29 Genetic parameters of latent variables related to main traits of lactation curve shape. N. P. P. Macciotta*[1], D. Vicario[2], and A. Cappio-Borlino[1], [1]*Università di Sassari*, *Sassari, Italia*, [2]*Italian Association of Simmental Cow Breeders*, *Udine, Italia*.

The genetic analysis of multivariate phenotypes has to cope with computational issues essentially due to the large number of parameters to be estimated. In recent years, dimension-reduction techniques have been proposed mainly based on principal component analysis. However, this multivariate technique does not always allow a simple and meaningful interpretation of the leading new variables. A different technique to extract latent variables able to reconstruct the (co)variance matrix of original data is the multivariate factor analysis, where extracted factors can be rotated to make their interpretation easier. In this work the factor analysis was used to extract two latent variables from Test Day milk yields, taken at different days in milk, related to peak yield and lactation persistency. A data set of 48374 lactations of 21721 Italian Simmental cows was used. Edits were on the number of Test Day records for cow (8), parity (<7), days in milk at first test (<30), lactation length (>200), n. cows per herd (>5). TD milk yields were arranged in a multivariate setting, latent factors were extracted. Factor scores were then used as new variables and analysed by a bivariate animal model that included the fixed effects of the herd, parity, year and calving season, and the random effects of the genetic additive value of the animal and of the permanent environment. Estimates of heritability are moderately low, (0.14 both for persistency and peak yield) confirming the results reported from other authors. Repeatabilities show values of 0.27 for peak and 0.29 for persistency. Particularly interesting is the moderate genetic correlation among these two variables (0.26) suggesting the possibility to select, at least to a certain extent, separately on different aspects of lactation curve shape.

Key Words: Lactation curve shape, Persistency of lactation, Latent factors

30 Simultaneous estimation of environmental values and genetic parameters in reaction norm model. G. Su*, P. Madsen, M. S. Lund, D. Sorensen, I. R. Korsgaard, and J Jensen, *Danish Institute of Agricultural Sciences, Department of Genetics and Biotechnology*, *Tjele, Denmark*.

The reaction norm model is becoming a popular approach for the analysis of G x E interactions. In a classical reaction norm model, the expression of a genotype in different environments is described as a linear function (a reaction norm) of an environmental gradient, such as overall effects of various environments (environmental values). An environmental value could be defined as the mean performance of all genotypes in a particular environment, which is typically unknown. One approximation is to estimate the mean performance based on data then treat this as a known covariate in the model (referred to as the approximate method below). However, a more satisfactory alternative is to infer the environmental values simultaneously with the other parameters of the model. The objectives of this study were (1) to describe a method and its Bayesian MCMC implementation that makes this possible and (2) to confirm the superiority of the present method relative to the approximate method by a simulation study. In the simulation study, data were generated using simulated herd-year effects as covariates of the reaction norm. The correlation between the estimates of herd-year effects from the present method and the true values was close to one. The genetic parameters inferred from the present method were similar to those estimated from a reaction norm model using true values of herd-year effects. On the other hand, using phenotypic mean of herd-year as a proxy for the environmental value resulted in biased estimates of genetic parameters.

Key Words: Reaction Norm, G x E Interaction, Genetic Parameters

Dairy Foods: Dairy Chemistry

31 Influence of lipolysis and proteolysis of calibration milks on infrared milk analyzer performance. K. E. Kaylegian* and D. M. Barbano, *Cornell University*, *Ithaca, NY*.

Lipolysis was measured weekly as the increase in free fatty acid (FFA, meq/kg milk) content and proteolysis was measured weekly as the decrease in casein as a percentage of true protein (CN%TP). Pasteurized, potassium dichromate preserved modified milk (MM) calibration sets had a shelf life of 4 wk and raw preserved producer milk (PM) calibration sets had a shelf life of 2 wk. Every day that the chemical analyses for lipolysis and proteolysis were performed, MM and PM calibration sets were used separately to calibrate a fixed-filter mid-infrared (MIR) analyzer. The experiment was replicated 3 times. The MM sets had a smaller ($P < 0.01$) mean and change in lipolysis over the first 2 wk of use than the PM sets. The mean FFA levels were 0.115 and 0.253 meq/kg milk for the MM and PM sets, respectively. The mean decrease in CN%TP was larger ($P < 0.01$) for the MM sets (1.96%) at the end of the first 2 wk than for the PM sets (1.54%). The mean level of proteolysis observed for the MM sets at the end of the 4 wk set life was a 4.06% decrease in CN%TP, and no effects on the calibration slope and intercept were observed . The highest FFA level observed was 0.589 meq/kg milk for an individual PM sample at the end of the 2 wk set life. Individual PM samples with high FFA levels did show a larger difference between IR predicted value and chemistry for those samples, as expected, but these did not affect the protein slope and intercept because they were in the middle of the protein concentration range of the calibration set. No significant differences were observed in the MIR calibration slope and intercept that were attributed to lipolysis or proteolysis for either type of calibration set.

Key Words: IR Milk analyzer Calibration, Lipolysis, Proteolysis

32 Comparing a gas chromatography/mass spectrometry technique with sensory evaluation in relation to the acceptability of fluid milk. A. A. Glueck-Chaloupka*[1], C. H. White[2], and W. E. Holmes[3], [1]*The Kroger Company*, *Cincinnati, OH*, [2]*Mississippi Agricultural & Forestry Experiment Station*, *Mississippi State, MS*, [3]*Mississippi State Chemical Lab*, *Mississippi State, MS*.

A gas chromatography/mass spectrometry (GC/MS) technique was evaluated for ability to detect changes in volatile compounds in reduced-fat milk over time. Pasteurized reduced-fat fluid milk samples were collected from 10 filler heads on a fluid milk packaging machine from one dairy plant (n=150). Expert sensory panelists evaluated samples for acceptability using the ADSA scorecard and a quality scale. Ten volatile compounds known to have an impact on the flavor and flavor intensities of reduced-fat fluid milk over the shelf life of the product were monitored with a GC/MS technique. The GC/MS technique was able to provide identification and quantification of compounds. It provides a qualitative and quantitative means of identifying metabolites present in both "good" and "bad" quality milk. Both sensory and GC/MS evaluations occurred on days 0, 1, 7, 11 and 15 at 7°C. Sensory data were subjected to analysis with Statistical Analysis Software (SAS) version 8.0. Response factors were determined by dividing the concentrations by the area counts and reported for the volatile compounds. By day 7 expert panelists were able to detect overall flavor intensity differences ($P<0.05$). Overall flavor scores and occurrence of "pleasant" flavor attributes decreased over shelf life. Concentrations on day 0 versus day 15 of ethanol (2.11ng, 2.50ng), ethyl butyrate (0.00ng, 0.01ng), ethyl hexanoate (0.01ng, 0.22ng), 3-methyl-1-butanol (0.00ng, 0.01ng), 2-nonanone (0.02ng, 0.12ng), and 2-heptanone (0.002ng, 0.014ng) increased as the milk aged. A similar trend over the 15 day evaluation period for both testing methods was shown, indicating that as the milk ages, concentration of volatile compounds increase, and overall flavor scores and occurrence of "pleasant" flavor attributes decrease. Therefore, GC/MS, when combined with sensory evaluation, shows promise in understanding flavor deterioration in reduced-fat milk.

Key Words: Reduced-fat milk, GC/MS, Flavor

33 Novel technique for the differentiation of caseins and whey proteins in confocal scanning laser microscopy. A. Dubert-Ferrandon*, A. Grandison, and K. Niranjan, *The University of Reading*, *Whiteknights, Reading, UK*.

This study aimed to understand the acid gelation process of milk following different heat treatments in Glucono-Delta-Lactone systems. A method was developed to allow separate observation of the incorporation of whey proteins and caseins into the gel structure. By combining a labelling technique with a purification step, the 2 proteins were tagged with different fluorescing dyes (Protein labelling kits Alexa Fluor 488 and 594, Invitrogen). Caseins (labelled in red) and whey proteins (in green) could thus be studied during gel formation on a real-time scale. Skim milk was subjected to 2 heat treatments: standard pasteurisation (72°C-15 seconds); and a heat treatment of 90°C-2 minutes. A series of images of exactly the same field was collected during gelation at 40°C using both fluorescent probes (allowing acquisition of gelation movies and snapshots). The confocal microscope used was an inverted motorised microscope (Leica DMIRE 2, Milton Keynes, UK) used with the Leica TCS SP2 AOBS computer system. A × 63 glycerol objective was used.

In the standard pasteurised milk, after addition of GDL, a green background colour was observed for up to 20 minutes, but no structure. From 20 to 40 minutes, the appearance of casein (red) aggregates was clearly observed and gaps (seen as a green background colour) were filled with whey proteins. As pH decreased, the gel network, which was coarse and made up of aggregates, became greener in appearance, implying that the whey proteins had coated the casein network subsequent to its formation.

At the higher heat treatment, after addition of GDL, formation of gel structure occurred much more rapidly (8 minutes). The appearance of the structure was quite different, being much less coarse, and the colour indicated that it consisted of both casein and whey protein. As pH decreased, the gel network became more and more finely structured, but the colour did not change, implying that the composition of casein and whey protein did not change.

It is apparent that the mechanism of gel formation is quite different for the 2 heat treatments. These structural observations will be linked to rheological studies in real time.

34 Effect of heat and homogenization pressure on activity of xanthine oxidase isolated from buttermilk. C. van den Berg and D. Everett*, *University of Otago*, *Dunedin, New Zealand*.

The effect of heat and homogenization pressure on activity of xanthine oxidase (XO), isolated by one of four methods, was examined: (1) precipitation from unpasteurized buttermilk with $(NH_4)_2SO_4$ (Ball, J. Biol. Chem. 128:51-67, 1939), (2) a two-step $(NH_4)_2SO_4$ precipitation (33% and 66% saturation) from unpasteurized buttermilk with batch-scale anion exchange chromatography (Spitsberg and Gorewit, Protein Expression and Purification 13:229-234, 1998), (3) a two-step adsorption from acid whey using charcoal and kaolin (Dixon and Kodama, Biochem J. 20:1104-1110, 1936), and (4) a modification of method 2 with with 35% and 50% saturated $(NH_4)_2SO_4$. XO preparations were dialyzed against deionized water using 10 kDa membranes, and freeze-dried. The protein-flavin (PF) absorbance ratio, A_{280}/A_{450}, was used to assess purity. XO from method 4 (22.7 mg/15 mL water) was heated to between 25°-75°C for 5 min, and at 70°C for 30 min. Activity was measured by monitoring conversion of xanthine to uric acid at 295 nm, and expressed as µmol uric acid per min per weight of dry matter (U/mg). XO (50.1 mg/50 mL pyrophosphate buffer, pH 8) was passed 40× through a Microfluidizer™ homogenizer at 38, 75, 100, 125 or 150 MPa and the activity measured. Turbidity of homogenized XO solutions was measured at 500 nm, and PAGE under reducing and non-reducing conditions. Methods 1 and 4 had PF-ratios of 7 and 8, close to maximum purity of 5, and activities of 5.5 and 4.5 U/mg respectively. Method 2 yielded low protein content and XO was not observed on the PAGE gel. Method 3 had a low purity of XO with a PF-ratio of 300 and activity of 0.06 U/mg. Molecular weight of XO was 148 kDa under reducing, and 290 kDa (dimer) under non-reducing conditions. Activity of XO in solution was 60% higher than in unpasteurized cream at 25°C. Activity dropped by 40% from 25°C to 70°C, and at 70°C dropped by 95% over 30 min. Turbidity of XO solutions decreased by 92% and activity decreased by 50% up to 150 MPa. Pressure had no discernable effect on the PAGE gel bands. Homogenization may reduce XO activity by a change in tertiary structure rather than reduction of the dimer.

Key Words: Xanthine Oxidase, Enzyme, Homogenization

35 Residues 69-74 of beta-lactoglobulin are responsible for a monoclonal antibody binding to thermal denatured lactoglobulin. C. Y. Song*, M. C. Yang, and S. J. T. Mao, *National Chiao Tung University*, *HsinChu, Taiwan*.

β-lactoglobulin (β-LG) is a bovine milk protein sensitive to thermal denaturation. Previously, we demonstrated that such structural change can be detected by a monoclonal antibody (mAb) specific to denatured β-LG. In the present study, we show a dramatic increase in β-LG immunoreactivity when heating raw milk between 70°C and 80°C. To map out the specific epitope of β-LG recognized by this mAb, we used a combined strategy including tryptic and CNBr fragments, chemical modifications (acetylation and carboxymethylation), peptide array containing in-situ synthesized peptides, and a synthetic soluble peptide for immunoassays. The antigenic determinant we defined was exactly located within the D strand (residues 66-76) of β-LG. The result suggests that a further disordered structure occurred in β-LG and thus rendered the mAb recognition. Mutations on each charged residue (three Lys and one Glu) revealed that Lys-69 and Glu-74 were extremely essential in maintaining the antigenic structure. Further deliniation on the antigenic site on the D strand shows the minimal residues of the epitope to the KKIIAE (residues 69-74) for the mAb recognition. In addition, we found an inverse relationship between the immunoreactivity in heated β-LG and its binding to vitamin D. Taken together, we concluded that strand D of β-LG participated in the thermal denaturation between the temperatures of 70°C and 80°C.

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Key Words: β-Lactoglobulin, Epitope Mapping, Thermal Denaturation

36 Properties of lactoperoxidase isolated from individual cow's milk by ion-exchange chromatography. A. Grandison*[1], F. Fonteh[2], and M. Lewis[1], [1]*The University of Reading*, *Reading, Berkshire, UK*, [2]*University of Dschang*, *Dschang, Cameroon*.

Lactoperoxidase (LP) was isolated from the milk of five individual cows and from a commercial powder, by ion exchange chromatography. The samples presented different elution patterns with varying peaks of LP activity, indicating that LP occurs in different forms. These could be isoenzymes, or the result of post-translational enzymic or chemical changes. The pH optima for the fractions varied between 5.0 and 6.5. SDS gel electrophoresis revealed three forms of the enzyme with molecular weights 92, 88 and 85 kDa respectively. The HPLC chromatographs of the eluents were also varied, presenting different peak shapes, heights, and double peaks in some samples.

These results show that LP exists as different chemical species with differing physicochemical properties. Such differences suggest that the different forms may also exert varying degrees of activity, depending on the prevailing environmental conditions. Therefore, the efficiency of the LP system will depend on the predominant enzymic form(s) in any batch of milk, and the prevailing environmental conditions.

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Key Words: Lactoperoxidase, Ion-Exchange, Isoenzymes

37 Evolution and regulation of the casein gene cluster region: A genomics approach. M. Rijnkels*[1], T. Le[1], and J. Thomas[2], [1]*Baylor College of Medicine*, *Houston, TX*, [2]*Emory University School of Medicine*, *Atlanta, GA*.

Multi-species sequence analysis and other genomics based approaches are being used in our studies of the evolution and regulation of milk protein genes.

Multi-species sequence analyses were performed on sequences from Bacterial Artificial Chromosome (BAC) clones isolated and sequenced for this purpose,

and from various genome-sequencing efforts. These sequences covered the casein gene cluster region or parts of it in 15 mammalian species: human, chimpanzee, macaque, marmoset, galago, mouse, rat, rabbit, shrew, bat, dog, cow, armadillo, elephant and opossum.

This and earlier studies indicated that the casein genes are located in a mammalian specific genomic domain. This domain contains besides the casein genes a number of non-casein genes that share evolutionary ancestry, spatial expression patterns (mammary and salivary gland), and functional properties (secreted (phospho)-proteins, involvement in mineral homeostasis, and immune modulation). The presence and structure of orthologous genes in the mammalian species studied was determined. Predicted transcripts were cloned from a number of species. Phylogenetic analyses showed that the divergence of the casein genes is not only due to a high rate of nucleotide substitutions but also to the differential use of exons. Genomic rearrangements were identified that result in deletions of genomic segments containing casein genes, e.g. lack of alpha-s2-like genes in shrew. Overall there is remarkable conservation in this region with regard to the genes present, gene structure, and gene order and orientation despite high divergence at the nucleotide level.

These studies also identified a number of non-coding conserved regions that might play a role in gene regulation. These included the upstream beta-casein enhancer, previously identified in human and cow and now shown to be present in most species studied albeit at different positions with respect to the beta-casein promoter. Computational analyses identified patterns of conservation in these regions and the proximal promoters that represent transcription-factor binding sites known to be involved in casein gene expression.

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Key Words: Milk Protein, Casein, Genomics

38 Distinguish between native and thermally denatured β-lactoglobulin using a monoclonal antibody as a probe. S. J. T. Mao*, W. L. Chen, M. C. Yang, and W. T. Liu, *National Chiao Tung University*, *Hsinchu, Taiwan*.

Previously, we have established a monoclonal antibody (mAb) line to study the thermal denaturation of β-lactoglobulin (LG) and have identified an epitope responsible for its biological functions (Chen et al., J Dairy Sci. 2004 87:2720-2729 and Song et al., J Biol Chem. 2004 Nov 9; [Epub ahead of print]). In the present report, we prepared a mAb that specific only to native LG, the immunoreactivity was totally abolished when LG was cross-linked to casein, lactalbumin, or other milk proteins upon the heating. Characterization of this native mAb shows that residue Cys-121 of LG was possibly involved for the antibody binding using carboxymethylated LG. Since heating is a necessary procedure in bovine milk processing, the loss of native LG is almost unavoidable. We than used this native mAb to develop a quantitative immunoassay to determine the residual LG in the commercially available milks. The result shows that the loss of native LG was from minor to sever levels. Because LG plays provocative roles in fatty acid, retinol, and vitamin D binding as well as in cell proliferation, determination of native LG in milk products becomes a subject of essence.

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Key Words: β-Lactoglobulin, Monoclonal Antibody, Thermal Denatureation

Dairy Foods: Extended Shelf Life of Fluid Milk

39 Influence of raw milk quality on fluid milk shelf life. D. M. Barbano*[1], Y. Ma[1], and M. V. Santos[2], [1]*Cornell University*, *Ithaca, NY*, [2]*Universidade de São Paulo*, *Pirassununga, SP, Brazil*.

Pasteurized fluid milk shelf life is influenced raw milk quality. The microbial and somatic cell count (SCC) of milk will determine the load of heat resistant microbial enzymes in milk. Generally, high levels of psychrotrophic bacteria in raw milk will contribute significant quantities of heat stable proteases and lipases that will break down protein and fat after pasteurization. Sanitation, refrigeration, and the addition of CO2 to milk are used to control of both total and psychrotrophic bacteria count. It is not uncommon for total bacteria counts of raw milk to be < 10,000 cfu/mL. In the past, fluid milk processors have not focused much attention on milk SCC. Increased SCC is correlated with increased amounts of heat stable protease (plasmin) and lipase (lipoprotein lipase) in milk that originates from the cow. When starting with raw milk that has low bacteria count, and in the absence of microbial growth in pasteurized milk, enzymes associated with high SCC will cause protein and fat degradation during refrigerated storage and produce off-flavors. As the ability to kill, remove, or control microbial growth in pasteurized refrigerated milk continues to improve, the original milk SCC will be the factor limiting the time of refrigerated storage before development of an off-flavor in milk. Most healthy cows in a dairy herd have a milk SCC < 50,000. Bulk tank SCC > 200,000 are usually due to the contribution of high SCC milk from a small number of cows in the herd. Technology to identify these cows and keep their milk out of the bulk tank could substantially increase the value of the remaining milk for use in fluid milk processing. To achieve a 60 to 90 day shelf life of refrigerated fluid milk, fluid processors and dairy farmers need to work together to structure economic incentives that allow farmers to produce milk with the somatic cell count needed for extended refrigerated shelf-life.

Key Words: Shelf Life, Raw Milk, Somatic Cell Count

40 Current status of commercial fluid milk quality. K. Boor*, N. Carey, S. Murphy, and R. Zadoks, *Cornell University*, *Ithaca, NY*.

Packaged fluid milk samples were collected from 23 dairy processing plants across New York State at least twice per year over a period of 10 years and subjected to shelf life analyses that included Standard Plate Count (SPC), coliform count and sensory evaluation. Products were tested initially and after storage at 6.1°C for 7, 10 and 14 days post-packaging. On an annual basis, the percent of samples that met the Pasteurized Milk Ordinance (PMO) standard of SPC < 20,000 CFU/ml after 7, 10 and 14 days ranged from 46% to 66%, 25% to 50% and 12% to 32%, respectively. Over the ten year period, SPC values across test-days: decreased in eight plants, including the four plants that had the lowest SPC scores among all 23 plants; increased in two plants; and did not change significantly in the remaining 13 plants. The percent of samples positive for coliforms in a given year ranged from 5% to 15% on initial testing and up to 34% after subsequent storage. The percent of samples scored as unacceptable from a sensory perspective (score < 6.0) after 7, 10 and 14 days ranged from 0% to 8%, 16% to 35%, and 41% to 67%, respectively. For the majority of plants, product flavor scores improved during this 10 year period. While some plants involved in the study can produce fluid milk products that are consumer acceptable when stored at 6.1°C for > 14 days, others consistently fall short of this goal.

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Key Words: Fluid Milk, Quality, Shelf Life