from the bull's first proof, all major selection decisions are already made. Average number of sons per sire has been quite constant over time for North America (15 sons per sire), while Europe has slowly increased from 9-10 in the 1980s to 13-14 sons per sire in recent years. Selection intensity has been increasingly higher over time also in the dam of sons pathway. Genetic level of most recent bulls was highest for milk and protein yield in North America, and for fat yield in Oceania. Genetic progress in the last 5 years was strongest for milk in North America, for fat in Oceania, and for protein yield in Other countries. Fast development of Interbull evaluations has allowed a rapid evolution from local to global selection. Superior genetics expressed on local scales is widely available and the best bulls have been chosen as sires of sons independent of their origin, thus providing strong genetic progress, even in countries with small breeding programs.

Key Words: Dairy bulls, International evaluations, Breeding strategies

135 The effect of producer goals on sire selection. P. R. Tozer*[1] and J. R. Stokes[1], [1] *The Pennsylvania State University.*

Information from an on-line survey of dairy producers was used to determine how important producers perceived three different objectives in the breeding problem. The objectives were: maximizing expected net merit of the progeny; minimizing the expected progeny inbreeding coefficient; and minimizing semen expenditure. Producers were asked to rank the three objectives and then weight the importance of each objective relative to the others. This information was then used to determine weights to be used in a multiple-objective integer program designed to select individual mates for a herd of 76 Jersey cows with known genetic background and cow net merit. The results of the multiple-objective models show that rank and relative importance of producer objectives can affect the portfolio of sires selected. Producers whose primary objective was to maximize expected net merit had a range of average expected progeny net merit of $306 to $310, but the level of expected progeny inbreeding was from 6.99% to 10.45% and a semen cost per conception of $35 to $41. Similar results occurred for producers who selected minimizing progeny inbreeding or minimizing total expenditure on semen as their primary goal in their breeding programs. The results of this research suggest that producer information and goals have a substantial impact on the portfolio of sires selected by that producer to attain these goals.

Key Words: Sire selection, Producer objectives, Mathematical programming

136 Effect of fitting dominance effects on the prediction of genetic values for beef cattle. K. A. Donoghue*, J. K. Bertrand, and I. Misztal, *The University of Georgia, Athens, GA, USA.*

The purpose of this study was to examine differences in direct and maternal breeding values under dominance and additive models using field data. The data consisted of 253,072 and 382,895 adjusted weaning weight records from the American Charolais and American Gelbvieh populations, respectively. In these data sets 10% and 9% of all animals had at least one full-sib, with the largest full-sib family consisting of 16 and 31 animals in the Charolais and Gelbvieh populations, respectively. The dominance model fit to both data sets accounted for dominance covariances, and included contemporary group, animal additive, animal maternal, maternal permanent environment and parental dominance (one-quarter of dominance variance) effects. In addition, a regression coefficient for inbreeding percentage was included. A reduced model was also fit, in which the parental dominance effect was removed. The correlations between direct and maternal breeding values in the two models were >0.99. However, changes were observed for parents whose proportion of total progeny that were full-sibs was large. Direct breeding values under the reduced model (ignoring dominance effects) were inflated for sires and deflated for dams as the proportion of total progeny that were full-sibs increased. For Charolais sires with a large proportion of full-sib progeny (>50%), direct breeding values were 0.65 kg higher and maternal breeding values were 0.35 kg lower under the reduced model, while changes observed for Gelbvieh sires were very small. For dams with a large proportion of full-sib progeny (>50%), direct breeding values were 0.13 and 0.06 kg lower under the reduced model, whereas maternal breeding values were 0.07 and 0.13 kg higher under the reduced model, for Charolais and Gelbvieh dams, respectively. The largest changes in direct (and maternal) breeding values in both breeds were 6.85 (3.98) and 2.53 (1.24) kg for Charolais and Gelbvieh populations, respectively, and were observed for sires with no individual records and many full-sib progeny or relatives. Ignoring dominance effects has the greatest impact on animals with little individual information who rely heavily on pedigree records from large full-sib progeny groups.

Key Words: Dominance, Mating system, Beef cattle

Dairy Foods
Micro

137 Production of Exopolysaccharides from *Streptococcus thermophilus* strains in Batch, Continuous and Fed-batch culture. F. Vaningelgem*[1], T. Adriany[1], M. Zamfir[2], and L. De Vuyst, [1] *Vrije Universiteit Brussel (VUB), Brussels, Belgium,* [2] *Institute of Biology Bucharest (IBB), Bucharest, Romania.*

Compared to dextran-producing lactic acid bacteria (LAB) or Gram-negative exopolysaccharide (EPS) producers, the low EPS production by thermophilic LAB is a constraint for both its use as additives (*ex situ*) and the *in situ* production of EPS during yoghurt fermentation. Both approaches implicate the optimisation of physical and chemical factors to enhance bacterial growth and EPS production during fermentation. Secondly, technological factors, like the use of (semi)-continuous fermentations, need to be considered for a higher EPS production. Two high-molecular-mass (MM > 1000 kDa) EPS-producing *S. thermophilus* strains were used to optimise bacterial growth and EPS production during batch, continuous and fed-batch fermentations. Besides the production of high-molecular-mass EPS, also low-molecular-mass EPS material was found in the fermentation broth, possibly degradation products. Physical (pH, T) and chemical (nitrogen source) optimisation of the milk medium resulted in an eleven-fold increase in maximum EPS yields. The influence of combined energy sources on the kinetics of bacterial growth and EPS production was investigated using this optimised milk medium. The EPS yield was clearly dependent on the energy source. Whereas the monomer composition of the EPS was not dependent on the type of monomer used, there was evidence that the molecular mass might be influenced. During batch and continuous fermentations it was further observed that EPS production by *S. thermophilus* followed the bacterial growth. The carbon/nitrogen ratio influenced the biomass formation, the EPS production and the relative concentrations of high-molecular-mass and low-molecular-mass EPS. Fed-batch fermentations using two different feeding strategies (constant feeding and acidification-controlled feeding) did not result in higher maximum EPS concentrations compared to batch fermentations. However, it was shown that a high (constant) lactose concentration was necessary to yield high concentrations (1130 ± 226 mg polymer dry mass per litre) of high-molecular-mass EPS and to prevent their degradation upon prolonged fermentation. Fed-batch fermentations can in this respect be interesting, not only to obtain high concentrations of high-molecular-mass EPS but also to improve their stability.

Key Words: Exopolysaccharides, Thermophilic Lactic Acid Bacteria, Fermentation

138 Effect of milk salts on autolytic behavior of lactococcal starter and wild *Lactobacillus paracasei* strains under cheese conditions. M.A. Bjurlin, A. Au-Yeung, K. Bies, and K.M. Polzin*, *Land O'Lakes, Inc.*

The autolysis of starter and, potentially non-starter bacteria, during the ripening process of cheese results in the release of intracellular enzymes into the curd matrix, consequently leading to enhanced flavor development. Previous research defining the autolytic behavior of cheese bacteria has focused on the effect of pH and NaCl concentration by assaying autolysis in various NaCl-adjusted buffer systems. This does not take into account, however, the ionic component of the cheese moisture

phase. In this study 4 commercial lactococcal starter strains, including reference strain AM2, and 8 wild *Lactobacillus paracasei* strains were examined for their autolytic behavior in a cheese milk salts (CMS) buffer and a sodium acetate (NaAc) buffer system. Autolysis was monitored by a decrease in cell suspension turbidity as measured by absorbance at 600/650nm. Viability was determined by colony enumeration on M17 or MRS plates. Cell plasma membrane permeability was monitored by fluorescent microscopy using the LIVE/DEAD BacLight® Bacterial Viability kit. Lactococcal starter strains displayed similar autolytic, viability and cell membrane permeability trends in either NaAc or CMS buffer. Conversely, wild *Lb. paracasei* strains displayed significant differences in viability and permeability trends in NaAc and CMS buffer, while autolysis was unaffected. Both starter lactococcal and wild *Lb. paracasei* strains entered a viable but non-culturable (VBNC) state when suspended in either buffer. The majority of the cells of the 4 starter strains and 5 of the 8 *Lb. paracasei* were VBNC by the end of the 28 day study. Our results suggest that the ionic environment may have a greater effect on the autolytic behavior of NSLAB than on lactococcal starters, that the viable but non-culturable state may account for a significant proportion of the cell population during cheese ripening, and that strains can vary greatly in the relative proportion of cells in the different autolytic stages.

Key Words: Lactococcus lactis, Lactobacillus paracasei, Autolysis

139 Survival and antimicrobial effect of bifidobacteria and yoghurt bacteria during refrigerated storage of yoghurt made from lactose hydrolysed milk.

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The viability of yoghurt bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus* LX and *Streptococcus thermophilus* S3) and four strains of bifidobacteria (*Bifidobacterium bifidum* DSM 20456, *B. bifidum* DSM BB12, *B. longum*DSM 20097 and*B. infantis* DSM 20090) during 15 days of refrigerated storage was assessed in yoghurt made from lactose hydrolysed or unhydrolysed milk. A reduction in milk lactose of 42.3% was achieved using Maxilact, 20000 . For all trials, milk was inoculated at a level of 2% (v/v) with a bacterial mixture of *Bifidobacterium* spp. *:Str. thermophilus :L. delbrueckii* ssp. *bulgaricus*using a ratio of 2:1:1 (v/v/v). Lactose hydrolysis resulted in a significant ($P<0.05$) reduction in fermentation time taken by such starter mixtures to reach pH 4.5. The production of organic acids is significantly ($P<0.05$) higher in standard yoghurt and lactose hydrolused yoghurt compared to bifidobacteria containing yoghurt and unhydrolysed yoghurt, respectively. At one day storage, the viability of yoghurt bacteria and bifidobacteria was significantly ($P<0.05$) higher in lactose hydrolysed than in unhydrolysed yoghurt. However, the decay in the viable counts of bifidobacteria between 3 and 15 days of storage period was faster in hydrolysed compared to unhydrolysed samples. Among tested strains of bifidobacteria, *B. longum* exhibited the highest rate of viability in both types of yoghurt during storage. Yoghurt samples with added bifidobacteria showed considerably variable antimicrobial activities. The neutralised supernatants prepared from lactose hydrolysed yoghurt had higher inhibition ($P<0.05$) than unhydrolysed samples. Yoghurt samples with added *B. bifidum* 20456 exhibited an inhibitory effect against *E. coli, Staph. aureus, P. aeruginosa* and *B. subtilis*. Samples with added *B. bifidum*BB12 or*B. longum* showed antimicrobial activities against *Staph. aureus* and *B. subtilis*, while those with *B. infantis* were active only against *Staph. aureus*. However, no inhibitory effect was found against *B. cereus, S. typhimurium and C. albicans*.In conclusion, the bifidobacteria used in this study had dietetic criteria for fermented dairy products.

Key Words: Antimicrobial, Bifidobacteria, Yoghurt

140 Analysis of the early promoter P1 of *Streptococcus thermophilus* bacteriophage DT1.

Genevive Lamothe*, Celine Levesque, Denise Tremblay, Frederic Bissonnette, Armelle Cochu, Michel Frenette, and Sylvain Moineau, *Universite Laval*.

The lytic bacteriophage DT1, a member of the group with cohesive genome extremities (*cos*-type), was isolated from a mozzarella whey sample obtained from a Canadian dairy factory using the strain *Streptococcus thermophilus* SMQ-301. The genome of phage DT1 contains 34,820 bp and 46 ORFs of more than 40 codons. In silico analysis of the nucleotide sequence revealed the presence of a putative early promoter that possess the -35 and -10 consensus sequences. This promoter, called P1, is positioned upstream from the predicted start codon of orf29. Primer extension analysis located the transcriptional start point at 6 nucleotides downstream from the last nucleotide of the inferred -10 box. Five minutes after infection of SMQ-301 with phage DT1, a transcript of approximately 6 kb was detected by Northern blot using an orf36-specific probe. In order to determine its relative strength, P1 was cloned upstream of the promoter-less chloramphenicol acetyltransferase-encoding gene (cat) of plasmid pBV5030. Its activity was then compared with that of the strong constitutive promoter of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) operon of *S. thermophilus*. The activity of both promoters were measured by their ability to confer chloramphenicol resistance in *S. thermophilus* SMQ-301 and in *Lactococcus lactis* MG1363. The minimum inhibitory concentrations (MIC) of chloramphenicol require to prevent the growth of a standardized inoculum of *S. thermophilus* and of *L. lactis* harboring pFB1 (P1 promoter cloned into pBV5030) were determined at 60 g/mL and 20 g/mL, respectively. The MIC of chloramphenicol was slightly lower for *S. thermophilus* containing pGA1 (PTS promoter cloned into pBV5030) whereas it was almost double for *L. lactis* (pGA1). These results indicate that P1 is a strong constitutive promoter in these two lactic acid bacteria and that it may be used for the development of expression vectors.

Key Words: Bacteriophage, *Streptococcus thermophilus*, Promoter

141 Identification of the melibiose carrier in *Lactococcus lactis* subsp. *cremoris* MG1363.

I. Boucher*, C. Vadeboncoeur, and S. Moineau, *Universite Laval, Quebec, Canada*.

Sugar metabolism by lactic acid bacteria plays a key role in dairy fermentations. Production of organic acids from sugar metabolism is initiated by the transport of mono- and disaccharides across the cytoplamic membrane through substrate-specific transporters. In *Lactococcus lactis*, lactose is transported into the cell by the phosphoenolpyruvate:sugar phosphotransferase system (PTS) via a cascade of phosphotransfer proteins. Melibiose, a galactoside analogous to lactose, is not a common fermentation substrate for *L. lactis*. However, melibiose fermentation is a distinctive characteristic of the non-dairy lactococcal species *Lactococcus raffinolactis*. We previously showed that the *L. raffinolactis aga* gene encoding alpha-galactosidase activity can confer the ability to metabolize melibiose to various *L. lactis* strains. Here, we report that melibiose is transported in *L. lactis* MG1363 through GalA, the permease encoded in the galactose operon. Inactivation of the gene encoding GalA effectively resulted in a loss of the Mel-positive phenotype conferred by *aga*. This altered phenotype could be complemented and restored by transformation with a plasmid encoding *gal*A. Interestingly, the inactivation of *gal*A did not affect the capacity of the cells to produce acid from galactose indicating that galactose may enter the cell through an alternative route. Accordingly, GalA can be regarded as the melibiose transporter in *L. lactis* MG1363 and its exact role in the galactose fermentation phenotype remains to be elucidated.

Key Words: *Lactococcus lactis*, Food-grade cloning vector, Alpha-galactosidase

142 Phage ul36 gene expression in sensitive and resistant *Lactococcus lactis* hosts.

J. D. Bouchard* and S. Moineau, *Universite Laval*.

The lactococcal bacteriophage ul36 has been used repeatedly as a reference lytic phage of the P335 species in a number of studies on the characterization of antiphage systems such as abortive infection mechanisms (Abi). The complete genomic sequence of this phage was also recently determined in our laboratory. Here, we investigated the gene expression of this phage in infected $AbiK^-$ and $AbiK^+$ cells by Northern blot analysis. Using probes located within the putative early region, a large transcript of over 10 kb, as well as several shorter mRNAs were detected 5 minutes after the infection of sensitive host cells ($AbiK^-$). An abondant 3.8-kb transcript indicated that the origin of phage DNA replication may act as a terminator. Interestingly, this virulent phage possesses a complete lysogeny module that was transcribed 30 minutes after the beginning of the lytic cycle. The late expression of these genes could explain the absence of chromosome integration events in this host. Transcripts of the late region were first detected 30 minutes after the adsorption step. A large mRNA that covered this region and the lysis module was only expressed after 45 minutes. During the ul36 infection

of AbiK$^+$ cells, expression of the early genes was normal but no late transcript was detected at any time. Together with previous results on phage DNA replication and late protein production, these results confirm that AbiK rapidly blocks macromolecule synthesis during ul36 infection.

Key Words: Bacteriophage, Gene expression, *Lactococcus lactis*

143 Monitoring endospores and endospore-forming bacteria populations in commercial skim milk powder production plants. C. Murillo* and Rafael Jimenez-Flores, *California Polytechnic State University, San Luis Obispo, CA.*

The microflora of milk powder consists of a wide array of microorganisms of which special attention is given to *Bacillus* endospores. *Bacillus* endospores survive pasteurization and spray drying and inhabit the final powder product in the dormant state indefinitely. Once the powder is reconstituted, endospores may germinate, and through their enzymatic activity become detrimental to quality. The objectives of this study are to 1) enumerate mesophilic and thermophilic endospore populations during commercial, low-heat skim milk powder production, and 2) characterize the microbial ecology of this process using Terminal Restriction Fragment Patterns (TRFPs) in conjunction with the Ribosomal Data Base, and 3) compare the changes in bacteria populations during processing of low-heat, skim milk powder. Our approach is to observe these changes in commercial operations and to use the DPTC pilot plant as a model system. Fluid and powder skim milk samples were collected from two commercial milk powder facilities. Sampling points included the raw milk silo, separator, evaporator, and spray dryer. Microbial evaluation was normalized based on total solids. Every sample was evaluated for total aerobic plate count and mesophilic and thermophilic endospore counts. For TRFPs community DNA was extracted, amplified by PCR using 16s rDNA probes, and digested with *Hae*III and *Dpn*II. Endospore formers are predominant in condensed and powdered milk, and tent to increase in the powder with increasing processing time. In raw milk mesophilic and thermophilic endospores ranged from <25CFU/g to 70CFU/g and <25CFU/g to 10^2 CFU/g, respectively. In powder they ranged from <25CFU/g to 10^3 CFU/g and <25CFU/g to 10^5 CFU/g, respectively. Both endospore counts from skim milk showed an increasing trend with run time and rendered the powder out of the 10^3 CFU/g limit. In commercial samples TRF patterns successfully described microbial populations and a drastic change was observed between raw and powder milk for most runs.

Key Words: Endospore, Milk Powder, Production

144 Influence of lactococcal cell envelope proteinases on accelerated Cheddar cheese ripening. S. I. Myaka*, L. E. Metzger, and L. L. McKay, *MN-SD Dairy Food Research Center, University of Minnesota, St. Paul, MN.*

The proteolytic enzymes produced by lactococcal starters have an essential role in cheese ripening. Secondary proteolysis is initiated by the cell envelope proteinase (CEP) and cheese quality has been linked to particular CEPs. Additionally, Cheddar cheese ripening can be accelerated through the use of a "quick lysis" strain of *L. lactis*. Previously, thermolytic, isogenic strains of *L. lactis* subsp. lactis were constructed to possess different lactococcal CEPs. The objective of this research was to investigate the influence of lactococcal CEPs type a, c, d, e, and g from five isogenic, thermolytic strains on Cheddar cheese ripening. Three replicates of stirred curd Cheddar cheese were produced using these strains. Since the cultures undergo lysis during cheese manufacture, the cheeses were acidified with Glucono-delta-lactone after the cooking step. Additionally a control cheese was produced at each time with a commercial direct vat set starter culture using a conventional stirred-curd Cheddar cheese making procedure. There were no significant differences ($p \geq .05$) in cheese composition among the treatments and the mean moisture, fat on a dry basis, salt to moisture ratio, and pH ranged from 36.6 to 38.1%, 50.5 to 51.6%, 4.9 to 5.1%, and 5.2 to 5.3 respectively. Proteolysis was determined at 3 weeks, 2 months, and 4 months of ripening whereas descriptive sensory analysis (15 judges) was performed at 2 and 4 months of ripening. As expected the level of pH 4.6 soluble nitrogen, 70% ethanol soluble nitrogen, and free amino acids increased in all treatments during ripening and at 4 months the level of free amino acids was 1.68, 1.30, 1.89, 1.56, 1.20, and .97 mg Leu/g cheese respectively for type a, c, d, e, g, and control. There were no significant ($p \geq .05$) differences in overall flavor among the cheeses. However, bitterness was significantly higher ($p \leq .05$) in the control cheese. These results indicate that the level of free amino acids produced during ripening can be increased using a thermolytic culture, but the different CEPs investigated had no significant ($p \geq .05$) effect on overall cheese flavor after 4 months of ripening.

Key Words: Accelerated ripening, Cell envelope proteinase

145 Exopolysaccharides from lactic acid bacteria: microbial physiology and fermentation kinetics. L DE VUYST*[1] and F VANINGELGEM[1], [1]*Vrije Universiteit Brussel.*

Exopolysaccharides (EPS) from lactic acid bacteria (LAB) can be subdivided into two groups, namely homopolysaccharides (HoPS) and heteropolysaccharides (HePS). Recently, HePS receive renewed interest, since they play an important role in the rheology, texture, and mouthfeel of fermented milk drinks. For instance, the creamy, smooth texture is one of the aspects of the quality of yoghurt that seems to be improved by the ability of the yoghurt bacteria to produce HePS, even though only small amounts of HePS are being synthesized in milk. Many different types of HePS are secreted by LAB strains with respect to sugar composition and molecular size. HoPS are polymerised extracellularly from sucrose as donor molecule and supplier of energy. HePS are made by the polymerization of repeating unit precursors formed in the cytoplasm. The repeating units are assembled at the membrane by specific glycosyltransferases (GTF) through the sequential addition of activated sugars (sugar nucleotides), followed by export and polymerisation into a final HePS. Several enzymes and/or proteins are involved in the biosynthesis and secretion of HePS; some of them are unique to HePS formation. Glucose-1-phosphate and fructose-6-phospate are the precursor molecules for HePS biosynthesis. A major difference between cells and strains grown on different carbohydrate sources is the capacity to synthesize sugar nucleotides. Instability of HePS production and variability of polymer yields are well-documented problems in the dairy industry. Therefore, a well-understood optimal carbon flux and supply of sugar nucleotides in stable, EPS-producing, industrial strains is a key issue for their economical exploitation. Whereas mesophilic LAB strains seem to produce maximum amounts of HePS under conditions not optimal for growth, for instance low temperatures, HePS production from thermophilic LAB strains appears to be growth-associated,*i.e.* maximum production during growth and under conditions optimal for growth. HePS degradation often takes place upon prolonged incubation of HePS-producing LAB strains due to glycohydrolase activity.

Key Words: Eps, Lactic acid bacteria, Physiology

Nonruminant Nutrition
Nutritional Values of Phytase and Other Enzymes

146 Impacts of site-directed mutations and expression systems on efficacy of *Escherichia coli* phytases in diets for weanling pigs. C. H. Stahl*, J. M. Gentile, T. W. Kim, K. R. Roneker, and X. G. Lei, *Cornell University.*

Our laboratory has previously expressed an *E. coli* phytase (ECAP) and its two variants (Mutant U and AppA2) in *Pichia pastoris* using an inducible promoter. To reduce the fermentation cost, we expressed AppA2 in a constitutive expression system and characterized the biochemical properties of the produced enzyme. The objective of this study was to compare the efficacy of these three enzymes produced from the two systems in a corn-soybean meal diet for young pigs. Thirty pigs (9.45 ± .95 kg BW) were fed the diet plus ECAP, Mutant U, and AppA2 produced by the inducible system, AppA2 produced by the constitutive system, or .16% inorganic P. All phytases were included at 500 U/kg diet. Growth performance, and plasma inorganic P concentrations were assessed weekly. At wk 1, pigs fed inorganic P had higher ($P < .05$) plasma inorganic P levels than all other groups, with the exception of the group receiving mutant U phytase. This difference disappeared by wk 2. There were no significant differences in overall ADG, ADFI, or gain:feed