Amino Acid Metabolism and Utilization in Bovine Mammary Gland and Their Signaling Pathways: Postprint

Authors: Liu Lan, Liu Hongyun, Liu Jianxin

Date: 2017-10-10T00:00:00+00:00

Abstract

The mammary gland is a crucial organ for milk synthesis in dairy cows, and nutrient metabolism within the mammary gland is closely associated with milk quality. Milk protein, as the most important nutrient component in milk, has consistently been a focus of research interest. Amino acids serve as important precursors for milk protein synthesis and also function as regulatory factors involved in the modulation of mammary metabolic pathways. Amino acid metabolism within the mammary gland encompasses numerous signaling pathways; in-depth investigation of these pathways can elucidate the regulatory mechanisms of milk synthesis at the cellular and molecular levels, thereby providing a theoretical foundation for the nutritional regulation of amino acid metabolism in dairy cow mammary glands. This review summarizes amino acid metabolism in dairy cow mammary glands and the associated signaling pathways, and discusses their relationship with nutrition.

Full Text

Preamble

Title: Amino Acid Metabolism and Signaling Pathways in Bovine Mammary Gland

Authors: LIU Lan, LIU Hongyun*, LIU Jianxin*

Affiliation: Institute of Dairy Science, College of Animal Science, Key Laboratory of Molecular Animal Nutrition of Ministry of Education, Zhejiang University, Hangzhou 310058, China

Abstract: The mammary gland is a crucial organ for milk synthesis in dairy cows, and nutrient metabolism within the mammary gland is closely related to milk quality. Milk protein, as the most important nutrient component in

milk, has long been a focus of research attention. Amino acids serve not only as essential precursors for milk protein synthesis but also as regulatory factors involved in modulating metabolic pathways in the mammary gland. Amino acid metabolism in the mammary gland involves numerous signaling pathways, and in-depth investigation of these pathways can reveal the regulatory mechanisms of milk synthesis at the cellular and molecular levels, providing a theoretical basis for nutritional regulation of amino acid metabolism in the dairy cow mammary gland. This review summarizes amino acid metabolism in the bovine mammary gland and its associated signaling pathways, and discusses their relationship with nutrition.

Keywords: bovine mammary gland; amino acid metabolism; signaling pathway

CLC number: S852.2 **Document code:** A **Article ID:**

Received date: 2015-08-25

Funding: National Key Basic Research Program of China (973 Program) "Study on the Formation and Regulation Mechanism of Important Milk Quality Traits" (2011CB100801); National Natural Science Foundation of China (31372336)

Author biography: LIU Lan (1990–), female, from Harbin, Heilongjiang, PhD candidate, engaged in molecular nutrition research. E-mail: liulan11279@163.com

Corresponding authors: LIU Hongyun, associate professor, doctoral supervisor, E-mail: hyliu@zju.edu.cn; LIU Jianxin, professor, doctoral supervisor, E-mail: liujx@zju.edu.cn

Introduction

Nutrient metabolism in the mammary gland has long been a focal point in dairy cow nutrition research. Milk protein represents the most critical nutrient in milk and serves as a key indicator of milk quality. As the fundamental building blocks of milk protein, amino acids are metabolically active within the mammary gland and intimately associated with lactation. The majority of free amino acids taken up from blood by the mammary gland are utilized for milk protein synthesis, while also playing important metabolic regulatory roles. Extensive in vivo and in vitro studies have been conducted on amino acid metabolism in dairy cows, yet the molecular mechanisms remain unclear. Signaling pathway research can elucidate nutritional regulatory mechanisms at the cellular and molecular level, providing a theoretical foundation for improving mammary gland function through nutritional interventions to enhance milk quality and yield.

The mammary gland is the primary site of milk synthesis, and its functional characteristics fundamentally determine the economic value of dairy cows. The

mammary gland of mammals is a unique organ that undergoes cyclical development throughout life in conjunction with pregnancy, exhibiting distinct metabolic characteristics at different lactation stages. The synthesis and secretion of milk in the mammary gland is a complex process, and its metabolic status influences nutrient uptake and absorption by the mammary gland [1]. During the metabolically active lactation period, protein synthesis in the dairy cow mammary gland accounts for 43% of whole-body protein synthesis, with 90% being milk protein [2]. Protein metabolism in the mammary gland involves both amino acid assembly and synthesis as well as degradation processes, representing a dynamic equilibrium. Amino acids, as precursors for milk protein synthesis, have consistently been a research priority in milk protein metabolism. In-depth investigation of mammary gland amino acid utilization and the regulatory mechanisms by which amino acids control milk protein synthesis is therefore essential.

2 Amino Acid Metabolism in the Mammary Gland

The mammary gland primarily takes up free amino acids from blood. Amino acids enter mammary epithelial cells via transport carriers to undergo protein synthesis and degradation. Amino acid metabolism within the mammary gland is complex, and the metabolic processes and efficiencies of various amino acids require further elucidation. The mammary gland increases milk protein yield and improves metabolizable protein utilization by enhancing the uptake of essential amino acids and selectively absorbing non-essential amino acids in balanced proportions, thereby minimizing the catabolism of absorbed amino acids [3].

Hormones influence amino acid metabolism; for instance, growth factors affect the transport of multiple amino acids [4], and lactogenic hormones can promote the expression of amino acid oxidases [5]. Studying amino acid metabolism through in vivo infusion of isotopically labeled amino acids provides valuable insights into amino acid metabolism in the whole body and mammary gland, though the specific mechanisms remain to be revealed. The wide variety of amino acids, numerous influencing factors, and differential metabolism under various physiological states substantially increase research difficulty. Only through comprehensive understanding of amino acid uptake and metabolic patterns in ruminant mammary tissue can we reasonably determine amino acid requirements and regulate them to achieve the goals of increasing milk yield and improving milk quality.

2.1 Methionine

The sulfur-containing amino acids methionine and cysteine play important roles in mammalian protein metabolism. Methionine is the first limiting amino acid for dairy cows fed corn-based diets, with mammary uptake being lower than milk secretion. Beyond its close association with protein synthesis, methionine also undergoes various tissue-specific metabolic conversions, such as generating phospholipids, carnitine, and polyamines [6]. Additionally, methionine serves as a

methyl donor, playing a crucial role in transmethylation reactions that regulate DNA activity and oncogene expression, while also providing sulfur for cysteine synthesis. Supplementing methionine alone or in combination with lysine significantly enhances milk protein synthesis in dairy cows fed corn-based diets [7]. Studies in cultured bovine mammary epithelial cells have found that methionine promotes β-casein gene expression, with the 60S ribosomal protein L35 subunit playing an important role in methionine-mediated regulation of β-casein gene translational elongation and secretion [8]. While L-amino acids constitute proteins in nature, Lapierre et al. [9] discovered that although D-methionine is not taken up or utilized by the mammary gland, it can be converted to L-methionine in other body tissues for milk protein synthesis. Whether methionine and cysteine serve functions beyond milk protein synthesis in the mammary gland, and whether the catabolic pathways identified for these amino acids in other tissues exist in the mammary gland, remain to be determined.

2.2 Lysine

Lysine uptake by the mammary gland exceeds its secretion in milk. Despite this oversupply, lysine remains a nutritionally limiting amino acid in dairy cows, not only because it can be directly used for milk protein synthesis but also due to its active metabolism within the mammary gland. Supplementing rumen-protected lysine and methionine increases milk protein yield [10], while related in vitro studies have further identified an optimal ratio of 3:1 for lysine to methionine in milk protein synthesis [11]. Lapierre et al. [12] conducted a series of studies on lysine metabolism, finding that lysine undergoes catabolism within the mammary gland and provides nitrogen for non-essential amino acid synthesis, a process that continues even when lysine is limiting; when lysine is in excess, mammary uptake increases and oxidation is enhanced. How oversupplied lysine is metabolized and how lysine limits milk protein production remain to be elucidated. Understanding the metabolic mechanisms of lysine would enable prediction of its requirements under different nutritional conditions, thereby achieving the goal of regulating milk synthesis.

2.3 Arginine

Arginine is a functionally essential amino acid that plays important roles in growth, reproduction, and lactation, with mammary uptake exceeding milk secretion. In the mammary gland of lactating dairy cows, arginine is converted to ammonia and ornithine via arginase catalysis; ornithine is then transformed into polyamines by ornithine decarboxylase, which regulates milk production. Nitric oxide (NO) generated from arginine metabolism acts as a vasodilator, helping maintain vascular permeability and ensuring normal amino acid blood supply to tissues. Arginine can also be converted into non-essential amino acids such as proline. When other essential amino acids are adequately supplied to the mammary gland, arginine deficiency reduces arginine uptake without decreasing milk protein or milk yield [13], indicating certain metabolic interconversions

exist between arginine and other amino acids. The multiple functions of arginine underscore its importance, and arginine metabolism in the mammary gland has been relatively well-studied among amino acids, though the specific quantities metabolized remain unclear and require further confirmation, providing a reference framework for subsequent studies on other amino acids.

2.4 Branched-Chain Amino Acids

Leucine, isoleucine, and valine are essential amino acids required for nutritional metabolism in dairy cows, with leucine being the most extensively studied. Research has shown that milking frequency can affect leucine metabolism in the mammary gland of dairy goats [14], and glucogenic nutrients can also regulate leucine metabolism [15]. Leucine is abundantly absorbed by the mammary gland, with uptake exceeding milk protein secretion.

In vitro experiments have demonstrated that keto acids generated from branched-chain amino acids are not oxidized to carbon dioxide but are instead released extracellularly [16]. The activity of branched-chain keto acid dehydrogenase is regulated by phosphorylation in response to branched-chain amino acids and insulin; enzyme activity is suppressed when plasma branched-chain amino acid concentrations decrease or insulin concentrations increase. Oxidation of branched-chain amino acid-derived keto acids can generate acetyl-CoA and succinyl-CoA, thereby initiating de novo synthesis of non-essential amino acids, fatty acids, and carbon skeletons. Valine may participate in metabolic processes including oxidation in the mammary gland beyond protein synthesis [17]. Altered oxidation of branched-chain amino acids may be associated with milk yield, though whether the quantity oxidized is sufficient to affect milk production remains unknown.

Amino acid metabolism in the mammary gland provides the foundation for lactation in dairy cows, and comprehensive understanding of individual amino acid metabolism can indicate overall animal status or even disease conditions. Metabolomics approaches also facilitate research on amino acid metabolism in the dairy cow mammary gland by providing a macroscopic view of amino acid metabolism, thereby offering insights into various metabolic pathways [18].

3 Signaling Pathways Related to Amino Acid Metabolism in Dairy Cow Mammary Gland

Genes involved in lactation are regulated not only by their own expression but also by external environmental factors such as nutrients, hormones, and temperature [19]. Amino acid metabolism in the dairy cow mammary gland involves numerous signaling pathways: the amino acid response (AAR) pathway senses amino acid balance; the Janus kinases (JAK)-signal transducers and activators of transcription (STAT) pathway primarily regulates milk protein synthesis; the AMP-activated protein kinase (AMPK) pathway controls energy balance; and the mammalian target of rapamycin (mTOR) pathway plays important roles

in amino acid metabolism, synthesis, and glucose-lipid metabolism, while being closely associated with multiple other pathways. These integrated pathways are illustrated in Figure 1 [Figure 1: see original paper].

Figure 1. Pathways of amino acid metabolism in bovine mammary epithelial cells [20-23]

Abbreviations: AMPK: AMP-activated protein kinase; AMPKK: AMP-activated protein kinase kinase; eEF2: eukaryotic elongation factor 2; eEF2k: eukaryotic elongation factor 2 kinase; NO: nitric oxide; IRS1: insulin receptor substrate 1; PI3K: phosphatidylinositol-3-kinase; Akt: protein kinase B; mTORC1: mammalian target of rapamycin complex 1; S6K1: translational regulators S6 kinase 1; eIF: eukaryotic translation initiation factor; 4E-BP1: eukaryotic translation initiation factor 4E binding protein; tRNA: transfer RNA; GCN2: general control nonderepressible 2; ATF4: activating transcription factor 4; JAK2: Janus kinase 2; STAT5: signal transducer and activator of transcription 5.

3.1 GCN2 (General Control Nonderepressible 2) Signaling Pathway

Cells maintain dynamic homeostasis by sensing intracellular and extracellular amino acids and allocating them for utilization. GCN2 is a signaling pathway that senses amino acid deficiency; when any amino acid becomes limiting, the AAR is activated, intracellular amino acids are recycled, and required proteins are synthesized under nutrient-restricted conditions. GCN2 has high affinity for uncharged transfer RNA (tRNA). When amino acids are abundant, they are charged onto their corresponding tRNAs; when amino acids are deficient, GCN2 binds to uncharged tRNA, triggering conformational changes that activate eukaryotic translation initiation factor 2α (eIF2α) [21], inhibiting most protein synthesis while specifically activating activating transcription factor 4 (ATF4) to promote translation of mRNA from genes regulating amino acid metabolism, such as amino acid transporters, amino acid metabolic enzymes, and energy regulatory factors, thereby controlling the cellular response to amino acid deficiency. Amino acid imbalance also affects glucose and lipid metabolism [24].

In Mac-T cells, histidine and branched-chain amino acid supply can reduce eIF2α phosphorylation, but in cultured mammary tissue, these amino acids do not affect eIF2α phosphorylation despite changes in milk protein synthesis rates [25]. In vivo studies have shown that deficiency of branched-chain amino acids and lysine also upregulates eIF2α expression. Although eIF2α expression is maintained under leucine and lysine deficiency conditions, the decrease in milk protein production indicates that other factors regulate lysine and leucine metabolism [26]. Maintenance of homeostasis is a critical process in amino acid metabolism, and understanding its mechanisms is essential for comprehensive knowledge of amino acid metabolism. GCN2 signaling pathway research is currently common in human diseases such as cancer, and dairy nutrition research may provide insights from the perspective of nutrient deficiency.

3.2 AMPK Signaling Pathway

The AMPK signaling pathway functionally regulates cellular metabolism and coordinates multiple metabolic responses in various cell types, controlling metabolic energy balance at both cellular and whole-organism levels. The mammary gland during lactation requires substantial energy supply, and the AMPK pathway plays a critical role while also regulating fat metabolism in dairy cows and performing important functions in the liver.

Mahmoudi et al. [27] identified mutation sites in the AMPK$ $1 gene affecting milk protein and milk yield using genomic approaches. AMPK inhibits protein synthesis through eukaryotic elongation factor 2 (eEF2) kinase and mTOR. Activated AMPK can suppress mTOR and its effectors [28], and activate eEF2 kinase, increasing eEF2 phosphorylation to inhibit protein synthesis and reduce ATP consumption. AMPK activation primarily occurs under stress conditions, with AMP/ATP ratio, hormones such as leptin and adiponectin, and AMPK kinase (AMPKK) all regulating AMPK activation [29]. Adding essential amino acids to cultured bovine mammary epithelial cells increases ATP concentration, inhibits AMPK phosphorylation, and affects milk protein synthesis [23]. Additionally, energy substrates such as glucose and acetate can regulate milk protein production through the AMPK-mTOR signaling pathway [30]. Energy can serve as an indicator of metabolic intensity, and studying the AMPK pathway provides a new perspective for revealing amino acid metabolic processes at the energy level.

3.3 JAK-STAT Signaling Pathway

The JAK-STAT pathway participates in various biological processes including apoptosis, growth, proliferation, and inflammatory responses. JAK and STAT comprise multiple kinases, with research in dairy cow mammary tissue focusing primarily on the JAK2-STAT5 signaling pathway [11]. This pathway is crucial for casein synthesis transcription: prolactin binds to its receptor to activate JAK2, which phosphorylates STAT5, subsequently initiating casein synthesis transcription. Over 30 cytokines, including prolactin, growth hormone, epidermal growth factor, and erythropoietin, have been found to activate STAT proteins in various animal cells [31], though the hormonal activation mechanisms remain to be investigated. Amino acids regulate milk production through the JAK2-STAT5 signaling pathway, primarily modulating milk protein expression via the STAT5a gene [32]. Studies have confirmed that methionine, leucine, and lysine play important regulatory roles in lactation of bovine mammary epithelial cells, regulating key protein expression in milk protein synthesis pathways and thereby enhancing proliferation of bovine mammary epithelial cells and synthesis of lactose, milk fat, and milk protein [33-34]. Recent research indicates that methionine-methionine dipeptide affects milk protein synthesis through the JAK-STAT signaling pathway [35]. The JAK-STAT pathway also importantly regulates immune responses, warranting further exploration.

3.4 mTOR Signaling Pathway

The mTOR signaling pathway is a critical pathway for amino acid metabolism in the dairy cow mammary gland, regulating not only milk protein synthesis but also sensing amino acid abundance while being interconnected with other metabolic pathways. mTOR complex 1 (mTORC1) is rapamycin-sensitive and primarily controls various cellular processes including protein synthesis, lipid synthesis, energy metabolism, and autophagy, serving as a key regulatory node in mammary amino acid metabolic pathways. Its activity is regulated by growth factors, hormones, energy, and amino acids. The regulatory mechanisms of growth factors and hormones have been established, both stimulating milk protein production through the phosphatidylinositol 3-kinase (PI3K)-Akt-mTOR pathway [36], while amino acid regulatory mechanisms remain to be further determined.

3.4.1 Amino Acid Sensing and Regulation mTORC1 exhibits differential sensitivity to various amino acids, being most sensitive to leucine. Unlike the GCN2 pathway, which can sense deficiency of any amino acid, mTOR only senses the presence of specific amino acids, meaning the pathway is activated only when amino acids are abundant. Studies have confirmed that isoleucine and threonine can affect mTOR phosphorylation, but their effects are opposite: isoleucine linearly increases S6K1 phosphorylation, while threonine inhibits isoleucine' s effect. Additionally, leucine and isoleucine can reduce eEF2 phosphorylation, whereas threonine decreases eEF2 phosphorylation in a curvilinear manner, with leucine and isoleucine negatively affecting eEF2 phosphorylation while threonine tends to suppress leucine' s effect on eEF2 [37]. Leucine, arginine, and histidine play important roles in regulating casein gene translation and expression of genes related to the mTOR pathway [38-39], though the molecular mechanisms of amino acid sensing and regulation of mTORC1 remain unclear. Although mTORC1 is highly sensitive to changes in amino acid levels, it is not an amino acid receptor. SLC38A9 functions as an amino acid transceptor, possessing not only the ability to transport amino acids across cell and organelle membranes such as lysosomes but also to sense amino acids, enabling mTORC1 to sense arginine [40]. In contrast, glutamine stimulation of mTORC1 activation depends on lysosome and vacuolar H+-ATPase (V-ATPase) activity, which differs from the mechanism sensing leucine [41], indicating that amino acid sensing by mTOR is complex. Amino acid enhancement of milk protein synthesis is associated with phosphorylation of mTOR and its downstream signals, eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) and translational regulators S6 kinase 1 (S6K1). Appuhamy et al. [42] found that essential amino acids enhance phosphorylation of mTOR, S6K1, 4EBP1, and insulin receptor substrate 1 (IRS1) in the mTOR pathway, and exert stronger effects than insulin on control points regulating translation initiation and elongation in protein synthesis. Related in vivo experiments also indicate that cellular signaling molecules regulating protein synthesis respond differently to nutritional stimuli [43].

3.4.2 Peptide Regulation In addition to amino acids, small peptides can also regulate milk protein synthesis through the mTOR signaling pathway. Yang et al. [44] found that adding methionine-methionine dipeptide to cultured bovine mammary tissue in place of methionine significantly increased αs1-casein gene expression and markedly enhanced expression of key genes in the mTOR pathway. Small peptides have gradually become a research hotspot in recent years, with their functional roles being increasingly recognized in dairy cow nutrition research. This experimental study also provides a theoretical basis for milk protein synthesis research. However, many aspects of the mechanism by which small peptides regulate the mTOR pathway remain unknown and require further investigation.

Amino acid metabolism in the mammary gland is extremely complex. Under different physiological conditions and environmental influences, signaling pathways including GCN2, AMPK, JAK-STAT, and mTOR all play important roles in mammary amino acid metabolism and utilization through corresponding sensing and feedback mechanisms. Additionally, signaling pathways such as cGMP-dependent kinase and cAMP-dependent kinase, as well as gas molecules like hydrogen sulfide, may also regulate dairy cow mammary metabolism. Elucidating the molecular mechanisms of these signaling pathways in dairy cow mammary amino acid metabolism will help clarify the mechanisms of milk protein metabolism in the mammary gland, provide a basis for optimizing nutritional regulation strategies to improve production efficiency in dairy cows and other livestock, and thereby exert profound impacts on dairy production.

References

[1] NICHOLS J R, SCHINGOETHE D J, MAIGA H A, et al. Evaluation of corn distillers grains and ruminally protected lysine and methionine for lactating dairy cows[J]. Journal of Dairy Science, 1998, 81(2): 482–491.

[2] THIVIERGE M C, PETITCLERC D, BERNIER J F, et al. Variations in mammary protein metabolism during the natural filling of the udder with milk over a 12-h period between two milkings: leucine kinetics[J]. Journal of Dairy Science, 2002, 85(11): 2974–2985.

[3] HAQUE M N, GUINARD-FLAMENT J, LAMBERTON P, et al. Changes in mammary metabolism in response to the provision of an ideal amino acid profile at 2 levels of metabolizable protein supply in dairy cows: consequences for efficiency[J]. Journal of Dairy Science, 2015, 98(6): 3951–3968.

[4] SCIASCIA Q L, PACHECO D, MCCOARD S A. Administration of exogenous growth hormone is associated with changes in plasma and intracellular mammary amino acid profiles and abundance of the mammary gland amino acid transporter SLC3A2 in mid-lactation dairy cows[J]. PLoS One, 2015, 10(7): e0134323.

[5] FUJII K, ZHANG H L, USUDA K, et al. Lactogenic hormone stimulation

and epigenetic control of L-amino acid oxidase expression in lactating mammary glands[J]. Journal of Cellular Physiology, 2015, 230(11): 2755–2762.

[6] MANJARIN R, BEQUETTE B J, WU G Y, et al. Linking our understanding of mammary gland metabolism to amino acid nutrition[J]. Amino Acids, 2014, 46(11): 2447–2462.

[7] WEEKES T L, LUIMES P H, CANT J P. Responses to amino acid imbalances and deficiencies in lactating dairy cows[J]. Journal of Dairy Science, 2006, 89(6): 2177–2187.

[8] JIANG N, HU L J, LIU C N, et al. 60S ribosomal protein L35 regulates β-casein translational elongation and secretion in bovine mammary epithelial cells[J]. Archives of Biochemistry and Biophysics, 2015, 583: 130–139.

[9] LAPIERRE H, HOLTROP G, CALDER A G, et al. Is D-methionine bioavailable to the dairy cow?[J]. Journal of Dairy Science, 2012, 95(1): 353–362.

[10] LEE C, HRISTOV A N, CASSIDY T W, et al. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet[J]. Journal of Dairy Science, 2012, 95(10): 6042–6056.

[11] NAN X M, BU D P, LI X Y, et al. Ratio of lysine to methionine alters expression of genes involved in milk protein transcription and translation and mTOR phosphorylation in bovine mammary cells[J]. Physiological Genomics, 2014, 46(7): 268–275.

[12] LAPIERRE H, DOEPEL L, MILNE E, et al. Responses in mammary and splanchnic metabolism to altered lysine supply in dairy cows[J]. Animal, 2009, 3(3): 360–371.

[13] DOEPEL L, LAPIERRE H. Deletion of arginine from an abomasal infusion of amino acids decreases milk protein yield in dairy cows[J]. Journal of Dairy Science, 2011, 94(2): 864–873.

[14] BEQUETTE B J, DOUGLASS L W. The frequency of unilateral milking alters leucine metabolism and amino acid removal by the mammary gland of lactating goats[J]. Journal of Dairy Science, 2010, 93(1): 162–169.

[15] CANTALAPIEDRA-HIJAR G, ORTIGUES-MARTY I, LEMOSQUET S. Diets rich in starch improve the efficiency of amino acid use by the mammary gland in lactating Jersey cows[J]. Journal of Dairy Science, 2015, 98(10): 6939–6953.

[16] LEI J, FENG D Y, ZHANG Y L, et al. Nutritional and regulatory role of branched-chain amino acids in lactation[J]. Frontiers in Bioscience: Landmark Edition, 2012, 17: 2725–2739.

[17] GUAN X, BEQUETTE B J, CALDER G, et al. Amino acid availability affects amino acid flux and protein metabolism in the porcine mammary gland[J]. The Journal of Nutrition, 2002, 132(6): 1224–1234.

[18] SUN H Z, WANG D M, WANG B, et al. Metabolomics of four biofluids from dairy cows: potential biomarkers for milk production and quality[J]. Journal of Proteome Research, 2015, 14(2): 1287–1298.

[19] WU G Y. Amino acids: metabolism, functions, and nutrition[J]. Amino Acids, 2009, 37(1): 1–17.

[20] BIONAZ M, LOOR J J. Gene networks driving bovine mammary protein synthesis during the lactation cycle[J]. Bioinformatics and Biology Insights, 2011, 5: 83–98.

[21] TSALIKIS J, CROITORU D O, PHILPOTT D J, et al. Nutrient sensing and metabolic stress pathways in innate immunity[J]. Cellular Microbiology, 2013, 15(10): 1632–1641.

[22] APELO S I A, KNAPP J R, HANIGAN M D. Invited review: current representation and future trends of predicting amino acid utilization in the lactating dairy cow[J]. Journal of Dairy Science, 2014, 97(7): 4000–4017.

[23] APPUHAMY J A D R N, NAYANANJALIE W A, ENGLAND E M, et al. Effects of AMP-activated protein kinase (AMPK) signaling and essential amino acids on mammalian target of rapamycin (mTOR) signaling and protein synthesis rates in mammary cells[J]. Journal of Dairy Science, 2014, 97(1): 419–429.

[24] GUO F F, CAVENER D R. The GCN2 eIF2α kinase regulates fatty-acid homeostasis in the liver during deprivation of an essential amino acid[J]. Cell Metabolism, 2007, 5(2): 103–114.

[25] APELO S I A, NAYANANJALIE W A D, APPUHAMY J A D R N, et al. mTOR independent model of protein synthesis regulation by essential amino acids in mammary epithelial cells[M]//CROVETTO M G. Energy and protein metabolism and nutrition. Wageningen, the Netherlands: Wageningen Academic Publishers, 2010: 247.

[26] DOELMAN J, KIM J J, CARSON M, et al. Branched-chain amino acid and lysine deficiencies exert different effects on mammary translational regulation[J]. Journal of Dairy Science, 2015, 98(11): 7846–7855.

[27] MAHMOUDI A, ZARGARAN A, AMINI H R, et al. A SNP in the 3' - untranslated region of AMPK$ $1 may associate with serum ketone body and milk production of Holstein dairy cows[J]. Gene, 2015, 574(1): 48–52.

[28] PARK S H, GAMMON S R, KNIPPERS J D, et al. Phosphorylation-activity relationships of AMPK and acetyl-CoA carboxylase in muscle[J]. Journal of Applied Physiology, 2002, 92(6): 2475–2482.

[29] HARDIE D G, ROSS F A, HAWLEY S A. AMPK: a nutrient and energy sensor that maintains energy homeostasis[J]. Nature Reviews Molecular Cell Biology, 2012, 13(4): 251–262.

[30] BURGOS S A, DAI M, CANT J P. Nutrient availability and lactogenic hormones regulate mammary protein synthesis through the mammalian target of rapamycin signaling pathway[J]. Journal of Dairy Science, 2010, 93(1): 153-161.

[31] CHAUDHARI N, ROPER S D. The cell biology of taste[J]. The Journal of Cell Biology, 2010, 190(3), 285-296.

[32] LIU X F, LI M, LI Q Z, et al. Stat5a increases lactation of dairy cow mammary gland epithelial cells in vitro[J]. Developmental Biology: Animal, 2012, 48(9): 554-561.

[33] WANG L N, LIN Y, BIAN Y J, et al. Leucyl-tRNA synthetase regulates lactation and cell proliferation via mTOR signaling in dairy cow mammary epithelial cells[J]. International Journal of Molecular Sciences, 2014, 15(4): 5952-5969.

[34] LU L M, LI Q Z, HUANG J G, et al. Proteomic and functional analyses reveal MAPK1 regulates milk protein synthesis[J]. Molecules, 2013, 18(1): 263-275.

[35] YANG J X. Uptake of Methionine Dipeptide in Dairy Cow Mammary Gland and Its Mechanism Affecting Milk Protein Synthesis[D]. Master' s thesis. Hangzhou: Zhejiang University, 2014: 125-133.

[36] HAY N, SONENBERG N. Upstream and downstream of mTOR[J]. Genes & Development, 2004, 18(16): 1926-1945.

[37] ARRIOLA A S I, SINGER L M, LIN X Y, et al. Isoleucine, leucine, methionine, and threonine effects on mammalian target of rapamycin signaling in mammary tissue[J]. Journal of Dairy Science, 2014, 97(2): 1047-1056.

[38] WANG M Z, XU B L, WANG H R, et al. Effects of arginine concentration on the in vitro expression of casein and mTOR pathway related genes in mammary epithelial cells from dairy cattle[J]. PLoS One, 2014, 9(5): e95985.

[39] GAO H N, HU H, ZHENG N, et al. Leucine and histidine independently regulate milk protein synthesis in bovine mammary epithelial cells via mTOR signaling pathway[J]. Journal of Zhejiang University Science B, 2015, 16(6): 560-572.

[40] REBSAMEN M, POCHINI L, STASYK T, et al. SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1[J]. Nature, 2015, 519(7544): 477-481.

[41] JEWELL J L, KIM Y C, RUSSELL R C, et al. Differential regulation of mTORC1 by leucine and glutamine[J]. Science, 2015, 347(6218): 194-198.

[42] APPUHAMY J A D R N, BELL A L, NAYANANJALIE W A D, et al. Essential amino acids regulate both initiation and elongation of mRNA translation independent of insulin in MAC-T cells and bovine mammary tissue slices[J]. The Journal of Nutrition, 2011, 141(6): 1209-1215.

[43] RIUS A G, APPUHAMY J A D R N, CYRIAC J, et al. Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids[J]. Journal of Dairy Science, 2010, 93(7): 3114–3127.

[44] YANG J X, WANG C H, XU Q B, et al. Methionyl-methionine promotes α-s1 casein synthesis in bovine mammary gland explants by enhancing intracellular substrate availability and activating JAK2-STAT5 and mTOR-mediated signaling pathways[J]. The Journal of Nutrition, 2015, 145(8): 1748–1753.

Note: Figure translations are in progress. See original paper for figures.