrogen (MUN) were explored to better understand protein and energy interactions during the grazing period. The overall objective of this observational study was to identify the significant factors associated with seasonal variation in milk urea nitrogen, in dairy cows grazing intensively or extensively managed pasture. Pasture management, stage of lactation, sample date and pasture supplementation on MUN levels, were examined during the summer 2000 grazing period. In total eighteen dairy herds were assessed. Ten of the herds were intensive grazing management (IGM) farms and 8 were extensive grazing management (EGM) producers. Each farm was visited within 48 hours after each Atlantic Dairy Livestock Corporation (ADLIC) test. During each visit, pasture and stored forages were sampled and a detailed questionnaire relating to nutrition and management was completed. Collected ration information was evaluated using a computerized ration evaluator (Spartan). Multilevel modeling (2327 records) was used to compute the relationships between the energy-protein ratio (EPR), a ratio which represents the protein and energy requirements relative to protein and energy delivery, grazing management, presence of rye grass, stage of lactation, milk production and their interactions on milk urea nitrogen. Stage of lactation, grazing management, milk yield, presence of ryegrass, the EPR and the interaction between intensive grazing management practices and the presence of rye grass were found to be significant predictors of MUN. Predicted MUN values were 3.9 units higher on IGM herds where rye grass was present when compared to EGM herds where ryegrass was absent.

Key Words: Milk Urea Nitrogen, Pasture, Dairy Nutrition

Graduate Paper Competition
Dairy Foods

61 Purification and characterization of two types of bile salt hydrolase from *Bifidobacterium* spp. GB Kim* and BH Lee, *Dept. of Food Sci. & Agri. Chemistry, McGill University*.

Previous research has indicated that bifidobacteria possess higher bile salt hydrolase (BSH) activity than other probiotics. To investigate the diversity of bile salt hydrolase activity and to understand the molecular organization, BSH activities from 30 strains (22 strains of human origin and 8 strains of animal origin) of bifidobacteria were screened using natural bile salts as well as a synthetic chromogenic substrate (a conjugate of cholic acid and 5-amino-2-nitro-benzoic acid). Among 30 strains tested, only two strains from honey bee hind gut (*Bifidobacterium aasteroides* ATCC 25910 and *B. coryneforme* ATCC 25911) did not show BSH activity. All positive strains contained constitutive intracellular BSH enzymes. From the profiles of native PAGE and BSH activity staining, two groups (group A and group C) of BSH enzyme were revealed. Most of bifidobacteria originated from ATCC was classified as group A, while many of commercial strains belong to group C. Group A and C showed different electrophoretic mobility and chromatographic profiles from anion exchange and hydrophobic interaction columns. This suggests that BSH enzymes from the same group have some similarities in their structure and amino acids composition. To investigate the biochemical characteristics of two enzymes, bile salt hydrolases were purified from *Bifidobacterium bifidum* ATCC 11863, *B. infantis* KL412, *B. longum* ATCC 15708, *B. longum* KL507, and *B. longum* KL515. The N-terminal amino acid sequences determined by Edman degradation were homologous to those of several lactobacilli as well as *Clostridium perfringens*. The native molecular weight of the enzyme in all five strains was estimated to be between 140 and 160 kDa and the subunit molecular weight determined as 35 kDa, indicating that the BSH enzyme is a tetramer. The isoelectric point (pI) determined by isoelectric focusing (IEF) was 4.4 and 4.6 for the BSH enzymes of group A and C, respectively. The relationship between the BSH types, bile tolerance and the molecular characteristics of group A and C enzymes is currently under investigation.

Key Words: Bile salt hydrolase, Bifidobacteria, Probiotics

62 Exopolysaccharide production by *Lb. rhamnosus* RW-9595M. D. Bergmaier*[1], C. Lacroix[1], and C.P. Champagne[2], [1]*Dairy Research Centre STELA,* [2]*Food Research and Development Centre, Agriculture and AgriFood Canada*.

Exopolysaccharides (EPS) synthesized by lactic acid bacteria (LAB) play an important role in the manufacturing of fermented dairy products. They contribute to the texture, mouthfeel, taste perception and stability of the final products. Furthermore, EPS could contribute to human health as prebiotics with positive effects on gut microflora. However the low production of EPS by LAB is a constraint for their commercial use as food additives. The immobilized cell technology (ICT) could be an attractive solution to enhance EPS production. The high biomass maintained in the reactor during repeated-batch or continuous culture could largely increase process productivity. In this study, bacterial growth and EPS production during batch and continuous cultures with *Lb. rhamnosus* RW-9595M, an efficient EPS producer, were compared to repeated-batch cultures with cells immobilized on solid supports (ImmobaSil®). Cultures were conducted at pH 6 in whey permeate medium (5% or 8% (w/w) WP) supplemented with 1% (w/w) yeast extract, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.05 g/L $MnSO_4 \cdot H_2O$ and 1 ml/L Tween-80. For free cell batch cultures in 8% WP medium, maximum cell counts ($1.4 \cdot 10^{10}$ CFU/ml) and EPS production (2374 mg/L) were measured after 20 and 32 h, respectively. This is one of the highest EPS productions reported in the literature for lactobacilli. For continuous cultures in 8% WP, maximum EPS production (1808 mg/L) and volumetric productivity (542.6 mg/L·h) were obtained for a low dilution rate of 0.3 h^{-1}. High immobilized biomass ($2.6 \cdot 10^{11}$ CFU/ml support) and EPS concentrations (1800 mg/L) were measured during repeated immobilized cell cultures for incubation periods of 8 h in 5% WP. The high biomass in the system increased EPS volumetric productivity (225 mg/L·h) compared to free cell batch cultures, even though this fermentation was limited by the low carbon source concentration. Our study clearly shows the high potential of *Lb. rhamnosus* RW-9595M and ICT for production of EPS as functional and nutraceutical food ingredients.

Key Words: *Lactobacillus rhamnosus*, exopolysaccharides, immobilization

63 The effect of lactic acid bacteria and bifidobacteria on interleukin-6 and interleukin-8 production by Caco-2 cells. C. Wong*[1], J.J. Pestka[1], and Z. Ustunol[1], [1]*Department of Food Science and Human Nutrition, Michigan State University*.

Probiotics and the milk products produced using these microorganisms have been reported to stimulate both non-specific and specific immune responses. However, the number of studies using human cell lines has been limited in the past. The objective of this study was to examine the interleukin (IL)-6 and IL-8 production by Caco-2 cells stimulated with *Lactobacillus acidophilus* LA2, *Lactobacillus bulgaricus* NCK 231, *Lactobacillus casei* ATCC 39539, *Lactobacillus reuteri* ATCC 23272, *Streptococcus thermophilus* St 133, *Bifidobacterium* Bf-6 or *Bifidobacterium adolescentis* M101-4. Caco-2 cells resemble normal human intestinal epithelial cells, and thus were chosen for this study. Lactic acid bacteria or bifidobacteria were added to 10% non-fat dry milk (NFDM) at concentrations of 10^6, 10^7, and 10^8 cfu/ml. Bacteria samples were either heat killed (95°C, 30 min) immediately after preparation or after fermentation (37°C, 4 hr). Cell numbers increased one log after fermentation. Bacterial samples were incubated with a monolayer of Caco-2 cells for 24 h. Uninoculated NFDM was used as the control. Cytokine, IL-1β, was used as the positive control for both IL-6 and IL-8 stimulation by Caco-2 cells. Supernatants of all treatments were collected and frozen at -80°C until assayed for IL-6, and IL-8 using ELISA. The complex effects of NFDM, probiotic cultures, probiotic dose, fermentation and their various interactions on the levels of IL-6 and IL-8 production will be presented. Since IL-6 and IL-8 are secreted by cells involved in inflammatory responses, their stimulation may or may not be desirable depending on the immune status of the individual.

Key Words: Lactic acid bacteria, Cytokine, Milk

64 Use of restriction fragment length polymorphism to isolate *Lactococcus lactis* strains producing novel EPS. Helene Deveau* and Sylvain Moineau, *Universite Laval*.

Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate strains from one another. Recently, van Kranenburg et al. (J. Bacteriol., 1999, 181:6347-6353) proposed a classification scheme to differentiate EPS-producing lactococcal strains based on RFLP of the *eps* operon. This grouping was based on the size of two *Sst*I fragments that hybridized with the *eps*B and *eps*D probe. Using this system, we were unable to classify three of the seven lactococcal eps^+ strains available in our laboratory. This dilemma is likely due to the presence of a *Sst*I site outside the *eps* gene cluster, which is responsible for the variable size of the hybridizing fragments. Thus, the hybridization signals are reflecting the variability of the nucleotide sequence upstream of the operon rather than the variability of the operon itself. These findings led us to the modification of the classification procedure, where two endonucleases (*Acy*I and *Hin*dII) and one probe (*eps*D) were used to classify the seven eps^+ strains. The strains *L. lactis* MLT3, SMQ-419 and SMQ-575 belong to group I, H414 to group II, MLT2 and SMQ-420 to group III. The strain SMQ-461 belongs to a fourth and novel group and the EPS produced by this strain is currently under investigation. The availability of such rapid cataloging system is likely to benefit research aimed at identifying lactococcal strains that produce novel EPS.

Key Words: Exopolysaccharides (EPS), *Lactococcus lactis*, Classification

65 The detection of *Bacillus* endospores during low heat skim milk powder processing using nucleic acid technology. Amy Rife*, Dr. Rafael Jimenez-Flores, Dr. Chris Kitts, and Dr. Mark Kubinski, *California Polytechnic State University*.

Detection of endospores in milk powder could be obtained by direct PCR tests plus terminal restriction fragment patterns (TRFP) based on amplification of the 16s rDNA gene for bacterial community analysis. In the DPTC endospore library, we have detected five specific endospores that contribute to the lipolysis, casein hydrolysis, starch hydrolysis, and acid production in milk. Optimal quality powder has to be free of specific detrimental endospores. The objectives of this work were to evaluate TRFPs for the efficient detection of the five detrimental Bacillus endospores during a low heat skim milk powder processing run using a pilot plant model, to perform an ecological study of the microorganisms during the processing run, and to evaluate endospore specific gene amplification using PCR as a detection method. In addition to being used as a detection system, TRFP allows knowledge of the composition of communities and the dynamics of individual populations within that community. By using standard peaks and the Ribosomal Database Project (RDP), we are able to identify the specific microorganisms present during the powder processing stages. Our results indicate that TRFPs have proven to be a sensitive endospore detection method during low heat milk powder processing when endospores are present in the range of 103 to 105 CFU/g of milk. TRFPs also prove to be effective in classifying specific microorganisms present throughout each processing step as well as observing community transformations occurring during normal milk powder production in a pilot plant. This can lead to microbial ecology studies dealing with contamination parameters during powder production at an industrial level. TRFP analysis allowed to observe how adding a high concentration of endospores altered the microbial community and the interactions that took place within each sample. Using this technique in place of current ecological study methods, culturing biases such as temperature, nutrients and oxygen concentration are not encountered. In addition, using PCR with a selected gene found only in endospores, we are able to specifically detect those endospore-formers associated with milk powder production.

Key Words: Endospore, Milk Powder, Terminal Restriction Fragment Pattern

66 Comparison of three media used to estimate psychrotrophic bacteria in milk. A.A. Glueck-Chaloupka* and C.H. White, *Mississippi State University, Mississippi State, Ms./USA*.

This study was designed to evaluate the ability of violet red bile agar without an overlay (VRBA/WO) to accurately and rapidly enumerate psychrotrophs in milk. A survey of 36 commercially produced reduced-fat milk samples was conducted. Samples were plated and evaluated the day they were collected from the retail outlet and the day following a preliminary incubation (PI) of 18h at 21C. Psychrotrophic bacteria counts were determined at 32C and 21C on violet red bile agar without an overlay, standard plate count agar, and crystal violet tetrazolium agar. Bacterial enumerations were compared among the three media and two temperatures. A correlation (r^2=0.73) was noted between violet red bile agar counts without an overlay and standard plate counts for samples incubated prior to testing. In addition, a strong correlation (r^2=0.87) was found between the violet red bile agar counts without an overlay and crystal violet tetrazolium counts incubated prior to testing. Violet red bile agar without an overlay (VRBA/WO) is a viable alternative method when enumerating psychrotrophic bacteria in fluid milk.

Key Words: Psychrotroph, Milk quality, Pasteurized milk

67 Sensory and instrumental measurements of the sensory properties of powdered buttermilk. M. Spill*[1], J.-X. Guinard[1], and R. Jimenez-Flores[2], [1]*Department of Food Science and Technology, University of California, Davis,* [2]*Dairy Products Technology Center, California Polytechnic University, San Luis Obispo*.

Buttermilk is the by-product of butter production. Over 40% of buttermilk is lost each year due to deterioration of quality, particularly sensory quality, yet sensory properties of buttermilk are not fully understood. To improve the knowledge of the sensory properties of buttermilk a descriptive language was developed. A panel of 10 judges developed a lexicon of sensory properties of reconstituted powdered buttermilk using industry samples. Thirty-three descriptors were defined including, for appearance: yellow and blue; for aroma: wheat, rice, grain, goat's milk, wet dog, cooked milk, powdered milk, cardboard, soy, butter, mac-n-cheese, white cheese, caramel, and egg; flavor attributes include: wheat, rice, grain, goat's milk, milk sugar, cooked milk, powdered milk, cardboard, soy, butter, mac-n-cheese, white cheese, caramel, and egg; and textural attributes include: thickness, mouthcoating, and astringency. References for each attribute were used to train panelists on these terms. This lexicon was validated by carrying out evaluations of 20 industry buttermilk samples. Sensory profiles were developed for the buttermilk samples using descriptive analysis techniques. Oxidation of the samples was examined using gas chromatography headspace analysis of hexanal after one and two weeks of storage at 30C. Susceptibility to oxidation and sensory attributes were compared. Preliminary work showed a positive correlation between susceptibility to oxidation and the following attributes: rice aroma, goat's milk aroma, wet dog aroma, egg aroma, egg flavor, grain flavor, soy flavor, and astringent/drying texture. A negative correlation was found between susceptibility to oxidation and caramel aroma and flavor. Having a standard sensory language for buttermilk will assist with training of sensory panels and communication between different industry and research groups.

Key Words: Buttermilk, Sensory, Descriptive analysis

68 Presence of an active phosphoenolpyruvate: glucose/mannose phosphotransferase system in *Streptococcus thermophilus* ATCC 19258. Armelle Cochu*, Christian Vadeboncoeur, Sylvain Moineau, and Michel Frenette, *Groupe de Recherche en Ecologie Buccale, Universite Laval, Quebec, Canada*.

Streptococcus thermophilus, which belongs to the lactic acid bacteria (LAB) family, is widely used in the dairy industry to produce fermented products. In most LAB, including streptococci, glucose is transported within the cell via the phosphoenolpyruvate:sugar phosphotransferase system (PTS). However, previous studies failed to detect any glucose-PTS activity in *S. thermophilus*. This is surprising considering that in *Streptococcus salivarius*, a species phylogenetically closely related to *S. thermophilus*, glucose is transported by the glucose/mannose-PTS and that a specific component for this PTS has been detected in *S. thermophilus*. We undertook this study with the goal of isolating and characterizing genes involved in the transport of glucose by the PTS in *S.*

thermophilus. The *ptsH* and *ptsI* genes, which code respectively for the PTS general proteins HPr and Enzyme I (EI), form the *pts* operon. An analysis of the amino acid sequence of these proteins in *S. thermophilus* showed that they shared a high level of identity with orthologues in other bacteria. Interestingly, unlike HPrs from other Gram-positive bacteria, the *S. thermophilus* HPr possessed a proline residue at position 68 that might interfere with HPr functions. Analysis of the transcription of the *pts* operon by Northern blot revealed the presence of a 0.6 kb transcript specific for *ptsH* and a 2.3 kb transcript covering the *ptsH* and *ptsI* genes. The *man* operon was composed of the *manL*, *M*, *N*, and *O* genes that coded for the $IIAB^{Man}$, IIC^{Man}, IID^{Man}, and ManO proteins respectively. The first three proteins shared significant levels of identity with members of the mannose-PTS family, while no function could be assigned to ManO. Quantification of the 3.5 kb transcript of the *man* operon showed that levels increased by 30% when cells were grown in the presence of glucose rather than lactose. PTS assays using purified recombinant *S. thermophilus* HPr, EI, and $IIAB^{Man}$ proteins and *S. thermophilus* membranes as sources of IIC^{Man} and IID^{Man} showed that glucose, 2-deoxyglucose, mannose, and fructose were phosphorylated by the glucose/mannose PTS. Our results indicated the presence of an active glucose/mannose-PTS in *S. thermophilus*.

Key Words: Lactic Acid Bacteria, Glucose transport, Gene transcription

69 Properties and substrate selectivities of esterases from *Lactobacillus casei* LILA, *Lactobacillus helveticus* CNRZ32, and *Lactococcus lactis* MG1363.

K.M. Fenster*, K.L. Parkin, and J.L. Steele, *University of Wisconsin-Madison, Madison, WI*.

Two esterases genes (*estB* and *estC*) were identified from a genomic library of *Lactobacillus casei* LILA. The *estB* and *estC* genes had open reading frames of 954-bp and 777-bp which could encode putative peptides of 35.7 kDa and 28.9 kDa, respectively. Recombinant EstB and EstC fusion proteins containing C-terminal six-histidine tags were constructed and purified to electrophoretic homogeneity using one step affinity chromatography. For comparison purposes, recombinant EstA (*Lactobacillus helveticus* CNRZ32) and tributyrin esterase (*Lactococcus lactis* MG1363) fusion proteins containing C-terminal and N-terminal six-histidine tags, respectively, were constructed and purified to electrophoretic homogeneity. Gel filtration of EstB and EstC suggest that they are hexameric and dimeric enzymes with native molecular masses of 219 kDa and 63.0 kDa, respectively. Characterization of EstB and EstC with various active-site inhibitors revealed that they are serine-dependent enzymes. Optimum temperature, NaCl concentration, and pH for EstB and EstC activities were determined to be 55 °C and 35°C, 15% and 1%, and 6.5-7.0 and 5.5-6.0, respectively. EstB and EstC had significant activity under conditions simulating those of ripening cheese (10°C, 4% NaCl, pH 5.1). Michaelis-Menten values (K_m and V_{Max}) were obtained for EstA, EstB, EstC, and the tributyrin esterase for a variety of ethyl esters and ester compounds consisting of substituted phenyl alcohol and short-chain fatty acids.

Key Words: Esterase, Substrate selectivity, Ethyl ester

70 Construction and evaluation of food-grade vectors for *Lactococcus lactis* using aspartate aminotransferase and α-galactosidase as selectable markers.

V. R. Sridhar*, V. V. Smeianov, and J. L. Steele, *University of Wisconsin-Madison, Madison, WI*.

Advances in genetic engineering have opened potential avenues for improving starter cultures; provided, that the genetic modifications are food-grade. Two food-grade vectors, pSUW611 and pSUW711, were developed for application in *Lactococcus lactis*. The vector, pSUW611 (4.0 kb), consists of a lactococcal $Gram^+$ theta replicon derived from pJW563 of *Lc. lactis* subsp. *cremoris* W56, a multiple cloning site, and utilizes a lactococcal aspartate aminotransferase gene (*aspC*) as a selectable marker. Selection for pSUW611 transformants is based on complementation of the *aspC* mutation present in *Lc. lactis* JLS400; this strain cannot grow or significantly acidify milk unless supplemented with aspartic acid or asparagine. A milk based medium containing bromocresol purple was developed for selection of pSUW611 transformants. The entire pSUW611 was re-sequenced to exclude appearance of any *de novo* mutations. The vector was found to be stable during 100 generations of growth in non-selective rich medium (0.2% loss per generation). The second vector, pSUW711 (5.0 kb), was constructed by replacing *aspC* with α-galactosidase (*aglL*) gene of *Bifidobacterium longum* VMKB44 under control of the Usp45 promoter of *Lc. lactis* MG1363. Transformants carrying pSUW711 utilized α-galactoside-containing carbohydrate such as melibiose and produced blue colonies on a melibiose-based medium with X-α-gal. Unlike complementation markers, dominant markers based on atypical sugar fermentation, such as the *aglL* gene are not dependent on specific vector-host combinations. Research is ongoing to determine the host range of pSUW711, compare transformation efficiencies of pSUW611 and pSUW711, and to determine their usefulness by over-expressing a peptidase gene of interest. Development of such food-grade starter systems will provide industry with the necessary tools for genetic-modification of industrially relevant *Lc. lactis* strains.

Key Words: food-grade vector and selectable markers, aspartate aminotransferase, α-galactosidase

71 Electrostatic effects on the yield stress of whey protein isolate foams.

J. P. Davis* and E. A. Foegeding, *North Carolina State University, Raleigh, NC/U.S.A.*

The foaming properties of whey protein ingredients are important in many of their functional applications. Structurally robust foams are often desirable, therefore, the vane method was used to characterize the yield stress (τ) of whey protein isolate foams (10% w/v) at three different levels of pH (3.0, 5.0 and 7.0) while in the presence of either NaCl or $CaCl_2$ (0 to 400 mM). While electrostatic forces are known to significantly affect other functional properties of whey protein ingredients, the relationship between τ of whey protein isolate foams and electrostatic variables had not been investigated. In the absence of salt, τ was approximately 20% greater (50 to 60 Pa) at pH 5.0 or 7.0 as compared to the pH 3.0 controls. No concentration of either NaCl or $CaCl_2$ significantly affected τ at pH 3.0. However, at pH 7.0, increasing concentrations of up to 400 mM NaCl or $CaCl_2$ progressively increased τ, with equivalent concentrations of $CaCl_2$, as compared to NaCl, increasing τ to greater magnitudes. For example, at pH 7.0, the addition of 400 mM NaCl increased τ by approximately 70% as compared to the control, while the addition of 400 mM $CaCl_2$ increased τ almost 100%. This suggested that specific divalent cationic effects were important to the mechanisms responsible for generating τ. At pH 5.0, which is near the isolectric point (pI) of the major whey protein β-lactoglobulin, the addition of NaCl up to 400 mM did not significantly change τ, while higher concentrations of $CaCl_2$ (100 and 400 mM) slightly increased τ. Measurements of foam overrun as well as dynamic surface tension measurements of the diluted foaming solutions were included to aid the data interpretation. A previously described theoretical model predicted that τ would increase with increasing overrun and τ would decrease with decreasing surface tension; however, these relationships did not hold. Dynamic surface tension measurements supported the importance of specific divalent cation effects to τ at pH 7.0.

Key Words: yield stress, foam, whey protein

72 Characterization of interactions involved in the gelation of hydrolyzed whey proteins.

D. Doucet*[1], S.F. Gauthier[2], and E.A. Foegeding[1], [1]*North Carolina State University,* [2]*Universite Laval*.

Previous work (Doucet et al., 2001, J. Food Sci., 66(5): 711-715) has shown that gelation occurs during extensive hydrolysis (DH >18%) of whey protein isolate (WPI) with Alcalase 2.4L®. This phenomenon is unexpected and creates a hurdle for the industrial production of whey protein hydrolysate, where high protein concentration is required to reduce the cost of drying. The objective of this study was to investigate the gelation mechanism and the type of interactions involved in this gelation process.

The enzyme-induced gel product obtained after 5 h of hydrolysis was studied by turbidity measurements at different pH, ionic strength and in the presence of various dissociating reagents. Electrophoresis (native and SDS-PAGE) and chromatography (HPSEC) were used to determine the size of the peptides causing aggregation. The enzyme-induced gel was stable over a wide range of pH (2.5-8.0) and therefore shows some similarities with plastein reaction products. Addition of NaCl, which is able to break weak ionic bonding, did not lead to the dissociation of the aggregates and did not salted-out the peptides suggesting that electrostatic interactions are not the main forces involved in aggregation. Chaotropic reagents such as urea, guanidine-hydrochloride and

SDS were effective at different levels to solubilize aggregates indicating that hydrogen bonds and hydrophobic interactions are involved in the gel network. The decrease in turbidity of enzyme-induced gels when the dielectric constant of the solvent was decreased also showed the importance of hydrophobic interactions. Reducing reagents such as β-mercaptoethanol and DTT did not break aggregates and suggests that disulfide bonds do not play a major role in the aggregation process. Analysis by native-PAGE and SDS-PAGE also demonstrated that most of the aggregates could be disrupted. HPSEC results have determined that 80% of the peptides present were $<$ 2000 Da. Therefore, physical aggregation via hydrogen bonds and hydrophobic interactions, with ionic bonding playing a minor role are the most probable type of interactions involved the gelation process.

Key Words: Enzymatic hydrolysis, Gelation, Interactions

73 Process analysis of skim milk microfiltration for selective concentration of casein. M Singh*, G Solakni, and S.S.H. Rizvi, *Institute of Food Science, Cornell University, Ithaca NY 14853*.

Cheese making from concentrated cheese milk has been of interest to the food industry for well over the past two decades. As more and more cheese plants incorporate membrane processing in cheese manufacture to standardize cheese milk and increase total solids, the need to analyze this process becomes more critical. In this study we report the effects of concentration factor (CF), cross flow velocity (CFV), and uniform trans-membrane pressure (UTMP) on energy requirements for selectively concentrating skim milk upto 8 times using cross flow microfiltration (CFM). CFM (0.2μm) of pasteurized skim milk was carried out at 50°C at three CFVs and three UTMPs. Volumetric CF of 8x was achieved at each combination of CFV and TMP, permeate flux and longitudinal pressure drop were recorded at each CF, and power consumption was calculated. Power consumption increased upto 1398% with CF (23.6 to 353.5 kJ/kg at 8x). A transition from turbulent to laminar flow was observed at 6x and retentate behavior became shear thinning. Increase in CFV from 5.3 to 6.3 m/s at 8x increased flux and reduced power consumption by 25% (353.1 to 263.1 kJ/kg). Although, increasing the UTMP (68.9 to 137.9 kPa) enhanced the starting flux (51.1 to 62.2 $kgm^{-2}h^{-1}$) and lowered the corresponding power consumption by 18% (28.8 to 23.6 kJ/kg), above 6x, higher UTMP caused excessive fouling, lowered the flux from 14.0 to 8.0 $kgm^{-2}h^{-1}$ and the power consumption increased from 179.9 to 353.1 kJ/kg. Overall power consumption is always lower due to shorter CFM process when skim milk is microfiltered to 8x at higher CFV (6.3m/s) as compared to 5.3m/s. Overall power consumption increased only marginally when UTMP was increased (68.9 to 137.9kPa). However at higher UTMP and above 6x, the permeate flux dropped precipitously below the minimally acceptable rate, which limited the performance of the CFM system.

Key Words: Microfiltration, Skim milk, Process Analysis

Graduate Paper Competition
ADSA Production Division and ADSA Southern Branch

74 Effects high wheat bran rations and different sources of protein on the milk constituents and production. Moslem Bashtani*, Abbasali Naserian, and Reza Valizadeh, *Ferdowsi University Of Mashhad, Mashhad, khorasan, Iran*.

The effect of including an increased amount of wheat bran on performance of Holstein dairy cows was investigated using a change over design with four treatment periods. Eight multiparous Holstein cow weighting 56319 kg and average days in milk of 48.5 26 and mean milk production of 30 1.94 kg/day were adapted to the experimental rations for 14 days and then entered into a collection period of 7 days. The proportion of concentrate and roughages in the total mixed ration were 60% and 40%. The treatments were 1) 25% wheat bran supplemented with cottonseed meal 2,3,4) 40% wheat bran supplemented with cottonseed meal, fish meal and urea respectively. The animals were individually kept indoors and had free access to fresh water and salt blocks. The daily feed intake was recorded and milk yield, blood, feces, and rumen liquor were sampled on a regular basis for analysis. There were no treatment effects on average daily dry matter and nutrient intakes. Dry matter, organic matter and crude protein digestibility were significantly increased with 40% level of wheat bran while NDF and ADF digestibility were similar in the different treatments. The digestibility values, which were calculated by AIA marker, were similar to the in vivo results. Feces, urine and ruminal pH were not affected by the different treatments. Changing the level of wheat bran did not significantly increase rumen ammonia-N, but urine utilization led to significant increases in rumen ammonia-N contents. A significant increase in blood glucose was observed in the supplemented fish meal diet. Daily milk production, percentage and daily yield of protein, lactose, casein, NPN and SNF milk were not significantly affected by different diets. It appeared that the level of wheat bran in dairy diets can be increased up to 40% with any adverse effects.

Key Words: Dairy cows, Wheat bran, Milk production and composition

75 Effect of lauric acid on ruminal fermentation, nutrient digestibility and milk yield of dairy cows. K. L. Grandeen*, A. N. Hristov, and J. K. Ropp, *Department of Animal and Veterinary Science, University of Idaho, Moscow, ID 83844-2330*.

Medium-chain saturated fatty acids have been shown to inhibit ruminal protozoa and ammonia production in the rumen in vitro. A cross-over design trial with four ruminally and duodenally cannulated lactating dairy cows (268±30.5 DIM; 768±36.9 kg BW) was conducted to study the effect of lauric acid-Na (LA) on ruminal fermentation, nutrient digestibility, and milk yield and composition. Cows were fed (DM basis) a 46% concentrate (barley, corn, cottonseed, soybean meal):52% forage (alfalfa hay, triticale silage) diet, twice a day (0600 and 1800). The daily dose of LA (0, Control or 240 g/cow, LA) was divided into two equal portions and introduced directly into the rumen through the cannula before the two feedings. Cows were treated with LA for 14 days before sampling and rumens were inoculated with ruminal contents (20% on weight basis) from donor cows fed on the same diet on day 1 of each study period. Ruminal samples (28 in 5 days) were analyzed for fermentation variables and protozoal counts. Digestibility was determined using acid insoluble ash as a marker. LA had no effect ($P > 0.05$) on ruminal pH (6.0 and 6.1), ammonia (12.0 and 11.8 mM), and VFA concentration (128.0 and 121.4 mM) and composition (Control and LA, respectively). Compared to Control, protozoal counts were reduced ($P < 0.05$) by LA (11.14 *vs* 0.98 $\times$ 10^5/ml, respectively). Carboxymethylcellulase and xylanase activities of ruminal fluid were lowered (by 40 and 36%, respectively; $P < 0.05$) and amylase activity was not affected ($P > 0.05$) by LA compared to Control. DM intake and DM, OM, CP, NDF, and ADF digestibility were not different ($P > 0.05$) between the two treatments. Milk yield (28.8 and 29.6 kg/d), FCM yield, milk fat (3.43 and 3.38%) and protein (2.92 and 2.79%) concentrations and yields and milk urea N content (24.6 and 21.1 mg/dl; Control and LA, respectively) were not affected ($P > 0.05$) by treatment. In conclusion, compared to untreated Control, lauric acid introduced into the rumen daily at approximately 0.3% of the rumen weight reduced protozoal numbers and fibrolytic activities of ruminal fluid but had no other effects on ruminal fermentation, total tract digestion of nutrients, or milk yield and composition.

Key Words: Lauric acid, Protozoa, Dairy cows

76 Production and metabolic responses to dietary conjugated linoleic acid (CLA) and trans-octadecenoic acid isomers in periparturient Holstein cows. KT Selberg*, CR Staples, and L Badinga, *University of Florida, Gainesville, FL*.

Thirty-nine multiparous Holstein cows were utilized in a completely randomized design to examine the effects of feeding ruminally protected CLA and trans-octadecenoic acid isomers on animal productivity and metabolism during the transition to lactation. Dietary treatments were initiated approximately 28 days (D) prior to expected calving date and continued through D 49 postpartum (PP). Treatments consisted of 1) a basal TMR diet (CON), 2) basal diet + 150 g/d CLA mix (CLAM), and 3) basal diet + 150 g/d trans-octadecenoic acid mix (TRANS). The amounts of CLA and trans-octadecenoic acid mixes fed were adjusted to 225 g/d during the seven-week (wk) PP treatment period. Liver biopsies