

## Dairy Foods: Milk Protein and Enzymes

**T94 Effects of prolactin on the expression of genes related to milk protein synthesis in bovine mammary epithelial cells.** X. Y. Li, J. Q. Wang\*, H. Y. Wei, X. M. Nan, D. P. Bu, P. Sun, 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 reveal the role of prolactin (PRL) on the expression of important genes related to milk protein synthesis in bovine mammary epithelial cells. Mammary epithelial cells were isolated from a 3 year old Chinese Holstein dairy cow in mid-lactation (ca.100 d relative to parturition) and cultured in the medium containing a combination of hydrocortisone, insulin, transferrin, bovine epithelial growth factor and PRL (5 $\mu$ g/m L). No PRL was added in medium of control groups. Expressions of genes were measured by RT-qPCR. The content of caseins and lactose were detected by Casein ELISA Kit and HPLC, respectively. All experiments repeated 3 times. The results showed that after treatment with PLR casein synthesis was increased by 20% and lactose synthesis was increased by 25%. The expression of prolactin receptor (PRLR) was significantly increased ( $P < 0.01$ ). *CSN3* and *LALBA* were the most highly expressed casein genes ( $P < 0.01$ ), followed by *CSN1S1*, *CSN2* and *CSN2S2*, but these genes were not increased significantly ( $P > 0.05$ ). The expression of genes related to *JAK2-STAT5* pathway were upregulated, and *JAK2* and *ELF5* expression increased significantly ( $P < 0.01$ ). This study revealed that PRL was necessary for high levels of milk protein genes expression and milk protein synthesis.

**Key words:** prolactin, bovine mammary epithelial cells, genes expression

**T95 The best ratio between lysine and methionine on milk protein synthesis in bovine mammary epithelial cells.** X. Y. Li, J. Q. Wang\*, H. Y. Wei, X. M. Nan, D. P. Bu, P. Sun, and L. Y. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

This study took the bovine mammary epithelial cells as a model to determine the best ratio between lysine and methionine, focusing on the effects of additional lysine and methionine on the regulation of genes related to milk proteins, genes related to amino acid transporters, and genes related to milk protein synthesis. The expression of important genes related to milk protein synthesis process included the genes of the *JAK2-STAT5* pathway and the *mTOR* signaling pathway. The experiment was conducted with a 5  $\times$  3 factorial design: with lysine (1.0, 1.2, 1.4, 1.5, 1.6 m mol/L in DMEM/F12 medium) and methionine (0.4, 0.5, 0.6 m mol/L in DMEM/F12 medium). All experiments repeated 3 times. The expression of genes and caseins content were detected by RT-qPCR method and Casein ELISA Kit, respectively. Data were analyzed with the PROC GLM and PROC ANOVA. The results showed that when the concentration of lysine was 1.2 m mol/L and the concentration of methionine was 0.4 m mol/L, the content of casein in medium increased significantly ( $P < 0.01$ ) and peaked at 2.95ppm. We measured the genes expression at this ratio (3:1). *CSN1S1* and *LALBA* were the most highly expressed genes encoding caseins and increased more than 2-fold ( $P < 0.01$ ), followed by *CSN2*, *CSN3* and *CSN2S2*, and these genes expression were also upregulated ( $P < 0.01$ ). The expression of genes related to *JAK2-STAT5* pathway were upregulated, and *JAK2* and *ELF5* expression increased significantly ( $P < 0.01$ ). The expression of genes related to *mTOR* signaling pathway was increased, but *S6K* was not significantly ( $P > 0.05$ ). *EIF4E-BP1*

expression was significantly downregulated ( $P < 0.01$ ). The expression of amino acid transporter *CAT-1* and *ASCT-2* had no change. These observations together with the fact that genes expression changed in the cells with the ratio between lysine and methionine revealed that the lysine, methionine and the ratio between lysine and methionine may directly regulate the expression of genes related to milk protein transcription and translation, leading to improved milk protein synthesis.

**Key words:** lysine, methionine, milk protein

**T96 Development of safe glue sticks containing whey protein.** G. Wang and M. Guo\*, *The University of Vermont, Burlington.*

The major whey proteins are small globular proteins that may be undesirable for application in adhesives. However, their structures can be modified using chemical and physical means such as heating. Commercial glue sticks on the market may contain toxic chemical components, which can pose potential health hazards to users. In this study, a new safe glue stick product was developed using whey protein isolate (WPI), polyvinylpyrrolidone K90, propylene glycol, stearic sodium, and sucrose. WPI was first dissolved in distilled water, and then other ingredients were added. The mix was heated to 93–95 $^{\circ}$ C with constant stirring for 30 min until it became homogenous, and then was filled into the push-up tubes. The new product was analyzed for bonding strength and hardness in comparison with a commercial product as a control. Bonding strength of the prototype was 247.63  $\pm$  11.73 N, which was higher than that of the control (241.60  $\pm$  9.49 N), but the difference was not significant ( $P > 0.05$ ). The bonding strength remained stable after 3 mo of storage at 23 $^{\circ}$ C or 40 $^{\circ}$ C. Hardness of the prototype (23.31  $\pm$  2.51 N) was comparable to that of the commercial glue (20.30  $\pm$  1.09 N). The content of total solids of the prototype (52.17  $\pm$  0.67%) was higher ( $P < 0.05$ ) than the control (36.46  $\pm$  0.29%). The new product was easy to be applied on paper, extended and retracted in push-up tubes. There was no growth of mold and yeast on the sticks after 3 mo of storage at 40 $^{\circ}$ C. In conclusion, the new safe glue stick prototype containing whey proteins was comparable to the commercial control in bonding strength and hardness. Shelf life tests are being carried out to determine the storage stability of this new product.

**Key words:** glue stick, whey protein, safe

**T97 Isolation and characterization of prosaposin from milk from four goat breeds.** A. Robertson-Byers\*, M. Worku, and S. Ibrahim, *North Carolina A&T State University, Greensboro.*

Increased scientific knowledge related to the nutritional, functional and biological activities and health benefits of goat milk is needed to further promote goat farming, goat milk and traditional or innovative nutraceuticals as a basis for socio-economic benefit. Prosaposin, a protein in goat milk is the precursor of the sphingolipid activator proteins. Saposins are small lysosomal proteins required for the hydrolysis of sphingolipids. Prosaposin is important in development and maintenance of the nervous system. Genetic variation may impact nutraceutical properties of milk by altering the biological function of bioactive peptides and antigens. The objectives of this study were to detect prosaposin and the sphingolipid activator proteins (saposins A, B, C, and D) in milk from different breeds of goats. Raw milk was collected at North Carolina A&T State University farm from Alpine, Spanish, Boer, Spanish  $\times$  Boer goats and from a Holstein Friesian

cow. Whey fractions were separated by centrifugation. Extracts were analyzed by electrophoresis and immunoblotting with anti-prosaposin and anti-saposin HRP conjugated antibodies. Specific proteins were identified using a tetramethylbenzidine substrate. Multiple proteins were observed in whey fractions from all animals tested. Antibodies detected a 65 kDa prosaposin band and a 29 kDa saposin C band. Sapo-

sins and their precursor prosaposin are present in milk from different breeds of goats. The possible effect of genetic variation on concentration need further study. These studies will contribute to our knowledge of the therapeutic benefits of goat milk to aid producers in maintaining breeds with the potential to produce prosaposin.

**Key words:** prosaposin, nutraceutical, goats