Effects of methionine and its ratio to lysine on expression of $\alpha_{s1}$ casein gene in cultured bovine mammary epithelial cells*

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ABSTRACT

Effects of methionine dose and its ratio to lysine on $\alpha_{s1}$ casein gene expression were evaluated in cultured bovine mammary epithelial cells. Methionine was added at levels of 0-100 $\mu$g/ml and ratio of lysine to methionine was set at 1-4.5:1. Expression of $\alpha_{s1}$ casein gene was enhanced by addition of methionine up to 60 $\mu$g/ml, and then leveled off. There was an increasing trend in $\alpha_{s1}$ casein expression with the increasing ratio of lysine to methionine and the highest level was at 3.5:1. These results indicated that adequate quantities and ratio of amino acids are required to promote milk protein synthesis.

KEY WORDS: mammary epithelial cell, methionine, lysine, $\alpha_{s1}$ casein gene expression

INTRODUCTION

Methionine (Met) and lysine (Lys) are often considered to be co-limiting amino acids (AA) for milk protein synthesis (Schwab et al., 1992). Most of the reported responses in milk and milk protein yields to infused Met and Lys have been in cows given diets prepared to be severely limiting in one, or both, AA. The influence of adequate quantities and ratio of Met and Lys on milk protein synthesis still remains uncertain. A number of models have been developed to examine AA metabolism in the mammary gland. Until recently, there has not been a comprehensive representation of AA metabolism in the udder of the lactating dairy cow (Hanigan et al., 2002). The epithelial cells derived from bovine

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mammary gland are suitable for \textit{in vitro} model to study mammary function (Wu et al., 2004; Zhao et al., 2005). In the present study, we adopted the cells to evaluate the effects of Met and its ratio to Lys on the expression of $\alpha_{sl}$ casein gene.

\textbf{MATERIAL AND METHODS}

\textit{Reagents}

DMEM/F12 was obtained from Gibco BRL Life Technologies (Carlsbad, CA). Reagents for reverse transcriptase (RT)-PCR assay were purchased from Promega (Madison, WI). Trypsin was obtained from Sigma (Louis, MO). All other reagents were of the highest purity commercially available, purchased from Sangon (Shanghai).

\textit{Culture of mammary epithelial cells}

Mammary tissues were obtained from two slaughtered Holstein dairy cows at the middle stage of lactation. Tissues were cut into 1 mm$^3$ pieces and incubated in culture plates (Nunc, Denmark) at 37°C in a water-saturated atmosphere of 95% air and 5% CO$_2$. The basal medium was supplemented with 2 mM glutamine, 100 IU/ml penicillin and, $\mu$g/ml: streptomycin 100, insulin 10, transferrin 5, prolactin 5, hydrocortisone 2 and foetal calf serum (FCS) 10. After the cells covered 80% of the bottom, the tissue and cells were digested with, %: trypsin 0.15 and EDTA 0.02 at 37°C in a shaking bath. The dispersed cells were filtrated through a 150-$\mu$m mesh and then seeded in 6-well culture plates. To ensure the quality of the cell culture, the cultured cells were identified by cytokeratin 18 because the mammary epithelial cells were immunostained positively for the cytokeratin.

\textit{Treatment of cultured cells with amino acids}

The amino acids were diluted with medium. At the beginning of culture, mammary epithelial cells were treated with different doses of Met (0-100 $\mu$g/ml) in medium. Using optimal dose of Met, cells were then challenged with different ratio (1:1-4.5:1) of Lys to Met.

\textit{RT-PCR assay}

Total cellular RNA was extracted by Trizol (In Vitrogen). The RNA purity was determined by optical density (OD260 nm/OD280 nm absorption ratio >1.80). The $\alpha_{sl}$ casein and GAPDH mRNA was amplify by RT-PCR according to the previous report (Wu et al., 2004). PCR products were analysed by electrophoresis in 1.2%
agarose gel. The net intensities of individual bands were measured using Image Master VDS Software (Pharmacia Biotech, Sweden). The ratio of the intensities of $\alpha_{s1}$ casein to GAPDH represented the relative mRNA level of $\alpha_{s1}$ casein. The average level of three repeats was used for statistical analysis.

**Statistical analysis**

All data were analysed by ANOVA and Duncan’s multiple range tests using the SAS 9.0 software. P<0.05 was considered as significantly different.

**RESULTS**

$\alpha_{s1}$ Casein mRNA expression was increased with the increasing level of Met up to 60 $\mu$g/ml, and then leveled off (Figure 1). From the fitted curve of optical density of the electrophoretic $\alpha_{s1}$ casein gene expression ($y$) and Met level ($x$, $\mu$g/ml): $y=-0.0071x^2+1.5139x+14.855$ ($R^2=0.8692$), it was estimated that the highest abundance of $\alpha_{s1}$ casein mRNA occurred at 57 $\mu$g/ml of Met (P<0.05).

![Figure 1](image1.png)

Figure 1. Effects of methionine (Met) levels on $\alpha_{s1}$ casein gene expression in cultured mammary epithelial cells. A - agarose gel electrophoresis. B - means of optical density of the electrophoretic $\alpha_{s1}$ casein gene expression influenced by adding Met

There was an increasing trend in $\alpha_{s1}$ casein mRNA expression with increasing ratio of Lys to Met in mammary epithelial cells (Figure 2). Highest $\alpha_{s1}$ casein mRNA expression was observed at ratio of 3.5:1 (P<0.05). However, no obvious changes were found among all groups at the ratio from 3:1 to 4.5:1.
DISCUSSION

The lactating mammary gland has a large demand for AA to meet the requirements for milk protein synthesis. Identification of AAs that limit milk protein synthesis has been a focus of ruminant nutrition studies for a long time. These studies have been interpreted collectively as suggesting that Met and Lys are generally limiting milk protein synthesis (Schwab et al., 1992). The most direct evidence of their limitation has been observed by infusing individual or combination of AAs into the abomasum or duodenum and measuring effects on N retention and milk protein production. Responses of lactating dairy cows to improved supplies of Met and Lys in metabolizable protein (MP) include variable increases in milk protein contents and yield (Garthwaite et al., 1998).

The mammary cells can respond to changes in nutritional adequacy of its external and internal AA environments by altering the activities of the AA transport systems. Therefore, this system was sensitive to the exogenous nutrients and could be used as a model for evaluating actions of AA and peptides on protein synthesis in vitro (Barnett et al., 2004; Zhao et al., 2004).

In this study, mammary epithelial cells were adopted to evaluate the effects of Met and its ratio to Lys on $\alpha_{s1}$ casein mRNA expression. The $\alpha_{s1}$ casein gene was found to most abundantly express at 57 $\mu$g/ml of Met. There was an increasing trend in $\alpha_{s1}$ casein mRNA expression when the ratio of Lys to Met increased from

![Figure 2](image_url). Effects of the ratio of lysine (Lys) to methionine (Met) on $\alpha_{s1}$ casein gene expression in cultured mammary epithelial cells. A - agarose gel electrophoresis. B - means of optical density of the $\alpha_{s1}$ casein gene expression influenced by Lys to Met ratio.
The αs1 casein gene expression showed the highest level at the ratio of 3.5:1 of Lys to Met. These results indicated that appropriate quantities and ratio of Met and Lys significantly increase the synthesis of milk protein. These results were in agreement with the viewpoints of Wu et al. (1999) and the NRC (2001) recommendations for the maximum use of Lys and Met for milk production. Imbalances created by excesses or deficiencies of dietary AA may limit milk protein synthesis and reduce lactation performance. Therefore, adequate quantities and ratio of AA are required to promote milk protein synthesis and its yield.

In summary, the mammary epithelial cell may be a suitable in vitro model to study AA metabolism. The αs1 casein mRNA expression was most abundant at the Met level of 57 μg/ml and showed the highest level in the ratio of 3.5:1 of Lys to Met.