Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/jpn.12246

ORIGINAL ARTICLE

Effects of phenylalanine and threonine oligopeptides on milk protein synthesis in cultured bovine mammary epithelial cells

M. M. Zhou[1,2], Y. M. Wu[1], H. Y. Liu[1] and J. X. Liu[1]

1 Institute of Dairy Science, Ministry of Education Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou, China, and
2 Weifang University of Science and Technology, Shouguang, China

Summary

This study was conducted to investigate the effects of phenylalanine (Phe) and threonine (Thr) oligopeptides on α_{s1} casein gene expression and milk protein synthesis in bovine mammary epithelial cells. Primary mammary epithelial cells were obtained from Holstein dairy cows and incubated in Dulbecco's modified Eagle's medium-F12 medium (DMEM/F12) containing lactogenic hormones (prolactin and glucocorticoids). Free Phe (117 μg/ml) was substituted partly with peptide-bound Phe (phenylalanylphenylalanine, phenylalanyl threonine, threonyl-phenylalanyl-phenylalanine) in the experimental media. After incubation with experimental medium, cells were collected for gene expression analysis and medium was collected for milk protein or amino acid determination. The results showed that peptide-bound Phe at 10% (11.7 μg/ml) significantly enhanced α_{s1} casein gene expression and milk protein synthesis as compared with equivalent amount of free Phe. When 10% Phe was replaced by phenylalanylphenylalanine, the disappearance of most essential amino acids increased significantly, and gene expression of peptide transporter 2 and some amino acid transporters was significantly enhanced. These results indicate that the Phe and Thr oligopeptides are important for milk protein synthesis, and peptide-bound amino acids could be utilised more efficiently in milk protein synthesis than the equivalent amount of free amino acids.

Keywords phenylalanine, threonine, oligopeptides, casein synthesis, bovine mammary epithelial cell

Correspondence Dr. Y. M. Wu and Dr. H. Y. Liu, Institute of Dairy Science, Ministry of Education Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou 310029, China. Tel: +86 571 889892930; Fax: +86 571 88982930; E-mail: ymwu@zju.edu.cn and Tel: +86 571 88982965; Fax: +86 571 88982930; E-mail: hyliu@zju.edu.cn

Received: 2 October 2013; accepted: 7 August 2014

Introduction

Milk protein is synthesised with 20 amino acids (AA) as precursors in the mammary gland. Free amino acids are the main forms in which amino-N is taken up by mammary tissue. However, it has been observed that the uptake of certain essential AA (EAA) across the mammary gland is insufficient for their output in milk protein in lactating dairy cows and goats (Metcalf et al., 1994; Mabjeesh et al., 2002). A contribution (2.5–23.8%) of circulating peptide-bound EAA to individual EAA has been found to be involved in mammary metabolism and milk protein synthesis (Tagari et al., 2008). The mechanism by which the mammary gland utilises peptides has not been well characterised. The peptide transporter 2 (PepT2) was detected in bovine mammary gland epithelial cells (Zhou et al., 2011). The dipeptides may be taken up in intact form to serve as a source of amino acids (Groneberg et al., 2002; Zhou et al., 2011).

In our previous studies, we investigated supplementation of methionine (Met)- or lysine (Lys)-containing dipeptide for maximal α_{s1} casein gene expression in cultured bovine mammary epithelial tissue/cells (Wu et al., 2007a; Wu, 2007b). Phenylalanine (Phe) and threonine (Thr) are other potential limiting AA after Met and Lys for lactating dairy cows (Bequette et al., 1998; Chamberlain and Yeo, 2003). In this study, we investigate the effects of Phe and Thr oligopeptides supplementation on milk protein synthesis in mammary gland epithelial cells.

Materials and methods

Preparation and culture of mammary gland epithelial cells

Mammary tissues obtained from three healthy mid-lactation Chinese Holstein dairy cows were cut into 1-mm^3 pieces and incubated in medium at 37 °C. The culture medium used was Dulbecco's modified Eagle's

medium (DMEM)-F12 (Gibco, Grand Island, NY, USA), which was supplemented with 1% glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml insulin (Bovine; Sigma, St Louis, MO, USA), 5 μg/ml prolactin (Bovine; Sigma), 1 μg/ml hydrocortisone (Sigma) and 10% foetal calf serum (FCS; Sangon, Shanghai, China). When cells that spread from the tissues covered 80% of the six-well plate bottom, they were dispersed and cultured following the method of Zhao et al. (2010). Briefly, the tissues were removed, and epithelial cells and fibroblasts were separated according to their different sensitivity to 0.25% or 0.15% trypsin plus 0.02% EDTA. The dispersed cells were seeded at a density of 5×10^4 cells/ml on six-well plates and incubated in 2 ml well-cultured medium.

Experimental design and treatments

Cells were subjected to the following experiments. In all treatments, media without FCS were used. Additional AA (Purity >98.5%, Bio Basic, Markham, Canada) were added in culture medium (Table 1). The AA contents were set according to our previous study (Lai, 2006; Liu et al., 2007; Wu et al., 2007a; Wu, 2007b). Except for Phe and Thr, the concentrations of all other free EAA were kept the same. All treatments were carried out in triplicate in each tissue for 48 h, followed by cell harvest for RNA extraction and medium collection for milk protein and/or AA analyses: (i) substitution of 0, 5, 10, 15 and 20% of total free Phe (117 μg/ml) with Phe–Phe dipeptide; and (ii) substitution of 10% of total free Phe (117 μg/ml) with different Phe-containing oligopeptides with equivalent peptide-bound Phe (Phe–Phe, Phe–Thr and Thr–Phe–Phe).

RNA extraction and cDNA synthesis

Total RNA was extracted from the cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The RNA purity was determined by optical density (OD260 nm/OD280 nm absorption ratio >1.80). RNA quality and integrity was checked by gel electrophoresis, and RNA with good quality was used for cDNA synthesis. Average RNA quantity was approximately 7 μg per sample. RNA was eluted and immediately used as template in first-strand cDNA synthesis. Reverse transcription was performed in 0.2-ml PCR tubes in a total volume of 10 μl containing 2 μl PrimeScript™ Buffer (5×), 0.5 μl PrimeScript™ RT, 0.5 ml Oligo (dT) primer (25 μM), 0.5 μl Random 6 mer (50 μM; Takara, Tokyo, Japan) and 6.5 μl RNA (total quantity <500 ng). The reaction was incubated at 37 °C for 15 min followed by 85 °C for 5 s, and the cDNA was then stored at −20 °C.

Table 1 Concentration of several essential amino acids in medium

	Several essential amino acids in medium						
	Phe	Thr	Met	Lys	Leu	Ile	Val
Concentration (μg/ml)	117	123	60	210	214	120	154

Phe, phenylalanine; Thr, threonine; Met, methionine; Lys, lysine; Leu, leucine; Ile, isoleucine; Val, valine.

qPCR

The mRNA expression of individual genes was determined by SYBR green method of quantitative real-time PCR following the MIQE guidelines (Bustin et al., 2009). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene. The primers of α_{s1} casein (GenBank Accession Number NM_181029), PepT2 (NM_001079582), dipeptidase 2 (DPEP2, XM_586714), sodium- and chloride-dependent neutral and basic amino acid transporter B(0+) (ATB^{0+}, NM_001098461), cationic amino acid transporter (y+ system, member 2-like isoform 2; y+CAT2, XM_865568), aromatic amino acid transporter (TAT1, NM_001192847), neutral amino acid transporter (ASCT1, NM_001081577) and GAPDH (AJ_000039) were specifically designed from their cDNA sequences (Table 2). Real-time PCR was performed in 96-well clear optical plates in triplicate. The reaction contains 10.0 μl of 1× SYBR® Premix Ex Taq™ (Takara), 0.4 μl of forward primer (10 μM), 0.4 μl of reverse primer (10 μM), 0.4 μl of ROX Reference Dye II (50×, Takara), 6.8 μl of ddH_2O and 2 μl of the template cDNA 2 μl of ddH_2O instead of template cDNA was used as negative controls. PCR cycle conditions were 10 s at 95 °C, followed by 40 cycles of 5 s at 95 °C and 34 s at 60 °C in an ABI 7500 Sequence Detector (Applied Biosystems, Grand Island, NY, USA). The Cq values of α_{s1} casein, PepT2, DPEP2, ATB^{0+}, y+CAT2, TAT1, ASCT1 and GAPDH were calculated under default settings of the real-time sequence detection software (Applied Biosystems). The Cq was defined as the quantification cycle at which a significant increase in the magnitude of the signal generated by the PCR was first detected. Amplification efficiencies of all detected genes were within 95%–105%. The proportional change detected in mRNA is calculated as $2^{-\Delta\Delta Cq}$.

Total protein content determination

Total protein content in culture medium was determined by the method of Bradford (1976), using the

Table 2 Oligonucleotide primer sets for real-time PCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Source
α_{s1} casein	CCTAAACATCCTATCAAGCACCAA	ATTGACCTTCTCCTTTCCAAACAC	NM_181029
PepT2	ATGGCAATGCCCAATGAAG	CACCAACACAGCAACAAACAAA	NM_001079582
GAPDH	GCCAAGAGGGTCATCATCTC	GGTCATAAGTCCCTCCACGA	AJ_000039
DPEP2	GACCAGAAACTAACCAGAACTCCAA	CACCAGAGCCAAAGAATGAGG	XM_586714
ATB^{0+}	TGGAAGTGAAGTTTATCAGACAGGA	GACTATGAGCCAAGCCAGAAGAA	NM_001098461
y+CAT2	TCCCAATGCCTCGTGTAATCT	CGAAAAGGAACGCCATCAC	XM_865568
TAT1	CTTCGTGTTTGTCCTCTTTCTGG	GTCTGTTGCCTCCTTTGGTTG	NM_001192847
ASCT1	TGTAGTTGTTGGTGTGTATGTGTGG	GAGGTAGGTAAAAGGCAGGTGATG	NM_001081577

PepT2, peptide transporter 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DPEP2, dipeptidase 2; ATB^{0+}, sodium- and chloride-dependent neutral and basic amino acid transporter B(0+); y+CAT2, cationic amino acid transporter (y+ system, member 2-like isoform 2; TAT1, aromatic amino acid transporter; ASCT1, neutral amino acid transporter.

BOSTER Protein Assay Kit according to the manufacturer's instructions (BOSTER, Wuhan, China). Bovine serum albumin (BSA) was used in making the standard curve.

Amino acid content determination

Concentrations of individual AA in culture medium were analysed using post-cation exchange column ninhydrin colorimetric method with commercial kits (Diasys Diagnostic Systems, Shanghai, China) according to the procedures described by Calder et al. (1999).

Statistical analysis

For every treatment, data of three different wells from the same cow was averaged. Three averaged data from different cows were subjected to one-way ANOVA using SAS statistical software package, and the maximum response was determined using the PROC RSREG in SAS (SAS Institute, 2000). For all the analyses, differences were considered significant at $p < 0.05$.

Results

Effect of partial replacement of free Phe by Phe–Phe dipeptide on milk protein synthesis

Replacement of free Phe with Phe–Phe dipeptide at 10% increased the α_{s1} casein mRNA expression quadratically ($p < 0.01$; Fig. 1a). The optimal replacement for the highest protein synthesis was estimated to be 7.06% (Fig. 1b). When 10% of free Phe were replaced by Phe–Phe, concentrations of Lys, Phe, leucine (Leu), isoleucine (Ile) and valine (Val) in culture medium decreased ($p < 0.05$; Fig. 2). It also significantly increased mRNA abundance of α_{s1} casein, PepT2, ATB^{0+} and y+CAT2 ($p < 0.05$), and had no effect on TAT1, ASCT1 and DPEP2 gene expression (Fig. 3).

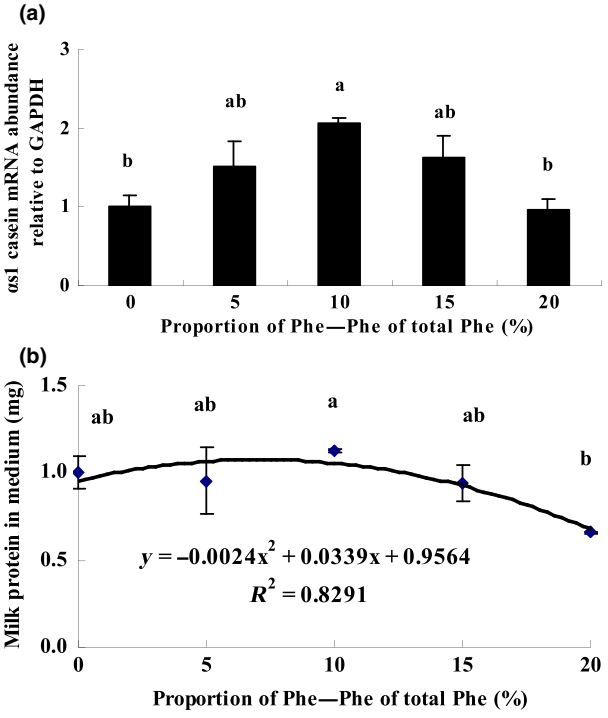

Fig. 1 Effects of partial replacement of free Phe with Phe–Phe dipeptide on α_{s1} casein gene expression (a) and protein secretion (b). Bovine mammary gland epithelial cells were incubated in DMEM/F12 medium containing substitution of free Phe by Phe–Phe for 48 h. A. Alpha s1 casein mRNA abundance relative to GAPDH was determined by real-time PCR. B. Total protein relative to DNA of attached cells was detected by Bradford method. Average values ±SE from three independent analyses are presented in the bar graphs. Bars with different superscripts (a and b) are statistically different ($p < 0.05$).

Effects of Phe–Phe, Phe–Thr and Thr–Phe–Phe on milk protein synthesis

Bovine mammary epithelial cells were cultured in medium in which 10% free Phe (117 μg/ml) were replaced by Phe–Phe, Phe–Thr or Thr–Phe–Phe for 48 h. The treatment with Thr–Phe–Phe increased α_{s1}

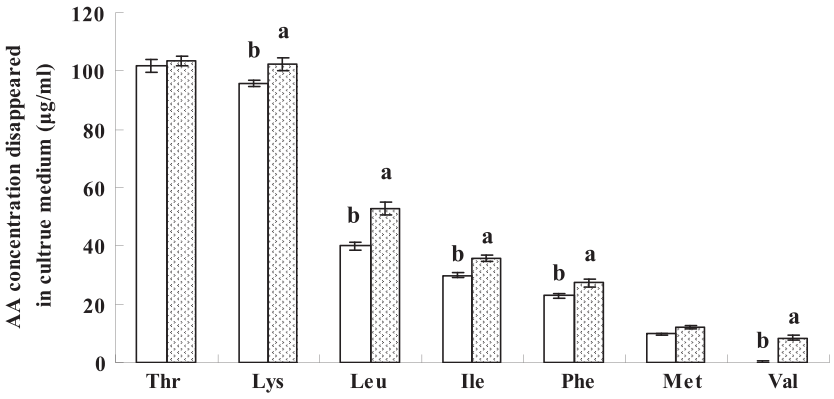

Fig. 2 Effects of replacement of 10% free Phe by Phe–Phe dipeptide on the utilisation of individual amino acids in culture medium. Bovine mammary gland epithelial cells were incubated in DMEM/F12 medium in which 0% (open bar) or 10% (filled bar) free Phe was replaced by Phe–Phe for 48 h. Amino acid concentrations in culture medium were analysed according to the procedure described by Calder et al. (1999). Bars with different superscripts (a and b) are statistically different ($p < 0.05$).

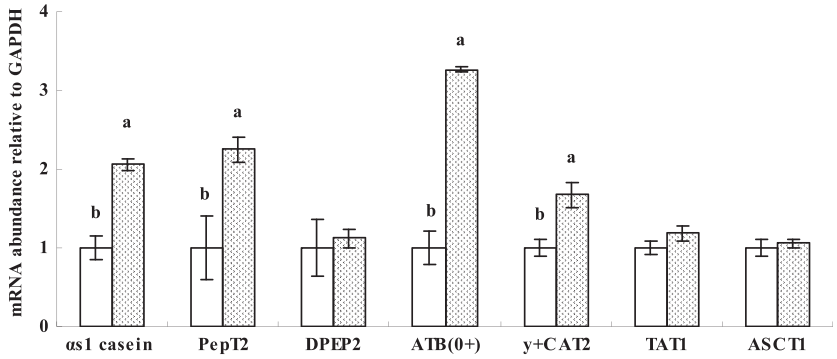

Fig. 3 Effects of replacement of 10% free Phe by Phe–Phe dipeptide on mRNA abundance of α_{s1} casein, PepT2, DPEP2 and amino acid transporter. Bovine mammary gland epithelial cells were cultured in DMEM/F12 medium in which 0% (open bar) or 10% (filled bar) by Phe–Phe for 48 h. The mRNA abundance of individual genes relative to GAPDH in the cells was determined by real-time PCR. Average values ±SE from three independent analyses are presented in the bar graphs. Bars with different superscripts (a and b) are statistically different ($p < 0.05$).

casein gene expression ($p < 0.01$; Fig. 4a). Compared with control, treatments with Phe–Phe, Phe–Thr and Thr–Phe–Phe all increased total protein in the media ($p < 0.05$; Fig. 4b).

Discussion

Peptide-bound amino acids (PBAA) have been observed to contribute to mammary gland metabolism and protein synthesis and secretion in different studies (Backwell et al., 1997; Remond et al., 2000; Tagari et al., 2008). In this study, substitutions of 10% free Phe with Phe–Phe significantly promoted the α_{s1} casein expression in cultured mammary epithelial cells. The optimal ratio of Phe–Phe to total Phe for milk protein synthesis was estimated to be 7.06%, in which Phe–Phe could be utilised with a higher efficiency than equivalent free Phe in milk protein synthesis. With the further increases in Phe–Phe, the α_{s1} casein gene expression and milk protein were depressed. It seems that along with increasing concentration of Phe–Phe and decreasing concentration of Phe, the free Phe alone may not satisfy the requirement for milk protein synthesis. The result is consistent with the finding that histidine (His)- containing dipeptide enhanced milk protein production compared with free His (Backwell et al., 1996). Arteriovenous balance studies across the mammary gland of dairy cows have indicated that the uptake of Phe is only 80–90% of that required for milk protein output (Bickerstaffe et al., 1974; Metcalf et al., 1994). Backwell et al. (1996) compared plateau enrichments of blood, plasma and casein of the lactating dairy goat and concluded that sources other than the labelled plasma-free amino acids (e.g. peptides or proteins) contribute l0–20% of Phe for casein biosynthesis. The present result is consistent with their conclusion.

The mechanism for the utilisation of dipeptides in mammary gland is still unclear. In the *in vivo* experiment with rats by Shennan et al. (1998), it was observed that dipeptides were hydrolysed extracellularly followed by the uptake of the constituent amino acids. However, it is hard to explain that the addition

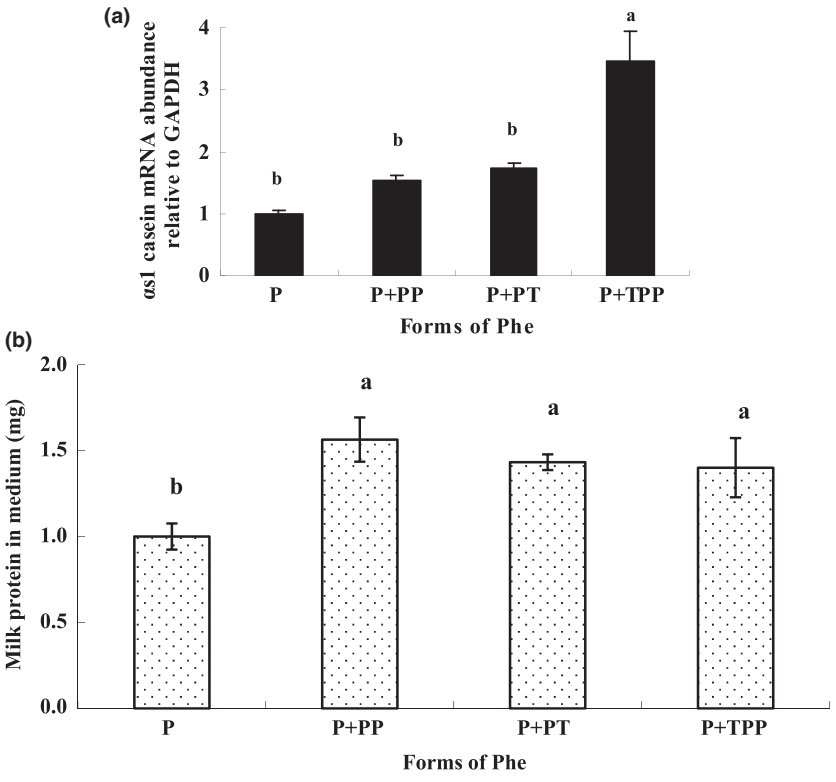

Fig. 4 Effects of Phe–Phe, Phe–Thr and Thr–Phe–Phe supplementation on α_{s1} casein gene expression (a) and protein secretion (b). Bovine mammary gland epithelial cells were cultured in DMEM/F12 medium containing Phe–Phe (PP), Phe–Thr (PT) or Thr–Phe–Phe (TPP) replacing 10% free Phe (P) by for 48 h. A. Alpha s1 casein mRNA abundance relative to GAPDH was determined in the cells by real-time PCR. B. Total protein relative to DNA was detected by Bradford method. Average values ±SE from three independent analyses are presented in the bar graphs. Bars with different superscripts (a and b) are statistically different ($p < 0.05$).

of 60 μg/ml of Met–Met dipeptide promoted the α_{s1} casein expression in cultured mammary epithelial cells as much as 60 μg/ml free Met treatment (Wu et al., 2007a). In the present study, α_{s1} casein and PepT2 gene expression were significantly increased when cells were incubated in medium containing Phe–Phe. However, the cytomembrane-located dipeptidase 2 mRNA abundance was not affected by Phe–Phe addition. This suggests that Phe dipeptide may be taken up in intact form by PepT2 in mammary epithelial cells.

It has been proven that the peptide transporter has a high affinity to oligopeptide consisting amino acid residues that are hydrophobic and bulky, such as Met and Phe, and a low affinity to oligopeptide with hydrophilic and charged amino acids residues, such as Thr (Matthews, 1991; Wang et al., 2004). However, in this study, although treatment with Thr–Phe–Phe significantly enhanced α_{s1} casein gene expression as compared with other groups, all three oligopeptides had similar utilisation efficiency for milk protein synthesis.

Amino acids are transported across the plasma membrane by many carriers, and there are many interactions among these carriers, involving inhibition or stimulation of AA transportation and even competition for energy supply. When a peptide is taken up, competition for transport between the constituent amino acids may be partly or completely avoided (Matthews et al., 1969). In the study, ATB^{0+} and y+CAT2 gene expression and total uptake of some amino acids were significantly increased when cells were incubated in Phe–Phe as 10% of total Phe. This may be due to reduced competition between AA. Thus, supply mammary gland with small peptides may be a way to increase milk protein synthesis.

In conclusion, the supply of the mammary gland with small peptides has the potential to influence milk protein synthesis to some extent. Mammary epithelial cells have highest α_{s1} casein abundance when peptide-bound Phe accounts for about 10% of total Phe in the medium.

Acknowledgements

This study was supported by the National Natural Science Foundations of China (Nos. 31372336, 30901034), the National Key Technologies R&D Program of China (2012BAD12B02) and the earmarked fund for China Agriculture Research System (CARS-37).

References

Backwell, F. R. C.; Bequette, B. J.; Wilson, D.; Metcalf, J. A.; Franklin, M. F.; Beever, D. E.; Lobley, G. E.; MacRae, J. C., 1996: Evidence for the utilization of peptides for milk protein synthesis in the lactating dairy goat in vivo. *American Journal of Physiology* **271**, R955–R960.

Backwell, F. R. C.; Hipolito-Reis, M.; Wilson, D.; Bruce, L. A.; Buchan, V.; MacRae, J. C., 1997: Quantification of circulating peptides and assessment of peptide uptake across the gastrointestinal tract of sheep. *Journal of Animal Science* **75**, 3315–3322.

Bequette, B. J.; Backwell, F. R. C.; Crompton, L. A., 1998: Current concepts of amino acid and protein metabolism in the mammary gland of the lactating ruminant. *Journal of Dairy Science* **81**, 2540–2559.

Bickerstaffe, R.; Annison, E. F.; Linzell, J. F., 1974: The metabolism of glucose, acetate, lipids and amino acids in lactating cows. *The Journal of Agricultural Science* **82**, 71–85.

Bradford, M. M., 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.

Bustin, S. A.; Benes, V.; Garson, J. A.; Hellemans, J.; Huggett, J.; Kubista, M.; Mueller, R.; Nolan, T.; Pfaffl, M. W.; Shipley, G. L.; Vandesompele, J.; Wittwer, C. T., 2009: The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry* **55**, 611–622.

Calder, A. G.; Garden, K. E.; Anderson, S. E.; Lobley, G. E., 1999: Quantitation of blood and plasma amino acids using isotope dilution electron impact gas chromatography/mass spectrometry with U-13C amino acids as internal standards. *Rapid Communications in Mass Spectrometry* **13**, 2080–2083.

Chamberlain, D. G.; Yeo, J. M., 2003: Effects of amino acids on milk production. In: J. P. F. D'Mello (ed.), *Amino Acids in Animal Nutrition*, 2nd edn. CABI Publishing, Wallingford, UK, pp. 367–387.

Groneberg, D. A.; Doring, F.; Theis, S.; Nickolaus, M.; Fischer, A.; Daniel, H., 2002: Peptide transport in the mammary gland: expression and distribution of PEPT2 mRNA and protein. *American Journal of Physiology. Endocrinology and Metabolism* **282**, E1172–E1179.

Lai, J. L., 2006: Effects of essential amino acids on as1 casein gene expression in cultured bovine mammary gland explants. Dissertation. Zhejiang University, China.

Liu, H. Y.; Yang, J. Y.; Wu, H. H.; Wu, Y. M.; Liu, J. X., 2007: Effects of methionine and its ratio to lysine on expression of α_{s1} casein gene in cultured bovine mammary epithelial cells. *Journal of Animal Feed Science* **16**, 330–334.

Mabjeesh, S. J.; Kyle, C. E.; MacRae, J. C.; Hanigan, M. D.; Bequette, B. J., 2002: Vascular sources of amino acids for milk protein synthesis in goats at two stages of lactation. *Journal of Dairy Science* **85**, 919–929.

Matthews, J. C., 1991: Absorption of carnosine and methionylglycine by sheep ruminal and omasal epithelia. Dissertation. Virginia Polytechnic Institution and State University.

Matthews, D. M.; Lis, M. T.; Cheng, B.; Crampton, R. F., 1969: Observations on intestinal absorption of some oligopeptides of methionine and glycine in rat. *Clinical Science* **37**, 751.

Metcalf, J. A.; Beever, D. E.; Sutton, J. D.; Wray-Cahen, D.; Evans, R. T.; Humphries, D. J.; Backwell, F. R. C.; Bequette, B. J.; MacRae, J. C., 1994: The effect of supplementary protein on in vivo metabolism of the mammary gland in lactating dairy cows. *Journal of Dairy Science* **77**, 1816–1827.

Remond, D.; Bernard, L.; Poncet, C., 2000: Free and peptide amino acid net flux across the rumen and the mesenteric- and portal- drained viscera of sheep. *Journal of Animal Science* **78**, 1960–1972.

SAS Institute, 2000: *SAS User's Guide: Statistics. Version 8.01*. SAS Inst., Cary, NC.

Shennan, D. B.; Calvert, D. T.; Backwell, F. R. C.; Boyd, C. A. R., 1998: Peptide aminonitrogen transport by the lactating rat mammary gland. *Biochimica et Biophysica Acta (BBA)-. Biomembranes* **1373**, 252–260.

Tagari, H.; Webb, K. Jr; Theurer, B.; Huber, T.; DeYoung, D.; Cuneo, P.; Santos, J. E. P.; Simas, J.; Sadik, M.; Alio, A.; Lozano, O.; Delgado-Elorduy, A.; Nussio, L.; Bittar, C. M. M.; Santos, F., 2008: Mammary uptake, portal-drained visceral flux, and hepatic metabolism of free and peptide-bound amino acids in cows fed steam-flaked or dry-rolled sorghum grain diets. *Journal of Dairy Science* **91**, 679–697.

Wang, Y. J.; Li, S. L.; Ou, Y.; Luo, Y. P., 2004: The advance on the absorption and utilization of small-peptide in ruminants. *Chinese Journal of Animal Nutrition* **16**, 1–5.

Wu, H. H., 2007b: Effects of amino acids pattern and methionylmethinoine on the synthesis of as1 casein in cultured bovine mammary tissue. Dissertation. Zhejiang University Hangzhou.

Wu, H. H.; Yang, J. Y.; Zhao, K.; Liu, H. Y.; Wu, Y. M.; Liu, J. X., 2007a: Effects of methionine-containing dipeptides on casein α_{s1} expression in bovine mammary epithelial cells. *Journal of Animal Feed Science* **16**, 7–12.

Zhao, K.; Liu, H. Y.; Zhou, M. M.; Liu, J. X., 2010: Establishment and characterization of a lactating bovine mammary epithelial cell model for the study of milk synthesis. *Cell Biology International* **34**, 717–721.

Zhou, M. M.; Wu, Y. M.; Liu, H. Y.; Zhao, K.; Liu, J. X., 2011: Effects of tripeptide and lactogenic hormones on oligopeptide transporter 2 in bovine mammary gland. *Journal of Animal Physiology and Animal Nutrition* **95**, 781–789.