Mechanism of Amino Acid Effects on Milk Fat Synthesis in Dairy Cow Mammary Gland: Post-print

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Abstract

Milk protein precursors primarily consist of free amino acids and small peptides. Amino acids not only influence milk protein synthesis within the mammary gland, but also exert certain regulatory effects on milk fat synthesis. This review primarily expounds on the modulatory role of amino acids in milk fat synthesis, and summarizes the potential mechanisms of amino acids on milk fat synthesis from the perspectives of mammary uptake patterns of milk fat precursors, milk fat synthesis-related gene expression, and the mammalian target of rapamycin and adenosine monophosphate-activated protein kinase signaling pathways, thereby providing a theoretical foundation for further investigation into milk fat synthesis mechanisms and enhancement of milk nutritional quality.

Full Text

Mechanism of Amino Acids Affecting Milk Fat Synthesis in the Mammary Gland of Dairy Cows

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Abstract: Free amino acids and small peptides serve as the primary milk protein precursors (MPP). Amino acids not only influence milk protein synthesis in the mammary gland but also exert regulatory effects on milk fat synthesis. This review elucidates the regulatory role of amino acids in milk fat synthesis and summarizes the potential mechanisms from three perspectives: the uptake patterns of milk fat precursors by the mammary gland, the expression of genes related to milk fat synthesis, and the mammalian target of rapamycin (mTOR)

and adenosine monophosphate-activated protein kinase (AMPK) signaling pathways. This synthesis provides a theoretical foundation for further investigation into milk fat synthesis mechanisms and improvement of milk nutritional quality.

Keywords: dairy cow; amino acid; milk fat; mammary gland; mechanism
Subject Classification: S823

Milk fat and protein constitute the primary material basis of milk nutritional quality, and research on milk quality and safety has become a focal point for nutritionists [1-3]. Multiple factors influence milk nutritional quality, among which the formation and utilization of milk component precursors (MCP) represent critical regulatory points. The content and composition of MCP directly affect the synthesis of milk components such as protein and fat in the mammary gland, thereby influencing milk quality [4]. As the main milk protein precursors (MPP), amino acids (AA) have been extensively studied regarding their effects on milk protein synthesis and underlying mechanisms [5-7]. However, research has revealed that AA not only affect milk protein synthesis but also influence milk fat and lactose synthesis [8]. The mechanisms through which AA affect milk fat synthesis remain unclear. This paper comprehensively reviews domestic and international research to elucidate the effects of AA on milk fat synthesis and the potential mechanisms, providing references for in-depth studies on milk fat synthesis mechanisms and milk quality improvement in dairy cows.

1. Effects of AA on Milk Fat Synthesis in the Mammary Gland

Research on the influence of AA on animal fat metabolism has been reported for decades. For instance, Ryzhenkov et al. [9] demonstrated that arginine reduces serum cholesterol and triglyceride levels in mice while inhibiting hyperlipidemia development. In high-fat diets, branched-chain amino acids (BCAA) affect obesity and fat metabolism balance, significantly reducing triglyceride content in mouse liver and muscle, suggesting that BCAA help maintain fat homeostasis [10], though the mechanisms underlying BCAA effects on lipid metabolism-related gene expression remain unclear. Recent studies have also found that AA, as primary MPP, can affect both milk protein and milk fat synthesis in the mammary gland, exerting a positive regulatory effect on milk fat synthesis. Adding methionine hydroxy analog to dairy cow diets increases milk fat content [11]. Chamberlain et al. [12] confirmed that intravenous methionine infusion in dairy cows elevates milk fat content. When dietary protein content increases from 12% to 18%, milk fat content decreases by 0.5% while milk protein and lactose remain unchanged; however, adding methionine hydroxy analog to dairy cow diets increases milk fat content [13]. This may occur because AA infusion increases energy carrier substances like glucose and acetate, with acetate being the primary substrate for milk fat synthesis, thereby promoting de novo fatty acid synthesis. Han Huina [14] reported that in cows fed corn straw as the sole roughage, infusion of mixed AA via the external pudendal artery significantly reduced dry matter intake and increased milk protein content, while also pro-

moting milk yield, standard milk production, milk fat content, milk fat yield, and milk protein yield. These effects may relate to altered uptake patterns of milk fat precursors by the mammary gland following AA infusion. After intravenous infusion of essential AA in dairy goats, blood glucose, triglyceride (TG), total cholesterol, milk protein, and lactose content increased, while urea nitrogen showed no significant change, though milk fat and solids-not-fat content tended to increase [15]. Blood concentrations of milk fat synthesis substrates and blood flow rate are primary factors affecting mammary uptake efficiency; AA supply promotes increased blood flow and acetate arteriovenous difference, enhancing mammary acetate uptake and uptake rate [14]. However, despite reports on AA effects on milk fat synthesis, no clear mechanism explains how AA addition affects milk fat synthesis in dairy cow mammary glands. Therefore, in-depth investigation of the regulatory mechanisms is necessary to explain AA effects on milk fat synthesis and provide theoretical basis for improving milk component synthesis and quality.

2.1. Affecting Uptake and Utilization of Fatty Acids by the Mammary Gland

Although no clear mechanism explains how protein infusion promotes milk fat synthesis, studies have found that AA infusion can increase arterial concentrations of milk fat synthesis precursors or enhance their uptake efficiency by mammary tissue, such as acetate, non-esterified fatty acids, and β-hydroxybutyrate [16-18]. Research shows that after essential AA infusion, mammary uptake of acetate, propionate, butyrate, and total volatile fatty acids (VFA) significantly increases, suggesting that AA infusion enhances mammary VFA uptake and oxidation for energy supply, facilitating milk formation [19]. Sun Manji [20] reported that infusing 49.2 and 65.6 g/d of AA mixtures into the external pudendal artery of Guanzhong dairy goats significantly increased mammary plasma concentrations of acetate, butyrate, and total VFA, as well as mammary acetate uptake. Weekes et al. [21] demonstrated that abomasal infusion of AA mixtures lacking lysine and histidine increased milk fat yield by 258 and 320 g/d, respectively. Duan Bin [15] found that infusing 6.2 g/d of AA mixture into the external pudendal artery of Guanzhong dairy goats increased mammary acetate uptake by 7.2%. Wang Qiang [22] used external pudendal artery infusion technology to administer different balanced AA patterns to dairy goats, finding that mammary uptake of milk fat precursors increased by approximately 50.0%–71.5%. In cows fed corn straw as roughage, AA infusion via the external pudendal artery increased acetate concentration in milk vein blood and acetate arteriovenous difference, narrowing the gap in mammary acetate uptake and uptake efficiency between straw-fed and alfalfa-fed cows [14]. These studies demonstrate that milk protein precursor AA can enhance mammary uptake efficiency of milk fat precursors, regulate mammary VFA content, and thereby affect milk fat synthesis. Furthermore, Maxin et al. [23] conducted a meta-analysis of seven studies on post-ruminal protein infusion effects on milk fat synthesis, finding that protein infusion increased milk fat yield, with medium- and short-chain fatty

acids (C6:0–C14:0) tending to increase, while C16:0, C18:0, and C18:2 tended to decrease. Collectively, MPP supply promotes milk fat synthesis primarily by increasing mammary uptake of milk fat precursors like acetate and butyrate, thereby increasing de novo fatty acid synthesis in the mammary gland while inhibiting uptake and utilization of long-chain fatty acids. However, relevant research remains scarce and requires further investigation.

2.2. Affecting Fatty Acid Ratios in Arterial Blood Entering the Mammary Gland

Milk protein precursor infusion can increase ratios of acetate/propionate, (acetate+butyrate)/propionate, and short-chain/long-chain fatty acids in arterial blood supplying the mammary gland [14]. Fatty acid ratios constitute important factors affecting milk fat synthesis. In vitro results show that when the acetate/butyrate ratio is 1:1 (5 mmol/L:5 mmol/L), transcription levels of acetyl-coenzyme A carboxylase α (ACACA), fatty acid synthase (FASN), and stearoyl-CoA desaturase 1 (SCD1) in bovine mammary epithelial cells (BMECs) significantly increase, while sterol regulatory element binding protein (SREBP) and peroxisome proliferator-activated receptor γ (PPARγ) mRNA expression remains unaffected [24]. When the acetate/β-hydroxybutyrate ratio is 2:1 (5.54 mmol/L:2.27 mmol/L), BMEC triglyceride content significantly increases, with FASN, ACACA, fatty acid-binding protein 3 (FABP3), lipoprotein lipase (LPL), PPARγ, and SREBF1 mRNA expression significantly upregulated [25]. Adding different ratios of unsaturated fatty acids (oleic acid:linoleic acid:linolenic acid at 0.75:4.00:1.00, 1.50:10.00:1.00, 2.00:13.30:1.00, 3.00:20.00:1.00, and 4.00:26.70:1.00) to cultured BMECs inhibited FASN and ACACA mRNA expression but upregulated FABP3 and cluster of differentiation 36 (CD36) mRNA expression [26]. Long-chain fatty acids (palmitic and stearic acid) downregulated expression of milk fat synthesis-related genes FASN, ACACA, FABP3, and SCD while upregulating CD36 expression [27-28]. Short-chain/long-chain fatty acid ratios of 1.0:2.0, 1.0:3.0, and 1.0:4.5 showed better promotion of triglyceride synthesis in BMECs; short-chain/long-chain ratios from 1.00:0.82 to 1.00:6.00 significantly promoted expression of ACACA, FASN, CD36, FABP3, LPL, PPARγ, and SREBF1 [29]. These findings suggest that milk protein precursor infusion may affect dairy cow milk fat yield by increasing the proportion of acetate and butyrate—fatty acids used for de novo synthesis—in arterial blood supplying the mammary gland, providing theoretical basis for explaining how milk protein precursors regulate milk fat synthesis. These studies also indicate that investigating the effects of AA and their interactions with milk fat precursors on milk fat and protein synthesis represents a promising area for future research.

2.3. Affecting Expression of Genes Encoding Milk Fat Synthesis-Related Enzymes

In the gene network regulating milk fat synthesis by precursors, ACACA and FASN are two key genes involved in de novo fatty acid synthesis in dairy cows, providing essential acetyl-CoA and butyryl-CoA for fatty acid de novo synthesis through the actions of FASN and ACACA [30]. SCD1 is a key regulatory factor in fatty acid synthesis, primarily responsible for desaturation during de novo synthesis. FABP, LPL, and CD36 are three important genes involved in intracellular transport of long-chain fatty acids (LCFA) in various mammalian tissues [31]. SREBP belongs to the nuclear transcription factor family and serves as an important transcriptional regulator of lipogenic genes, modulating expression of fatty acid synthesis-related genes. SREBP1 regulates numerous target genes, including ACACA, FASN, SCD1, and FABP3, thereby controlling fatty acid transport, de novo synthesis, and desaturation. PPARγ likely regulates SREBP activity in mammary tissue [32], and fatty acid transport genes such as LPL and CD36 are target genes of PPARγ [33]. Milk fat precursors regulate milk fat synthesis by modulating these related genes and regulatory factors.

During milk fat synthesis, animal fat biosynthesis and decomposition are directly regulated by enzyme activities catalyzed by various enzymes. All factors affecting enzymatic reactions, such as enzyme activity and content, influence fat synthesis. In vitro studies have found that different AA patterns significantly affect activities of key enzymes in fat metabolism and synthesis (FAS, malate dehydrogenase, and glucose-6-phosphate dehydrogenase) and triglyceride secretion in mammary cell cultures, enhancing triglyceride secretion and affecting fat synthesis by increasing activities of key fatty acid synthesis enzymes [34]. Methionine and lysine at appropriate concentrations promote ACACA mRNA expression in BMECs [35]. Since excess AA are converted to glucose, which both provides substantial energy for milk fat synthesis and yields glucose intermediate metabolites that serve as substrates for acetyl-CoA carboxylase (ACC) synthesis, triglyceride content in BMECs increases with glucose content [36]. Currently, research on AA effects on milk fat synthesis-related enzyme activities and gene expression in the mammary gland is limited, but given their important role in milk fat synthesis, examining these parameters is crucial in studies investigating AA effects on milk fat synthesis.

2.4. Regulating Milk Fat Synthesis via the Mammalian Target of Rapamycin (mTOR) Signaling Pathway

mTOR is an atypical serine/threonine protein kinase that forms two distinct complexes when bound to different proteins: mTORC1 and mTORC2 [37]. The mTOR signaling pathway senses and integrates multiple nutrient and environmental signals, participating in animal growth and internal homeostasis regulation. Mammary AA uptake is regulated not only by nutrient supply in arterial blood but also by the mTOR signaling pathway. Regulating protein translation

is crucial for determining milk yield [38], and AA effects on protein translation are mediated by the mTOR signaling pathway [7]. Proud [39] demonstrated through animal and cell model experiments that AA and intracellular available energy serve not only as substrates for protein synthesis but also as signals to protein synthesis tissues. While regulating milk protein synthesis, the mTOR pathway can also regulate milk fat synthesis. Leucine (Leu), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and lysine (Lys) can all upregulate expression of SREBP1 and PPARγ genes in dairy cow mammary epithelial cells [8]. mTORC1 regulates insulin effects on de novo fat synthesis by activating SREBP1 [40]. Additionally, the mTOR signaling pathway promotes blood lipid regulation, fat accumulation, and fat uptake through PPARG [41]. Luyimbazi et al. [42] reported that adding 100 nmol/L rapamycin reduced SCD1 and SREBP1 protein expression in human breast cancer cells without affecting SCD1 protein stability. In the same study, silencing eukaryotic initiation factor 4E (eIF4E) via small interfering RNA significantly reduced SCD1 and SREBP1 protein synthesis, suggesting SCD1 may be regulated by the mTOR/eIF4E pathway. Sheng Ran [43] proposed that acetate activates downstream factor eIF4E via the mTOR signaling pathway, which then activates SREBP1 to regulate milk fat synthesis-related genes such as FASN, ACACA, and SCD1, thereby controlling milk fat synthesis. Thus, eIF4E may be a convergence point through which acetate regulates milk fat and protein synthesis via the mTOR signaling pathway. Therefore, AA may affect milk fat synthesis through the mTOR signaling pathway, though related research is currently rare and requires further investigation.

2.5. Regulating Milk Fat Synthesis via the AMP-Activated Protein Kinase Signaling Pathway

AMP-activated protein kinase (AMPK) is a heterotrimeric protein kinase complex composed of a catalytic α subunit and regulatory β and γ subunits. Binding of 5' -AMP to the β and γ subunits activates AMPK, triggering conformational changes. Upstream kinases including transforming growth factor-β activated kinase 1 (TAK1), serine/threonine kinase 1 (LKB1), and calmodulin-dependent protein kinase kinase (CaMKK) catalyze phosphorylation of Thr172 in the AMPK subunit active center [44-46]. In mouse liver, AMPK phosphorylation inactivates SREBP1c, thereby downregulating expression of fat synthesis genes including ACC, FAS, and SCD1 [47]. High-protein diets can inhibit AMPK activity and promote mTOR activation in a Leu-dependent manner [48]. Essential AA addition reduces AMPK (Thr172) phosphorylation while increasing mTOR (Ser2448) phosphorylation levels in mammary epithelial cells [49]; activated AMPK inhibits downstream mTOR activity [50]. However, research on AA regulation of downstream gene expression via AMPK in dairy cow mammary cells has primarily focused on milk protein synthesis-related genes, with limited studies on milk fat synthesis-related genes (FASN, ACACA, SCD, SREBP1, and PPARG). Current research on AMPK mechanisms in fat synthesis mainly targets adipose tissue and adipocytes [51-52]. In chicken preadipocytes, AMPK

activator AICAR treatment reduced PPARα and PPARγ gene expression, while inhibitor Compound C treatment increased cellular lipid accumulation capacity and PPARα and PPARγ expression, indicating AMPK regulates fat deposition [53]. Therefore, whether AA can regulate fat synthesis via the AMPK/mTOR signaling pathway in BMECs requires investigation.

AA affect not only milk protein synthesis but also regulate milk fat synthesis in dairy cow mammary glands. Currently, numerous studies have investigated single or mixed AA effects on milk protein synthesis, but research on AA regulatory roles and mechanisms in milk fat synthesis remains limited. Comprehensive domestic and international studies suggest AA may influence milk fat synthesis through mammary uptake patterns of milk fat precursors, expression of milk fat synthesis-related genes, and mTOR and AMPK signaling pathways, though definitive mechanisms still require further exploration.

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