***Cite this as*:** Hai-na GAO, Han HU, Nan ZHENG, Jia-qi WANG. 2015. Leucine and histidine independently regulate milk protein synthesis in bovine mammary epithelial cells via the phosphorylation of the translation factors of the mTOR signaling pathway. *Journal of Zhejiang University-Science B (Biomedicine & Biotechnology)*, 16(6):560-572. [doi:10.1631/jzus.B1400337]

Leucine and histidine independently regulate milk protein synthesis in bovine mammary epithelial cells via the phosphorylation of the translation factors of the mTOR signaling pathway

Key words: Bovine mammary epithelial cells, Leucine, Histidine, Western-blotting, mTOR, Casein

Introduction

- Dairy cow nutrient requirement systems regard essential amino acid (EAA) requirements in aggregate as metabolizable protein (MP), and presume a fixed efficiency of MP use for milk protein (Arriola Apelo *et al*., 2014). The composition of EAAs in MP vastly affect milk protein synthesis. Since the exact amount of limiting amino acids (LAAs) in dairy cow's diets were unknown, cows were easily overfed to meet the balance of MP requirements, which lead to a waste of AAs and poor N efficiency (Bionaz *et al*., 2012 ; Arriola Apelo *et al*., 2014).
- AAs not only serve as a precursor for protein synthesis but also as signaling molecules that regulate the protein synthesis in mammalians (Arriola Apelo *et al*., 2014). A great number of reports have shown that AA-induced stimulation of milk protein synthesis is partially mediated by the mammalian target of rapamycin (mTOR), a protein kinase present in the rapamycin-sensitive mTOR complex 1(mTORC1) (Appuhamy *et al*., 2012; Arriola Apelo *et al*., 2014).
- These studies have shown that Leu and His in addition to Lys and Met are main LAAs in forage diets (Kim *et al*. 2001;Korhonen *et al*., 2002). Our study investigated the regulatory effects of different concentrations of Leu and His on the mTOR signaling pathway and the relationships between these signaling protein activities with milk protein synthesis.

Results

The WB results showed that, compared to the control group:

- The expression of caseins and the phosphorylation of mTOR (Ser2481), Raptor (Ser792), eIF4E (Ser209) and eEF2 (Thr56) ($P < 0.01$) increased with the Leu concentrations ranging from 0.45 to 10.8 mM. The P-4EBP1 (Thr37) and GβL at 10.8 mmol/L, and P-RPS6 (Ser235/236) from 5.4 to 10.8 mmol/L all decreased.

- The His supplementation from 0.15 to 9.6 mmol/L increased the expression of αs2-, β- , κ-caseins, P-mTOR (Ser2481), P-Raptor (Ser792), P-S6K1 (Thr389), P-4EBP1 (Thr37), P-eIF4E (Ser209) and P-eEF2 (Thr56) ($P < 0.01$) in CMEC-H. Whereas the αs1-casein expression was only reduced at 9.6 mmol/L, GβL at 0.15 and 9.6 mmol/L and P-RPS6 from 4.8 mmol/L to 9.6 mmol/L.

Our linear regression model assay suggested that :

- the αs1-casein expression was positively correlated with P-mTOR ($P<0.01$), P-S6K1 ($P<0.01$) and P-eEF2 ($P<0.01$) for the addition of Leu. While the expression of β-casein ($P<0.01$) and κ-casein ($P<0.01$) were positively correlated with P-eEF2 for the addition of His. In conclusion, the milk protein synthesis was up-regulated through activation of the mTOR pathway with the addition of Leu and His in CMEC-H.

Innovation points

- Although the effects of EAAs on mTOR cell signaling and milk protein synthesis have been extensively explored (Moshel, *et al*., 2006; Burgos, *et al*., 2010; Toerien, *et al*., 2010), effects of different levels of Leu or His on phosphorylation of Raptor on Ser792, mTOR on Ser 2481 and GβL expression involved in casein protein synthesis were not clear in bovine mammary cells.

- We can exclude the possibility that phosphorylation of mTOR on Ser 2448 was not the only one regulating mTOR activity in CMECs. The P-mTOR at Ser2481 was also a good biomarker for mTOR pathway activation in CMECs.

- Our results provide basic information for further study to clarify the regulation mechanism of Leu and His on casein expression through the mTOR pathway in the dairy cattle mammary gland.