Research Advances on Amino Acid Supply and Endocrine Crosstalk Regulating Milk Protein Synthesis: Postprint

Authors: Xu Lianbin, Lin Xueyan, Hu Zhiyong, Wang Yun, Wang Zhonghua

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Abstract

Research on limiting amino acids represents a key focus in studies of lactating dairy cows. A series of in vivo and in vitro investigations into their effects on milk protein synthesis pathways have indicated that substrate effects may not be the primary cause; more importantly, amino acids and their composition function as signaling factors influencing neuroendocrine and intracellular signaling pathways. This article addresses the factors affecting milk protein synthesis, summarizing the regulatory roles of amino acid supply, hormone secretion, and their interactions in milk protein synthesis, thereby providing theoretical references for elucidating the mechanisms by which amino acid supply influences milk protein synthesis pathways.

Full Text

Recent Advances in Regulating Milk Protein Synthesis by Interaction of Amino Acid Supply and Endocrine

XU Lianbin, LIN Xueyan, HU Zhiyong, WANG Yun, WANG Zhonghua*

(College of Animal Science and Technology, Shandong Agricultural University, Tai' an 271018, China)

Abstract: Limiting amino acid research represents a prominent focus in dairy cow studies. A series of in vivo and in vitro investigations into its pathways affecting milk protein synthesis indicate that substrate effects may not be the primary mechanism; rather, amino acids and their profiles function as signaling factors that influence neuroendocrine and intracellular signaling pathways. This review examines the factors affecting milk protein synthesis and summarizes the regulation of milk protein synthesis by amino acid supply, hormone secretion, and their interactions, aiming to provide theoretical reference for elucidating the pathways through which amino acid supply influences milk protein synthesis.

Keywords: amino acid; endocrine; interaction regulation; milk protein synthesis; research advances

*Corresponding author, professor, E-mail: zhwang@sdau.edu.cn

Amino acid supply encompasses three dimensions: type, ratio, and level, all of which significantly influence milk protein synthesis. Experiments manipulating essential amino acid (EAA) supply have confirmed this relationship with milk protein yield [1-2]. Traditional amino acid nutrition theory employs the "barrel principle" to explain how amino acid profile affects milk protein synthesis, positing that before the most deficient EAA relative to requirement reaches a certain supplementation threshold, adding other amino acids proves ineffective. However, both growing and lactating animals exhibit a "co-limiting" amino acid phenomenon, where supplementation of different EAAs each enhances milk protein synthesis, thereby demonstrating the limitations of this theory [3–5].

Unlike growing animals, lactating ruminants show limited sensitivity to changes in single EAA supply. Bequette et al. [6] found that omitting histidine (His) from mixed amino acids infused into the abomasum of dairy goats reduced arterial concentration by nearly 90% without significantly altering milk protein yield. Nevertheless, the substrate function of amino acids as fundamental building blocks of milk protein cannot be ignored. Appuhamy et al. [7] observed that methionine (Met) and threonine (Thr) did not affect signaling pathways in mammary epithelial cells but did influence casein synthesis rate, likely through substrate effects.

Eif et al. [8] demonstrated that limiting a single EAA supply reduces the cellular receptor tRNA loading of that specific EAA to zero while other amino acid receptor tRNAs remain fully loaded, causing substantial variation in translation speed across codons due to changes in available substrate quantity. Studies on mammary amino acid metabolism indicate that deficiency of a single EAA can increase mammary blood flow (MBF) and extraction efficiency of the deficient EAA to enhance supply to mammary tissue [6]. Therefore, reduced supply of limiting amino acids to the mammary gland may not be the primary cause of decreased milk protein yield, and research has identified two alternative pathways. First, amino acid supply affects signaling pathways regulating protein synthesis in mammary epithelial cells, primarily including: 1) the mammalian target of rapamycin (mTOR) pathway, where improved cellular amino acid nutrition stimulates phosphorylation of ribosomal protein S6 kinase 1 (rpS6K1) and eukaryotic translation initiation factor 4E binding protein 1 (4EBP1), thereby promoting translation [9]; and 2) the integrated stress response (ISR) network, where amino acid deficiency increases uncharged tRNA, stimulating phosphorylation of eukaryotic translation initiation factor 2 (eIF2) and inhibiting translation initiation [10]. Second, limiting or increasing EAA supply alters plasma hormone concentrations, which regulate milk protein synthesis at multiple levels including tissue metabolism, cell proliferation, and transcription-translation. A consistent pattern emerges where limiting single or groups of EAAs simultaneously elevates plasma insulin (INS) and glucagon (GLN) concentrations, observed across six studies published between 2006–2012 [1,11-15]. Thus, endocrine changes constitute an important factor affecting milk protein synthesis, making the EAA supply-endocrine change-milk protein synthesis pathway worthy of attention, which this review addresses.

1 Effects of Amino Acid Supply on Endocrine Hormones and Their Mechanisms

The hypothalamic-pituitary system and hormones secreted by mammary cells in lactating dairy cows influence free amino acid utilization, while free amino acids may conversely exert feedback regulation on the neuroendocrine system. Chen [16] adjusted the ratios of Met, lysine (Lys), and leucine (Leu) in culture medium based on amino acid balance index and found that different amino acid patterns affected hormone synthesis and secretion in isolated mammary tissue. A series of in vitro EAA infusion experiments support this conclusion; for example, intravenous infusion of amino acid mixtures deficient in Met, Lys, His, or branched-chain amino acids (BCAA) significantly altered plasma hormone concentrations in lactating dairy cows [1], and intraperitoneal arginine (Arg) injection in mice increased both INS and GLN secretion [12]. These findings indicate that the stimulatory effects on hormone secretion relate to amino acid type. Kuhara et al. [17] observed through sheep venous infusion experiments that acidic amino acids primarily promote growth hormone (GH) secretion, neutral straight-chain amino acids mainly stimulate INS and GLN secretion, while BCAA tend to inhibit GLN secretion.

Beyond affecting secretion, amino acids also influence tissue hormone sensitivity and receptor expression. Xiao et al. [18] found that BCAA deficiency enhanced hepatic INS sensitivity. EAA deficiency may not affect plasma GH and insulin-like growth factor I (IGF-I) concentrations but can influence expression of GH and IGF-I receptors in hepatocytes [19].

Both deficiency and excess of EAAs can cause amino acid imbalance, suggesting that amino acid imbalance rather than single amino acid signals may stimulate hormone secretion. Reports on mechanisms of amino acid-regulated hormone secretion are limited, but based on previous research can be summarized as follows: First, certain specific amino acids serve as substrates for small-molecule hormone synthesis. Tyrosine (Tyr) and phenylalanine (Phe) are precursors for epinephrine and thyroid hormone synthesis. Second, amino acids may directly regulate hormone secretion through intracellular signaling pathways. Studies show that over 50% of the stimulatory effect of amino acid mixtures on INS secretion occurs through glutamine (Gln) via the cyclic adenosine monophosphate-protein kinase A (cAMP-PKA) pathway [20-21]. Ma [22] noted that Arg can regulate hepatic IGF-I synthesis at transcriptional and translational levels, potentially involving transcription rate, nuclear-cytoplasmic transport, and post-transcriptional processing. Xiao et al. [18] found that BCAA deficiency increased hepatic INS sensitivity through mTOR and adenosine monophosphate activated protein kinase (AMPK) pathways. During cellular amino acid sensing, amino acid carriers may play a key role as "transceptors" [23]; for example, neutral amino acid carriers like Leu can interact with intracellular mTOR pathways to transmit signals [24]. Finally, single amino acids or amino acid balance may indirectly regulate through plasma metabolites. Wang [25] observed in cultured dairy cow pituitary cells that Arg promoted extracellular Ca2+ influx through nitric oxide (NO) generation to regulate GH secretion, while Tyr generated dopamine to inhibit prolactin (PRL) secretion via autocrine or paracrine mechanisms. Compared with increased exogenous infusion rates, the liver utilizes EAAs at a greater rate, and glucose produced through gluconeogenesis directly stimulates INS and GLN secretion [26-27]. Additionally, research reports that amino acid supply may promote INS and GH secretion by increasing ghrelin secretion from intestinal cells [11]. Ghrelin, a brain-gut peptide secreted by gastric mucosal endocrine cells, binds to growth hormone secretagogue receptor (GHSR) on GH-secreting cells to activate protein kinase C (PKC) and increase phosphorylation of nuclear cAMP response element binding protein (CREB), which specifically binds to the GH gene promoter region to promote GH gene transcription [28].

2 Effects of Endocrine Hormones on Milk Protein Synthesis and Their Mechanisms

Cant et al. [29] reviewed mammary amino acid metabolism research, noting that milk protein synthesis rate depends on physiological state, nutritional status, and milking regimen rather than extracellular amino acid concentration. Extensive data confirm that lactating dairy cows possess mechanisms that prioritize milk secretion during amino acid deficiency, with the endocrine system playing a crucial role. Integrating previous findings, we find that hormonal regulation of milk protein synthesis occurs at multiple levels—cellular, tissue, organ, and whole-body—through potential pathways including: 1) regulating whole-body metabolism and promoting nutrient allocation to the mammary gland; 2) promoting mammary gland development and maintaining lactation; 3) regulating amino acid carrier expression and activity [31]; and 4) promoting transcription and translation of milk protein synthesis-related genes, as shown in Figure 1 [Figure 1: see original paper]. The following sections discuss specific mechanisms of PRL, INS, IGF-I, GH, and GLN at molecular, cellular, tissue, organ, and whole-body levels.

Figure 1 Actions of IGF-I, PRL, GH and selected growth factors in a bovine mammary epithelial cell [42]

IGFBP: insulin-like growth factor binding protein; TGFα: transforming growth factor α; TGFβ: transforming growth factor β; Leptin; FGF: fibroblast growth factor; stromal cells; IRS-1: insulin receptor substrate 1; RAS: rat sarcoma pro-

tein; β-Catenin; Cell survival; Cell proliferation; SOCS: suppressors of cytokine signaling; JAK/STAT5 Signaling; Prl: prolactin; GH: growth hormone; Milk Protein Gene Expression; KEY; Prl or GH receptor; IGF-I receptor; Hybrid receptor; Insulin receptor; Fibroblast or adipocyte; ECM protein: extracellular matrix proteins.

2.1 PRL

PRL is a single-chain polypeptide hormone synthesized and secreted by pituitary lactotrophs, composed of 199 amino acids and widely distributed in tissues beyond the pituitary. PRL plays vital roles in initiating and maintaining lactation, stimulating mammary gland growth, and promoting milk protein synthesis. Yang et al. [32] found that increased milk protein content in lactating dairy cows was accompanied by elevated plasma PRL concentration. PRL supplementation significantly increased milk protein secretion in isolated dairy cow mammary epithelial cells [33]. Boutinaud et al. [34] treated lactating dairy cows with a PRL-release inhibitor and observed significantly decreased mRNA expression of -casein and PRL receptor (PRLR).

PRL regulates milk protein synthesis through several mechanisms: First, PRL promotes mammary development and maintains lactation. During late pregnancy, PRL controls lobuloalveolar development and epithelial cell proliferation through increased gene transcription and extended mRNA lifespan [35], mediated by the mitogen-activated protein kinase (MAPK) signaling pathway. Quinagolite, a PRL inhibitor, significantly reduced milk yield in peak-lactation cows [36], possibly by inhibiting mammary epithelial cell proliferation and reducing the number of normal lactating cells. Second, PRL binds to its receptor and induces milk protein gene transcription through the phosphatidylinositol 3 kinase-serine/threonine kinase (PI3K-Akt) pathway and Janus kinases-signal transducer and activator of transcription 5 (JAK-STAT5) pathway [37]. PRLR is a transmembrane protein with characteristics of the cytokine receptor superfamily, comprising extracellular, transmembrane, and intracellular domains. Upon PRL binding, PRLR dimerizes, and its intracellular domain undergoes trans-phosphorylation with tyrosine kinase, activating JAK and STAT. Activated STAT then enters the nucleus and binds to specific sequences on milk protein genes to initiate transcription [38]. Third, PRL can regulate amino acid transport [39]. Lacasse et al. [36] found that PRL modulates L-amino acid transporter activity. Studies show that treating peak-lactation rats with the PRL blocker bromocriptine altered mammary arteriovenous concentration differences of several amino acids, differences that could be restored by exogenous PRL treatment [40]. Additionally, PRL synergistically interacts with INS, progesterone, glucocorticoids, and estrogen to jointly regulate milk protein synthesis [41].

2.2 INS

INS is a protein hormone secreted by pancreatic β-cells with a molecular mass of 5,808 ku. As the only hormone that lowers blood glucose, INS significantly impacts milk protein synthesis. Research indicates that endocrine regulation of milk protein synthesis is primarily mediated by INS [42]. Mackle et al. [43] used hyperinsulinemic-euglycemic clamp techniques to increase plasma INS concentration fourfold while maintaining constant blood glucose, finding that milk protein yield increased by 15%, with further improvement when casein was additionally infused. Intravenous Leu infusion in mice transiently elevated plasma INS concentration, which promoted Leu-induced protein synthesis [44]. Menzies et al. [45] identified 28 genes directly related to milk protein synthesis that are stimulated by INS through whole-genome expression analysis, confirming that INS stimulates milk protein synthesis at multiple cellular levels. These results demonstrate that INS can promote mammary amino acid utilization and further exploit dairy cows' lactation potential.

INS promotes protein synthesis through two main mechanisms. At the cellular-molecular level, INS stimulates insulin receptor substrate 1 (IRS-I) and promotes generation of INS receptor protein sites through ligand binding and phosphorylation, thereby enhancing intracellular protein synthesis [46]. First, INS binds to INS receptors on mammary epithelial cells, activating the PI3K-Akt-mTOR signaling pathway to promote protein translation [47]. mTOR affects downstream pathways by: stimulating rpS6K1 phosphorylation to promote translation initiation and elongation [48]; stimulating 4EBP1 phosphorylation to promote formation of the eukaryotic translation initiation factor 4F (eIF4F) complex, thereby initiating translation of capped mRNA [49]; and stimulating eukaryotic elongation factor 2 (eEF2) phosphorylation to promote translation elongation [50]. After lactation initiation, mammary milk protein gene mRNA expression stabilizes while Akt gene expression upregulates, enhancing INS-mediated mRNA translation efficiency [51]. Second, INS induces transcription of related genes through the JAK-STAT5 signaling pathway [52]. As an important lactation signal transduction factor, STAT5 couples with hormone-mediated and phosphorylated JAK pathways, and activated STAT enters the nucleus as a dimer to bind promoters on milk protein genes, linking extracellular signals directly to gene expression regulation [53]. At the tissue-organ level, INS indirectly affects milk protein synthesis by regulating whole-body metabolism, energy balance, and substrate concentration and transport to the mammary gland [39]. MBF changes represent an important mechanism; Mackle et al. [43] reported that INS can increase MBF. Cant et al. [29] suggested that MBF changes may represent the mammary gland' s attempt to restore intracellular ATP balance when energy substrate concentration in circulating blood is insufficient. Wu et al. [30] confirmed that INS can promote milk protein synthesis by increasing hepatic glucose synthesis to meet lactation energy demands. Additionally, INS exhibits cross-talk with other hormones such as GH and PRL, forming a signaling network that jointly regulates milk protein synthesis.

2.3 IGF-I

IGF-I is a multifunctional basic polypeptide with high homology to INS, composed of 70 amino acids with a molecular mass of 7.5 ku, and serves as an important regulator of cell proliferation and differentiation. Approximately 95% of circulating IGF-I is secreted by the liver, with synthesis dependent on pituitary GH and nutritional status. IGF-I is transported to peripheral tissues after binding to insulin-like growth factor binding proteins (IGFBP) in blood. Rose et al. [54] found that blood IGF-I concentration typically correlates positively with milk yield, suggesting a promotional effect on milk protein synthesis. Prosser et al. [55] observed a 25% increase in milk secretion rate after injecting IGF-I into the external pudendal artery of lactating goats.

The presence of IGF-I receptors in mammary tissue confirms its direct action on the mammary gland [56]. IGF-I regulates lactation through several mechanisms: 1) Regulating mammary epithelial cell proliferation, differentiation, and apoptosis. IGF-I accelerates cell cycle progression, promotes cell division, and stimulates growth [34]. Transgenic overexpression of IGF-I in mice from puberty to maturity promoted mammary ductal branching and alveolar formation [57]. Forsyth et al. [58] found that IGF-I regulates cell growth and differentiation through endocrine, autocrine, and paracrine mechanisms. IGF-I binding to the receptor' s α subunit causes β subunit phosphorylation and activates downstream signaling pathways including PI3K-Akt and MAPK [59]. MAPK exists in a dephosphorylated state in quiescent cells; IGF-I binding relieves tyrosine kinase inhibition on the β subunit, activating MAPK and transmitting signals to the nucleus to initiate mitosis [60]. 2) Promoting transcription and translation of milk protein synthesis. Burgos et al. [61] confirmed that IGF-I promotes intracellular mRNA translation through the PI3K-Akt pathway in a concentration-dependent manner. Ji et al. [62] supplemented IGF-I in serum-free dairy cow mammary epithelial cell culture and found it could regulate milk protein synthesis by affecting transcription of key kinases and regulatory factors. 3) Promoting nutrient allocation to the mammary gland. For example, IGF-I can promote milk secretion in lactating dairy cows by increasing MBF [63].

2.4 GH

GH is a single-chain polypeptide produced by acidophilic cells in the anterior pituitary, composed of 191 amino acids. GH also promotes mammary gland development and lactation maintenance in mammals. Eppard et al. [64] found that pituitary GH concentration correlates positively with dairy cow milk yield. GH significantly promotes expression of αs1-casein and α-lactalbumin mRNA in isolated dairy cow mammary epithelial cell lines [65]. Treating early-lactation dairy cows with GH increased milk yield by 36% compared with controls [66]. Flint et al. [67] simultaneously inhibited PRL and GH in rats while providing exogenous GH treatment, finding that milk yield increased significantly, confirming GH' s independent regulatory role in promoting milk protein synthesis.

GH promotes lactation through two main pathways: First, GH directly acts on mammary epithelial cells by binding to GH receptors (GHR) [68-69]. GHR is a single-pass transmembrane protein belonging to the class I cytokine receptor superfamily. Glimm et al. [70] used Northern blotting to confirm GHR presence in dairy cow mammary glands, primarily expressed in alveolar epithelial cells. Second, GH stimulates IGF-I production in liver cells, which then exerts effects through endocrine and paracrine mediation [71]. Kleinberg et al. [72] found that GH can promote IGF-I mRNA expression in mammary tissue itself. Therefore, GH' s mechanisms for promoting milk protein synthesis are similar to IGF-I, including: 1) Promoting mammary cell proliferation and development [73]. GH can induce mouse mammary alveolar development [74], and heifers injected with GH showed nearly 50% more mammary cells [75]. 2) Promoting transcription and translation of milk protein synthesis-related genes. Hayashi [53] found that GH affects intracellular mTOR phosphorylation through PI3K-Akt and extracellular signal regulated kinases (ERK) pathways. Malewski et al. [76] found that GH promotes milk protein gene expression through the JAK-STAT5 signaling pathway. 3) Promoting nutrient allocation to the mammary gland [77]. On one hand, GH meets lactation demands by regulating metabolism in other tissues; Knapp et al. [78] noted that GH can increase hepatic gluconeogenesis and oxidation rates to support energy needs for milk protein synthesis. On the other hand, GH promotes precursor extraction by the mammary gland through increased MBF. Chaiyabutr et al. [79] found that GH injection altered hematological parameters in dairy cows.

2.5 GLN

GLN is a straight-chain polypeptide of 29 amino acids secreted by pancreatic α-cells. Opposite to INS, GLN is a catabolic hormone that increases blood glucose by stimulating phosphorylase to promote hepatic uptake and utilization of circulating amino acids. Research confirms GLN inhibits milk protein synthesis; for example, Bobe et al. [80] found that intravenous GLN infusion significantly reduced milk protein yield in lactating dairy cows under both restricted and ad libitum feeding, and She et al. [81] observed significantly reduced milk yield after 14-day GLN infusion in cows at 21 days postpartum.

Reports indicate that lactation initiation correlates with increased circulating GLN concentration [82]. Donkin et al. [83] noted that GLN promotes hepatic amino acid uptake for gluconeogenesis and urea cycling, thereby reducing liver free amino acid concentration and decreasing milk protein yield. Previous studies have rarely focused on GLN' s specific effects on milk protein synthesis, but interestingly, single EAA deficiency experiments have consistently observed simultaneous elevation of INS and GLN concentrations. Whether elevated GLN interferes with INS' s promotional effects on milk protein synthesis remains unclear and warrants further investigation.

3 Summary and Prospects

In summary, amino acids and their profiles function as signaling molecules participating in various metabolic processes. PRL, INS, IGF-I, GH, and GLN play important roles in regulating milk protein synthesis, with their secretion modulated by amino acid supply. While current research has established a foundation for understanding how amino acid profile affects hormone secretion and how hormones influence milk protein synthesis, the mechanisms remain incompletely defined. Future research should employ in vitro methods to investigate these two aspects at the molecular level, focusing on reported signaling amino acids. Such findings will help construct a neuroendocrine regulatory network of amino acid effects on milk protein synthesis and provide insights for exploring ideal dietary amino acid profiles and improving nitrogen conversion efficiency to milk protein in dairy cows.

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