(11%) had ulcers located in the nonglandular squamous epithelial mucosa. The mathematical mean of the lesion number score was 2.22 and the mathematical mean of the lesion severity score was 2.67. For all age groups, the occurrence of ulcers was not different than expected. Occurrence of ulcers was not different in Morgans, Quarter horses, and Warmbloods. Thoroughbreds and grade horses had fewer ($P<0.05$) ulcers than expected. The occurrence of ulcers among all breeds was lower ($P<0.01$) than expected. Geldings had fewer ($P<0.01$) ulcers than expected, while the occurrence of ulcers was not different than expected in mares. The occurrence of ulcers for both genders was lower ($P<0.01$) than expected. Occurrence of ulcers was less than expected in horses used in lessons ($P<0.01$) and in polo ($P<0.05$). The occurrence of ulcers was not different in horses in training. For all three use categories, the occurrence of ulcers was lower ($P<0.01$) than expected. Among all categories, lesion number and lesion severity was not different than expected. Since overall percent and occurrence of ulcers in this population was lower than in previous studies, it may be suggested that such things as management practices, length of turnout, and overall contact may play an important role in the occurrence of ulcers.

Key Words: Gastric Ulcers, Horse, Breed

79 Evaluation of feeding bee pollen to horses in training. K. K. Turner*, B. D. Nielsen, C. I. O'Connor, and J. L. Burton, *Michigan State University, East Lansing*.

"Bee pollen" is often fed to horses but few studies have evaluated its use. Our hypothesis was feeding bee pollen would positively influence nutrient digestibility, and indices of immunological status and physical fitness in horses in training. Ten Arabians (6 geldings, 4 mares) underwent a standardized exercise test (SET). Horses were pair-matched by sex and fitness and randomly assigned to P (receiving 118 g of bee pollen (Dynamic Trio 50/50) daily) or C (control receiving 73 g of a placebo) for a supplementation period of 42 d. A total collection period was conducted on geldings from d 18 to 21 to determine effects of bee pollen on nutrient retention and digestibility of NDF and ADF. Horses were exercise conditioned during the 42-d trial and completed another SET on d 42. V_{160} and V_{200} were calculated from SET heart rates (HR). Lactate, glucose, hematocrit (HT) and hemoglobin (HB) concentrations were determined from SET blood samples. Total and percent leukocytes, T and B lymphocytes, monocytes, neutrophils, and eosinophils, as well as IgG, IgM and IgA concentrations were determined from rest and recovery samples from both SETs. Geldings in P ate more feed than C on every day of the collection period. P had 80 % less phosphorus excretion ($P=0.003$), and tended to retain 90 % more nitrogen ($P=0.08$). P tended to digest more NDF ($P=0.06$) and ADF ($P=0.09$). P had lower NDF digestibility ($P=0.002$) and tended to have lower ADF digestibility ($P=0.07$). Although no treatment differences existed, V_{160} and V_{200} increased ($P<0.05$), and HR ($P=0.002$), blood lactate concentration ($P<0.0001$), HT ($P<0.0001$) and HB ($P=0.0003$) decreased from d 0 to 42. Few treatment differences were seen in immunological parameters. There was a trend for percent monocytes to increase in C while decreasing in P ($P=0.1$) from rest to recovery on d 42. Percent neutrophils increased from rest to recovery on d 42, with P increasing more ($P=0.04$). Percent neutrophils was increased on d 42, compared to d 0, with the greatest increase in P ($P=0.04$). Bee pollen supplementation, while not affecting measured fitness variables, may have a positive effect on performance by helping meet increased metabolic demands of equine athletes by increasing nutrient retention and feed intake.

Key Words: Pollen, Horse, Exercise

80 Potassium supplementation affects plasma [K^+] during an 80 km endurance exercise test on the treadmill. T. M. Hess*[1], K. Treiber[1], D. S. Kronfeld[1], J. N. Waldron[2], C. A. Williams[1], M. S. Freire[1], A. M. G. B. Silva[1], L. S. Gay[1], D. A. Ward[1], and P. A. Harris[3], [1]*Virginia Polytechnic Institute and State University, Blacksburg,* [2]*Rectortown Equine Center, Rectortown, VA,* [3]*Equine Studies Group, Waltham Centre for Pet Nutrition, Melton-Mowbray, UK*.

During exercise plasma [K+] increases and can lead to increased neuromuscular excitability and related clinical signs. Supplementation of K during exercise can further increase plasma [K+]. A K-free electrolyte mixture (EM-K) was tested and compared to a K-rich mixture (EM+K) during an 80 km simulated endurance exercise test (EET) on a treadmill. Twelve horses were tested in a cross over design performing four bouts (B) of 20 km at 45% of their maximum heart rates with three 30-minutes of rest (R) between bouts. Before the start of EET and during each R horses were supplied with EM-K or EM+K. Blood samples were collected before (PRE), at 10 km of each B, at 20 minutes of every R and 10 minutes after B4 (REC) and analyzed for hematocrit (Hct), and plasma for pH, PCO_2, lactate ($[La^-]$), phosphate ($[PO_4^-]$), albumin (alb), CK and AST and electrolytes. Horses were weighed and electrocardiograms done at PRE, every R, and REC; weights were also measured in the morning after (MA) EET. Effects of stage (PRE, B1, B2, B3, B4, R1, R2, R3, R4, REC, MA) treatment (EM-K vs EM+K) and their interactions were evaluated by ANOVA in a mixed model with repeated measures. Body weight losses during EET increased up to 4.96% at REC, and were 2.3% below PRE at MA ($P<0.001$). Hct increased during B, returned to PRE at every R ($P<0.001$). Plasma alb increased during EET ($P<0.001$), returning to PRE values at REC. Plasma CK and AST increased progressively during EET ($P<0.001$). Plasma pH increased with exercise, decreased with R and REC ($P<0.001$). Plasma PCO_2 and $[Ca^{++}]$ decreased during exercise and increased during R periods, however $[Ca^{++}]$ was lower than PRE at REC ($P<0.001$). Plasma $[Mg^{++}]$ decreased initially with exercise, but then was higher than PRE at REC ($P<0.001$). Plasma $[Na^+]$, $[Cl^-]$, $[La^-]$, and PO_4^- increased progressively during the EET ($P<0.0001$). No abnormalities were observed on the EKG results. A treatment effect was found for plasma $[K^+]$ ($P=0.014$), where horses that received EM-K had lower values than EM+K. Lower plasma $[K^+]$ during exercise could help maintain resting membrane potential and prevent signs of neuromuscular hyperexcitability.

Key Words: Potassium, Equine, Exercise

Milk Protein and Enzymes

81 Composition of the air interface in ice cream as affected by protein and emulsifier content. Z. Zhang* and D. Goff, *Department of Food Science, University of Guelph, Guelph, ON, Canada*.

Emulsifiers and proteins interact during the production of ice cream to form the fat interface, which in turn controls the extent of partial coalescence and hence fat structure formation in the frozen product. The interaction of these ingredients at the air interface, however, has not been as well investigated. We have examined the effect of saturated or unsaturated mono-glyceride and the effect of protein from skim milk powder or whey protein isolate on the composition of the air interface by immuno-gold labeling and transmission electron microscopy. When ice cream was made from skim milk powder in the absence of emulsifier, casein micelles, non-micellar b-casein and b-lactoglobulin were found at the air interface. When emulsifiers were used, more fat was seen at the air interface, especially with unsaturated mono-glyceride. When whey protein isolate was used, fat globules were seldom found at the air interface, regardless of emulsifier presence. As the fat interface is formed first during ice cream processing, the composition of the air interface was found to be a direct consequence of the composition of the fat interface.

Key Words: Milk Proteins, Air Interface, Immuno-Labeling

82 Evidence that the major novel disulfide bond in heated cows milk is between β-lactoglobulin Cys160 and κ-casein Cys88. E. K. Lowe[1], S. G. Anema[1,2], M. J. Boland[1], R. Jiménez-Flores[3], and L. K. Creamer*[1], [1]*Fonterra Research Centre, Palmerston North, New Zealand,* [2]*Riddet Centre, Massey University, Palmerston North, New Zealand,* [3]*Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo*.

Heat treatment of milk causes the milk proteins to interact irreversibly with one another, primarily caused by disulfide bond interchange. Early studies have shown that heating β-lactoglobulin (BLG) in solution invokes Cys-mediated disulfide bond interchange to give BLG polymers and BLG molecules with non-native disulfide bonds. Some of these molecules and polymers contain Cys66 or Cys160 in the reduced state.

It has long been known that both BLG and κ-casein (KCN) are present in the complexes formed as a consequence of heat treatment. BLG contains five Cys residues and KCN contains 2 Cys residues, and thus there is a large number of combinations of possible disulfide bondings. Reacting reduced KCN with DTNB (dithiobisnitrobenzoic acid) gives KCN molecules containing activated Cys groups. Reacting partially reduced BLG with these derivatives gave a range of products. These were separated and the fraction that contained material with a mass of about 35 KDa was hydrolyzed with trypsin. Among the products were peptides with masses corresponding the peptides BLG148-162 SS KCN87-97 and BLG148-162 SS KCN11-16. The identity of the peptides was verified by partial sequencing of both the intact peptides and the reduced peptides using ESI-MS-MS. A heated milk sample was analyzed and these peptides were identified in a trypsin-treated sample of the mixture of proteins and there was significantly more disulfide-bonded KCN87-97 than KCN11-16 in the mixture. The existence of other possible disulfide bonding patterns involving Cys 66, 106, 119, or 121 of BLG, and Cys 11 or 88 of KCN are being explored.

Key Words: Heat-Induced Complex, Whey Protein, Casein

83 Application of novel gel electrophoresis to analyze native, heat-, and pressure-treated milk protein systems. H. A. Patel[1,2], H. Singh[3], and L. K. Creamer*[1], [1]*Fonterra Research Centre, Palmerston North, New Zealand,* [2]*Institute of Nutrition, Food and Human Health, Massey University, Palmerston North,land,* [3]*Riddet Centre, Massey University, Palmerston North, New Zealand.*

The invention of gel electrophoresis presaged great advances in all protein sciences and has been particularly useful in dairy protein science. Incorporation of urea into the gels allowed clear separation of the caseins from one and reduction of the disulfide bonds allowed the clarification of the polypeptides that comprise the native α_{s2}- or κ-casein (KCN). Addition of other solutes, such as magnesium chloride or SDS, meant that the basis of separation could be changed. The advent of two-dimensional electrophoresis in polyacrylamide gels gave even greater power because the two separations could be under very different conditions; for example alkaline urea PAGE followed by SDS PAGE. The best-known 2D method uses isoelectric focussing followed by SDS PAGE, is very powerful technique and is used extensively in proteomics research. Our group has been using electrophoresis to study some particular problems, such as: Does β-lactoglobulin (BLG) bind preferentially to α_{s2}-CN or KCN during heat treatment carried out in non-native conditions? In the whey protein scene, 2D PAGE as modified by our group allowed the identification of the pathways leading to BLG and whey protein aggregation and gelation during heat treatment. These techniques, with some added refinements, have now been applied to a range of dairy protein problems of varying complexity. For example, the examination of heat and pressure treated milks which has lead to an even greater clarification of the relative roles of α_{s2}-CN or KCN in their reaction with the aggregating whey proteins

Key Words: PAGE Analysis, Protein-Protein Interaction, Caseins and Whey Proteins

84 Effects of genetic modification, pressure, and heat on the binding of various probes to β-lactoglobulin. K. A. N. S. Ariyaratne[1], G. B. Jameson[1], T. S Loo[2], G. E. Norris[2], H. A. Patel[4,3], T. Considine[4,5], H. Singh[5], L. K. Creamer*[4], and R. Jiménez-Flores[6], [1]*Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand,* [2]*Institute of Molecular Biosciences, Massey University, Palmerston North, New Zealand,* [3]*Institute of Nutrition, Food and Human Health Massey University, Palmerston North, New Zealand,* [4]*Fonterra Research Centre, Palmerston North, New Zealand,* [5]*Riddet Centre, Palmerston North, New Zealand,* [6]*Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

Native β-lactoglobulin (BLG) has been shown to bind a number of hydrophobic and amphipathic molecules within the central calyx. Examples are: hexane, retinol (vitamin A), cholesterol, 12 bromo-dodecanoic acid, palmitic acid, and cis-parinaric acid (CPA) but not anilinonaphthalene sulfonate (ANS). The binding of retinol and CPA can be followed by induced circular dichoism or increased fluorescence. This has allowed the determination of the displacement of CPA or retinol by dodecyl sulfate (SDS) or palmitate, but not by ANS. Heat treatment denatures the BLG, and the binding of CPA and retinol decreases in proportion with the extent of denaturation while ANS fluorescence increases. Pressure treatment has a similar effect. Addition of these ligands of BLG increases the stability of the protein under increased temperature and pressure. ANS does not have this effect. Polynucleotides were synthesized and expressed to give fusion proteins, corresponding to both the A and B variants of BLG. These were subsequently cleaved to obtain synthetic BLG A and BLG B. Modification of the polynucleotide allowed the preparation of a number of different BLG mutants. Some of these were modifications close to the binding site of retinol and the fatty acids. In particular the change of lysine to glutamic acid at residues 60 and 69 showed dramatic differences in behavior. These two residues are close spatially but the change at residue 60 prevents binding of retinol or CPA while that at residue 69 does not.

Key Words: Lactoglobulin Binding, Mutant Protein, Pressure Treatment

Nonruminant Nutrition: Finishing Pigs - Additives & Energy

85 Effect of L-lysine-HCl addition in late finishing gilts fed ractopamine HCl (Paylean®). B. W. Ratliff*[1], A. M. Gaines[1], P. Srichana[1], G. L. Allee[1], and J. L. Usry[2], [1]*University of Missouri, Columbia,* [2]*Ajinomoto Heartland LLC, Chicago, IL.*

The objective of this 21 d experiment was to evaluate the effect of L-lysine-HCl addition on growth performance of pigs fed ractopamine HCl (Paylean®). A total of 966 gilts (TR-4 × C22; 98.2 ± 0.2 kg) were randomly allotted to one of six dietary treatments with seven replicate pens/treatment and 23 pigs/pen. Dietary treatments included five levels of L-lysine-HCl that corresponded to concentrations of 0.10, 0.20, 0.30, 0.40, and 0.50% lysine, respectively. With the exception of L-threonine and Alimet® feed supplement (88% L-methionine activity), additional synthetic amino acids were not supplied to meet the minimum amino acid profile. To evaluate the effect of maintaining a minimum amino acid profile, additional synthetic amino acids (L-tryptophan, L-isoleucine, and L-valine) were supplemented to a diet containing 0.50% L-lysine-HCl. All diets were corn-soybean meal-based with 2% choice white grease formulated at a 0.93% TID lysine and contained 7 ppm Paylean®. Increasing the L-lysine-HCl inclusion resulted in a decrease (quadratic, $P < 0.04$) in ADG (1,016, 1,016, 1,016, 1,043, and 966 g/day, respectively) and lowered (quadratic, $P < 0.02$) G:F (0.352, 0.357, 0.353, 0.352, and 0.338, respectively). There were no differences ($P = 0.40$) in ADFI. Use of two slope-broken line analysis indicated a maximum L-lysine-HCl inclusion of 0.41% for ADG and 0.39% for G:F. The addition of synthetic amino acids to the diet containing 0.50% L-lysine-HCl did not restore growth performance. Collectively, these data demonstrate that up to 0.40% L-lysine-HCl can be added in Paylean® diets without compromising growth performance, provided the diets are supplemented with L-threonine and a methionine source. Furthermore, maintaining a minimum amino acid profile in Paylean® diets containing 0.50% L-lysine-HCl does not restore growth performance indicating that another nutrient may be limiting.

Key Words: Ractopamine, Lysine, Pigs

86 Threonine to lysine ratio in ractopamine treated pigs. D. M. Fernandez*[1], N. Rosas[2], A. I. Soria[1], and J. A. Cuaron[3], [1]*Universidad Nacional Autonoma de Mexico,* [2]*Patronato de Apoyo a la Investigación y Experimentacion Pecuaria en Mexico A.C. Queretaro, MX,* [3]*Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias Queretaro, MX.*

Previously we titrated the digestible lysine requirement of finishing pigs in the presence or absence of 5 ppm ractopamine (RAC). The objective of this study was to determine the threonine to lysine ratio (T:L, %) required to maximize growth performance of finishing pigs fed diets containing either 5 or 10 ppm RAC. One hundred individually penned crosbred pigs (Landrace×Duroc) were used in a 28-d trial. Initial body weight averaged 77.2±4.04. Dietary treatments included 1) a control diet (CON) formulated to .67% digestible lysine (DL) and 3.20 Mcal