

## Dairy Foods: Protein

**W78 The effect of lysine and methionine on milk protein mRNA expression of bovine mammary epithelial cells in vitro.** X. Y. Li, J. Q. Wang\*, D. P. Bu, H. Y. Wei, H. Hu, and L. Y. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

The objective of this study was to examine the effect of lysine and methionine supplementation on milk protein of bovine mammary epithelial cells in vitro. The lysine was added to the DMEM/F12 culture medium containing 10% fetal bovine serum (FBS) at concentrations of 0, 0.05, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 mmol/L and the methionine was also added to culture medium at concentrations of 0, 0.025, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 mmol/L. The cell proliferation and milk protein mRNA expression were detected by MTT colorimetric assay and RT-qPCR. Experiment with 48 h. This experiment was repeated 2 times. Within each experiment were repeated 3 replicates for each treatment. The results showed that 0.8 to 1.6 mmol/L lysine and 0.4 to 0.8 mmol/L methionine at 48 h significantly improved the proliferation of bovine mammary epithelial cells ( $P < 0.0001$ ), the mRNA expression level of  $\alpha_{s1}$ -casein,  $\beta$ -casein were all significantly higher than other group at these concentrations. Thus, the lysine and methionine may improve milk protein synthesis.

**Key Words:** lysine, methionine, bovine mammary epithelial cells

**W79 Identification of bovine casein phosphorylation using TiO<sub>2</sub> enrichment in combination with nano-ESI-MS/MS.** S. S. Li, Y. X. Yang, J. Q. Wang\*, D. P. Bu, H. Y. Wei, L. Y. Zhang, and L. Y. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Protein phosphorylation is an important post-translational modification that regulates milk protein structure and function. The objective of this study was to analyze the presence of phosphorylated casein. Bovine milk proteins were first separated by sodium dodecyl sulfate PAGE. After in gels digestion and extraction, phosphorylated peptide were enriched by titanium dioxide and identified by ultra performance liquid chromatography coupled with nano electrospray ionization tandem mass spectrometry. This method ensured the identification of 20 phosphorylated peptides, including 7 phosphorylated forms of  $\alpha_{s1}$ -casein, 8  $\alpha_{s2}$ -casein, and 5  $\beta$ -casein. Eight phosphorylated sites derived from 3  $\alpha_{s1}$ -caseins, 3  $\alpha_{s2}$ -caseins and 2  $\beta$ -caseins were also identified. Phosphates group were localized on residues Ser<sup>61</sup>, Ser<sup>63</sup> and Ser<sup>130</sup> in  $\alpha_{s1}$ -casein; Thr<sup>145</sup>, Ser<sup>146</sup> and Ser<sup>158</sup> in  $\alpha_{s2}$ -casein; and Ser<sup>50</sup> and Thr<sup>56</sup> in  $\beta$ -casein. These findings provide valuable information for the investigating of the bovine milk casein phosphorylation.

**Key Words:** bovine milk casein, phosphorylation, mass spectrometry

**W80 Developmental changes in the bovine whey proteome during the transition from colostrum to milk.** L. Y. Zhang<sup>1,2</sup>, J. Q. Wang<sup>\*1</sup>, Y. X. Yang<sup>1</sup>, S. S. Li<sup>1</sup>, D. P. Bu<sup>1</sup>, and L. Y. Zhou<sup>1</sup>, <sup>1</sup>State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agriculture Science, Beijing, China, <sup>2</sup>Department of Animal Science, College of Agriculture, Hebei University of Engineering, Handan, China.

Bovine milk whey proteins in colostrum are of more significance for newborn than whey proteins in milk, but no studies on the difference

in whey protein patterns during the transition from colostrum to milk have been reported to date. This study separated whey proteins from d 1, day3, d 7 and d 21 after calving using 2-dimensional electrophoresis. Differentially expressed proteins in different collection time were identified using high-performance liquid chromatography tandem mass spectrometry (LC/MS/MS) and validated by enzyme linked immunosorbent assay (ELISA) to understand the developmental changes in the bovine whey proteome during the transition from colostrum to milk. Whey proteins from d 1 and d 3 were found to be the same except for immunoglobulin G. Seven proteins were found to be lower in d 7 and d 21 milk whey, including immunoglobulin G, immunoglobulin M, albumin, and lactotransferrin, which are involved in immunity and molecule transport. These proteins were detected using an advanced proteomic method, 2-dimensional electrophoresis coupled with LC/MS/MS, which confirmed that the changes in the differential expression proteins of bovine whey fraction did occur with increased concentrations of serum protein in colostrum whey.

**Key Words:** bovine milk whey protein, colostrum, mass spectrometry

**W81 Formation of nanofibers and hydrogels from a milk-derived peptide.** M.-M. Guy<sup>1</sup>, N. Voyer<sup>2</sup>, S. F. Gauthier<sup>1</sup>, and Y. Pouliot<sup>\*1</sup>, <sup>1</sup>STELA Dairy Research Center, Institute of Nutraceuticals and Functional Foods (INAF), Université Laval, Québec, Canada, <sup>2</sup>Department of Chemistry and PROTEO Protein Structure, Function and Engineering Research Network, Université Laval, Québec, Canada.

It has been previously shown that the N-terminal fragment of  $\beta$ -lactoglobulin, namely  $\beta$ -LG f1–8, isolated from a flocculated tryptic peptide mixture of whey protein isolate, undergoes self-assembly by a secondary structure transition to  $\beta$ -sheet conformation at basic pH. The objective of the present work was to investigate the physicochemical conditions that trigger self-assembly of peptide  $\beta$ -LG f1–8 and therefore lead to nanofibers and hydrogel formation. Nanostructures formed by self-assembly of peptide  $\beta$ -LG f1–8 in the pH range of 2.0 to 11.0 were studied by transmission electron microscopy (TEM). Hydrogel formation was studied as a function of pH and results evidence a link between hydrogel formation and charge distribution carried by the peptide structure. Finally, circular dichroism (CD) spectropolarimetry was used to characterize the effects of peptide concentration (0.4 to 2.0 mg/mL), temperature (20 to 80°C), and ionic strength (0 to 1 M NaCl) on the secondary structure of peptide  $\beta$ -LG f1–8. Hydrogels were obtained at peptide concentrations above 2.5 mg/mL. Peptide concentration and pH adjustment were shown to trigger self-assembly of  $\beta$ -LG f1–8 whereas temperature and ionic strength had only limited effects. Overall, results emphasize the role of particular molecular interactions in  $\beta$ -sheets self-assembly of peptide  $\beta$ -LG f1–8 and pH-dependent electrostatic interactions occurring between  $\beta$ -LG f1–8 units explain its propensity to self-assembly and flocculation in complex media such as whey protein tryptic hydrolysates.

**Key Words:** whey peptide, hydrogels, nanofibers