625 Effect of method and time of hydration on structure of dried milk proteins. B. S. Oommen*[1] and D. J. McMahon[1], [1]*Utah State University.*

Microstructure of caseins in non-fat dried milk, calcium caseinate, and sodium caseinate were studied using transmission electron microscopy. Solutions of all the dried products were made to a casein concentration of 2.4% and the pH of the solution adjusted to 6.7. The powders were hydrated at 40 °C, and allowed to stabilize for 4, 10, and 18 h. Another set of these powders were hydrated at high shear for 5 min using a hand held high speed blender and subsequently stabilized for 55 min with moderate mechanical stirring. These solutions were diluted 100 times and the casein micelles were adsorbed on to parlodion coated copper grids. Parlodion coated copper grids were coated with poly-L-lysine to improve the adsorption of protein on to the film. These grids were stained using uranyl acetate and oxalic acid, quick frozen in liquefied Freon 22, and freeze dried so that whole casein micelles in a form as close to their native state was imaged. Images were photographed at 50,000x, 85,000x and 140,000x. After 4h of hydration and moderate mechanical stirring, small casein micelles in non-fat dried milk were seen as agglomerates, calcium caseinate micelles were seen connected with proteins, and sodium caseinate appeared to be a mesh of proteins. After longer hydration of 10 h or with high shear the agglomeration of the micelles were not seen. Micelles appeared as individual units distributed randomly in the field. In case of sodium caseinate where micellar structure is minimal, the proteins formed strands or small aggregates of strands when hydrated longer or sheared at a higher rate. These structural differences show the effect of hydration time and method on the structure of milk proteins.

Key Words: caseinates, TEM, structure

626 Aggregation reactions of apo- and holo-α-lactalbumin at neutral pH. M.K. McGuffey* and E.A. Foegeding, *North Carolina State University, Raleigh, NC.*

α-Lacalbumin (LA) is the most thermostable whey protein to aggregation and consequently has tremendous potential in thermally-processed beverages. The objective of this research was to characterize NaCl-dependence (0-100 mM) of aggregates formed by heating (75C) apo- (A; calcium free) and holo- (H; calcium bound) LA at pH 6.8. A commercial preparation of LA was obtained from Davisco Foods and contained 96% total protein (w/w) (91% LA and 5% β-lactoglobulin). Dispersions of the LA were extensively dialyzed and ALA was prepared by calcium chelation with EDTA. The transition temperature, denaturation enthalpy and % reversibility were determined by differential scanning calorimetry and were consistent with literature values. The fraction of native-like protein was determined by absorbance at 280 nm with size-exclusion chromatography. The concentration dependence of turbidity was used to determine the apparent molecular weight (M_w) and the second virial coefficient (A_2). Dispersions of both ALA and HLA (4% w/v) demonstrated first order reaction kinetics for all NaCl levels. The rate constant for ALA loss was 1.46 x 10^{-4} s^{-1}, which increased dramatically (80%) when 50 mM NaCl was added, but only increased 11% from 50 to 100 mM NaCl. The rate constant for HLA loss was 1.85 x 10^{-4} s^{-1}, which increased 36% from 0 to 50 mM NaCl and 31% from 50 to 100 mM NaCl. The turbidity data indicated the apparent M_w of ALA and HLA were $\sim$1,200 kDa and $\sim$330 kDa, respectively, and were relatively insensitive to NaCl addition. The value for ALA may be overestimated because a high degree of polydispersity caused a few very large aggregates to scatter disproportionately. This polydispersity was demonstrated in native PAGE where the ALA aggregates were smeared throughout the stacking and resolving gels whereas HLA aggregates yielded two sharp bands. For all NaCl levels, ALA had an $A_2>0$, which indicates a net repulsive interaction between proteins causing them to swell and interact with the solvent. Aggregates of HLA had an $A_2<0$, which indicates the Ca^{2+} binding results in a net attractive interaction between proteins. The aggregates are primarily disulfide bonded according to reducing SDS PAGE.

Key Words: alpha-lactalbumin, aggregation, second virial coefficient

627 The change of insulin-like growth factor-1 (IGF-1) in bovine milk during lactation period. S. H. Kang*[1], J. W. Kim[2], J. Y. Imm[3], S. J. Oh[4], and S. H. Kim[2], [1]*Seoul Dairy Cooperatives,* [2]*Korea University, Division of Food Science,* [3]*Kookmin University, Dept. of Food & Nutrition,* [4]*Korea Yakult Co. Ltd.*

The objectives of this study were to examine the change of Insulin-Like Growth Factor-1 (IGF-1) content in bovine milk during an year lactation period and to identify parameters affecting IGF-?content in bovine milk. Individual milk was collected from 70 lactating Holstein cows at Kyong-Ki province in Korea. The IGF-1 content was determined by radioimmunoassay using 125I after acid-ethanol treatment. The proximate composition of milk was determined by near infrared milk analyzer. The data were analyzed using GLM and CORR procedures of SAS to examine significant differences ($p < 0.05$) within groups (lactation period, season, parity and dairy farms). Tukey's test was used for multiple comparison. Different feeding pattern between dairy farms may have influenced the IGF-1 content in milk. IGF-?content (36.6 ng/ml) in the middle lactation period (91 180 days) was higher than that of early (28.7 ng/ml) and late lactation period (30.3 ng/ml). Parity did not significantly affect IGF-1 content. Although it was not significant positive correlation was found between IGF-1 and total protein content.

Key Words: IGF-1, Milk, Lactation period

628 The effect of stage of lactation on milk protein composition. J. A. Maas*[1], [1]*Department of Animal and Food Sciences, University of Delaware.*

The effects of stage of lactation on milk protein composition were tested in milk samples of four individual lactating Holstein cows managed in a standard TMR fed, free stall dairy. AM and PM milk samples were obtained at four points in the lactation corresponding approximately to days 61, 122, 183 and 244 of lactation respectively and analyzed for content of 6 individual mammary-origin milk proteins (α-lactalbumin, β-lactoglobulin, αS_1-, αS_2-, β-, and κ-casein) by capillary electrophoresis. Stage of lactation significantly affected the concentration of the whey proteins α-lactalbumin, and β-lactoglobulin ($P>.05$). Stage of lactation did not affect the concentration of the individual caseins as a proportion of the sum of total of the 6 mammary origin proteins. The concentration of each of the individual caseins, as a proportion of total casein protein was also not altered by stage of lactation. There was a nonsignificant trend towards increased αS_2-casein content at the two mid lactation sampling dates. The concentration of whey proteins was highest in milk from the first and last sampling dates and lowest in milk from the two mid lactation sampling dates. These data suggest that there are effects of stage of lactation on milk protein composition, however, most of the effects are manifested through changes in the concentration of whey proteins only. Milk casein composition appears to be quite stable, within cow, over the entire lactation irrespective of changes in milk volume. This suggests that the limitation to increased milk protein synthesis and secretion is at stage other than substrate nutrient supply and absorption by the mammary gland.

Key Words: protein, casein, composition

629 Antihypertensive effect of milk-based media fermented by *Lactobacillus helveticus* R-211 and R-389. P.-L. Leclerc*[1], S. F. Gauthier[1], H. Bachelard[2], and D. Roy[3], [1]*Laval University, Quebec, Canada,* [2]*Hypertension unit, Laval University, Quebec, Canada,* [3]*Agriculture and Agri-Food Canada, St-Hyacinthe, Canada.*

Antihypertensive activity of milks fermented by *Lactobacillus helveticus* has been demonstrated both with spontaneously hypertensive rats (SHR) and human subjects. The effects have been related to the release of milk protein-derived peptides inhibiting angiotensin I-converting enzyme (ACE) as a result of the action of microbial proteinases. The objective of this study was to assess the potential of two strains of *Lactobacillus helveticus* (R-211 and R-389) for the production of antihypertensive fermented milks from reconstituted skim milk media enriched or not with milk proteins (caseins and whey proteins). Results indicated that bacterial growth of both strains was similar in all type of culture media, whereas the proteolysis (free NH_3 groups) and ACE-inhibitory

activity (IC_{50}) were higher in casein-enriched media. Hence, the antihypertensive activity of casein-enriched media fermented by R-211 and R-389 strains was evaluated *in vivo* in SHR rats (0.5, 1.0 and 2.5 g/kg of body weight), and compared to unfermented milk. A significant decrease ($p<0.05$) in arterial blood pressure was measured in SHR rats for the three doses of milk fermented by both strains of *L. helveticus* when compared to PBS control (phosphate buffer saline). However, a lowering effect on arterial blood pressure was also measured in rats given the two highest doses (1.0 and 2.5 g/kg of BW) of unfermented casein-enriched milk. Antihypertensive effect of unfermented medium could be explained by the release of ACE-inhibitor peptides from caseins during gastrointestinal digestion process. In conclusion, these results suggest that milk-based media fermented by *L. helveticus* may have an antihypertensive effect, which can be accentuated by the enrichment of milk with caseins.

Key Words: Fermented milk, Antihypertensive effect

630 Molecular structure and interactions of β-lactoglobulin studied by Fourier transform infrared spectroscopy. T Lefèvre[1] and M Subirade[2], [1]*Universite Laval CERSIM,* [2]*Universite Laval STELA.*

Fourier transform infrared (FT-IR) spectroscopy is a powerful and versatile tool used to determine the molecular structure of biomolecules. This technique is now widely used in biochemistry to study the conformation of biopolymers in aqueous solutions and complex systems. However, its enormous potential in the study of food biopolymers has yet to be reached. The aim of this paper is principally to provide information on biopolymers using FT-IR spectroscopy. β-Lactoglobulin (β-Lg), the major whey protein in the milk of ruminants, is chosen as a model. New aspects of β-Lg structure which have not previously been found by this technique are presented. First, it is shown that FT-IR spectra are sensitive to the quaternary structure of β-Lg, as deduced from the study of the protein amide I band as a function of concentration, pH, and temperature. Second, β-Lg fine-stranded and particulate thermal gels have been investigated. We revealed differences in the denaturation mechanism, in the unfolded state of the protein, in the aggregate formation, and in the strength of the intermolecular interactions as a function of pH. These specificities of each gel could be associated to the macroscopic and microstructural properties of each gel network. Finally, interactions between β-Lg and two zwitterionic phospholipids have been analyzed. Dipalmitoylphosphatidylcholine bilayers are unaffected in the presence of β-Lg, suggesting that no interaction occurs. In contrast, β-Lg increases the lipid chain conformational disorder of milk sphinglomyelin (SM) as a consequence of hydrophobic interactions of β-Lg with SM. Since this effect occurs at and above the gel-to-liquid-crystalline phase transition, it is suggested that membrane fluidity plays an important role in these interactions. These conclusion is supported by other recent works that emphasized the important role of the lipid chain fluidity in the protein-phospholipid interactions.

631 Effects of Beta-Lactoglobulin enriched colostrum on IgG transport in neonatal piglets. L.F. Sutton*[1] and B. Alston-Mills[1], [1]*North Carolina State University.*

The effects of the bovine milk whey protein Beta-Lactoglobluin (BLG) were investigated in the neonatal, colostrum deprived piglet. Objectives were to determine if BLG had stimulatory properties on mucosal growth, enzymatic activity, DNA proliferation and IgG uptake and endogenous Ig production. Two experiments were done, the first lasting 5 days (Exp 1), the second 28 days (Exp 2). For each, a total of 18 piglets were taken from three sows following parturition and divided into three experimental groups: two removed from the sow immediately (colostrum deprived) and one group remaining on the sow to serve as controls (n=6). The colostrum deprived piglets were further divided into two experimental groups: one receiving commercial bovine colostrum supplemented with an extra 10% BLG derived from whey protein concentrate (WPC)(Treatment 1, n=6) and the final group receiving only bovine colostrum (Treatment 2, n=6). After 36 hours, all piglets on the bovine colostrum were placed onto a liquid neonatal diet without additional supplementation. After the 5th day of Exp.1, animals were sacrificed, blood and intestinal samples were taken for enzymatic and DNA analysis. For Exp.2, all piglets were weaned at 21 days and placed onto a dry feed piglet diet. Blood samples were collected daily for the first five days and then every third day for 28 days. All blood samples were cast against both porcine and bovine anti-IgG,M,and A for sera concentrations. Intestinal tissue was also taken from the small bowel for total DNA, enzymatic activity, and morphology. For both experiments, animals receiving the WPC with 10% BLG suppplementation had highest uptake of IgG within the first 5 days as compared to those of other diets ($p<.01$). Treatment 1 also showed higher DNA proliferation after 5 days in Exp.1. BLG did not stimulate endogenous production of IgG, however, endogenous production was highest in controls ($p<.001$). Villi heights were highest in controls on day 28 ($p<.01$), although treatment 1 tended to have slightly higher villus height than treatment 2. These results suggest that BLG from WPC may facilitate uptake of IgG prior to gut closure, induce DNA proliferation, and affect intestinal morphology.

Key Words: Beta-Lactoglobulin, IgG, colostrum

632 Withdrawn. , .

Nonruminant Nutrition
Nutrient Metabolism, Evaluation, and Modeling

633 Effects of feed restriction and subsequent refeeding on energy utilization in growing pigs. P. A. Lovatto*[1], J. van Milgen[2], J. Noblet[2], and D. Sauvant[3], [1]*Universidade Federal de Santa Maria, Santa Maria, RS, Brasil,* [2]*INRA, UMR sur le Veau et le Porc, Saint Gilles, France,* [3]*INAPG/INRA, UMR Physiologie de la Nutrition et Alimentation, Paris, France.*

An experiment was carried out to evaluate the metabolic utilization of energy in crossbred barrows during feed restriction and re-feeding. Ten animals, initially weighing 52 kg, were used in five blocks of two littermates each. A 7-d adaptation period (P1) was used in which pigs were fed a diet containing (on a DM basis) 18 MJ ME and 1% lysine at 2.60 MJ ME.$kg^{-0.60}.d^{-1}$. Following the adaptation period, one animal of each block continued to receive feed at this level for 7 d (P2), whereas its littermate received the same feed at 1.55 MJ ME.$kg^{-0.60}.d^{-1}$ (40% restriction). During the subsequent 7-d period (P3), both animals were offered feed at 2.60 MJ ME.$kg^{-0.60}.d^{-1}$. Heat production (HP) was measured in each pig using an open-circuit respiration chamber, and energy and nitrogen balances were determined for P1, P2 and P3. The HP was decomposed in HP from physical activity (HPact), short-term thermic effects of feeding (TEFst) and resting heat production (RHP). Feed restriction during P2 decreased total HP (1.16 vs 1.45 MJ.$kg^{-0.60}.d^{-1}$, for restricted and control animals, respectively), RHP (0.84 vs 0.99 MJ.$kg^{-0.60}.d^{-1}$), TEFst (0.14 vs 0.27 MJ.$kg^{-0.60}.d^{-1}$) and retained energy (0.41 vs 1.13 MJ.$kg^{-0.60}.d^{-1}$). The HPact (0.17 MJ.$kg^{-0.60}.d^{-1}$) was not affected by feed restriction. Likewise, fecal N losses (5.2 vs 7.7 g.d^{-1}), urinary N losses (12.6 vs 21.0 g.d^{-1}), protein gain (107 vs 189 g.d^{-1}), lipid gain (62 vs 239 g.d^{-1}), and average daily gain (0.33 vs 1.07 kg.d^{-1}) were reduced during feed restriction. There were no differences in components of HP and energy utilization between both groups during P1 and P3. Nevertheless, urinary N loss was decreased (20.1 vs 23.6 g.d^{-1}) and ADG increased (1.45 vs 1.03 kg.d^{-1}) during P3 for animals that were fed restrictively in P2. Compensatory growth after a period of feed restriction does not appear to be related to a change in efficiency of energy utilization for energy gain, but may be due to gain of water, protein and gut contents.

Key Words: Pig, Energy, Feed restriction

634 Previous feeding level influences fasting heat production in growing pigs. C.F.M. de Lange*[1], J. van Milgen[2], J. Noblet[2], S. Dubois[2], and S.H. Birkett[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*Institut National de la Recherche Agronomique, St. Gilles, France.*

Factorial approaches to estimate energy requirements for growing pigs require estimation of maintenance or basal energy expenditure (BE) that is related to animal characteristics only. Heat production (HP) in fasted pigs (FHP) may provide a direct estimate of BE. However, FHP may be influenced by nutrient intake. Six barrows were used to determine