Regulatory Effects and Mechanisms of Rumen-Protected Methionine on Metabolism and Health in Periparturient Dairy Cows: A Postprint

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Abstract

Influenced by multiple factors, periparturient dairy cows experience negative balance of energy and other nutrients, predisposing them to nutritional and metabolic diseases. Studies have shown that methionine can regulate energy and lipid metabolism, improve hepatic function, and enhance systemic antioxidant capacity and immune function in periparturient dairy cows. This review summarizes recent advances in methionine regulation of metabolism and health during the periparturient period, discusses optimal supplementation levels of rumen-protected methionine, and aims to provide a reference for fundamental research and practical application of methionine in periparturient dairy cows.

Full Text

Rumen-Protected Methionine' s Regulatory Effects and Mechanisms on Metabolism and Health of Transition Dairy Cows

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Abstract: The transition period in dairy cows is characterized by negative balances of energy and other nutrients due to multiple factors, leading to a high incidence of nutritional metabolic diseases. Research has demonstrated that methionine can regulate energy and lipid metabolism, improve liver function, and enhance antioxidant capacity and immune function in transition dairy cows. This review summarizes recent advances in methionine' s regulation of metabolism and health during the transition period and discusses appropriate supplementation levels of rumen-protected methionine, aiming to provide references for both fundamental research and practical application of methionine in transition dairy cows.

Keywords: methionine; transition dairy cow; metabolic regulation; health regulation; mechanism

The transition period, comprising the prepartum (21 days before calving) and postpartum (21 days after calving) phases, represents a critical stage in the lactation cycle of dairy cows. During this period, cows undergo physiological transitions from pregnancy to parturition to lactation, involving coordinated changes across multiple tissues. Nutritional requirements increase significantly while dry matter intake (DMI) declines sharply, resulting in severe nutrient deficiencies and negative balances of multiple nutrients, particularly negative energy balance (NEB) [1]. To alleviate NEB, transition cows mobilize body fat reserves, producing nonesterified fatty acids (NEFA) that are primarily metabolized in the liver through three pathways: (1) complete oxidation to CO and H O for efficient energy supply; (2) incomplete oxidation to ketone bodies with low energy efficiency, predisposing to ketosis; and (3) esterification to form triglycerides (TG), whose accumulation in the liver can lead to fatty liver disease [2]. Both ketosis and fatty liver seriously threaten cow health during the transition period and can trigger secondary metabolic disorders, leading to premature culling.

Methionine (Met) is an essential sulfur-containing amino acid required for animal growth, development, and physiological activities, and serves as a crucial methyl donor in the one-carbon cycle and related metabolic processes in the liver [3]. Studies have shown that Met can regulate energy and lipid metabolism, maintain liver health [4], improve antioxidant capacity and immune function [5-6], and some maternal regulatory effects may be manifested in calves [7]. This review outlines the physicochemical properties, biological functions, and rumen degradation characteristics of Met, summarizes research progress on rumen-protected methionine (RPM) in regulating metabolism during the transition period, and discusses appropriate dietary supplementation levels of RPM for transition cows, aiming to provide scientific evidence and technical references for Met application in dairy cows.

1 Nutritional Overview of Methionine

Methionine has the molecular formula C H O NS, a chemical structure shown in [Figure 1: see original paper], and a relative molecular mass of 149.21. It is a sulfur-containing essential amino acid in humans and animals, closely related to numerous physiological and biochemical processes including cell signal transduction and synthesis of nucleic acids and proteins. Balanced Met nutrition is crucial for optimal growth, physiological metabolism, health, and lactation performance in dairy cows. Beyond its role as a component of cellular proteins, Met performs several key functions: (1) as a substrate and regulatory substance, it participates in protein synthesis throughout the body, particularly in mammary tissue where Met and lysine (Lys) are the two most limiting amino acids, with their limiting order determined by basal diet composition [8]; (2) its metabolite S-adenosylmethionine (SAM) serves as an important methyl donor involved in regulating multiple physiological and biochemical processes [9]; (3) similar to choline, Met promotes synthesis of very-low-density lipoprotein (VLDL) and carnitine in the liver, regulating hepatocellular lipid metabolism [2,10]; (4) it synthesizes antioxidant substances (e.g., taurine) to maintain redox balance [11]; and (5) it regulates immune cell activity and function to enhance immunity [11-12]. Additionally, in wool-producing animals, Met can be converted to cysteine to promote wool growth and increase wool yield [13].

Under the action of rumen microorganisms, Met is extensively degraded in the rumen and participates in related metabolic processes, resulting in limited Met reaching the small intestine and restricting its utilization from the diet [10]. In the rumen, Met is partially used for microbial protein synthesis and partially enters other metabolic pathways. For example, Met can act as a methyl donor, combining with hydrogen produced by rumen microbes to generate methane, causing losses of both energy and amino acids. Therefore, RPM must be added to dairy cow diets to ensure sufficient Met absorption in the small intestine and delivery via blood circulation to target organs for physiological functions [3].

2 Regulation of Physiological Metabolism and Health by Methionine in Transition Dairy Cows

In dairy cows, the metabolic pathways of Met and choline are interrelated and interconvertible. Based on the one-carbon cycle theory, both Met and choline promote liver health and metabolism, enhance antioxidant and immune functions, and reduce metabolic diseases during the transition period [11,14]. Met can also regulate digestive function, improve dietary nitrogen utilization, reduce nitrogen excretion, and enhance postpartum lactation performance [15-16]. As a substrate and regulatory substance, Met promotes protein synthesis through pathways such as the mammalian target of rapamycin (mTOR) signaling pathway [8,17].

2.1 Regulatory Effects of Met on the Liver of Transition Dairy Cows

RPM can regulate liver function in transition dairy cows by promoting lipid and carbohydrate metabolism in hepatocytes and increasing the output of energy and other substances from the liver. Abnormal or overloaded lipid metabolism is a primary threat to liver health and function during the transition period. Nutritional strategies that promote complete NEFA oxidation, TG transport, and gluconeogenesis represent important approaches to ensure efficient energy conversion and metabolism and to support synthesis of essential proteins (e.g., albumin and metabolic enzymes) in the liver.

Due to Met's crucial role in the one-carbon cycle (Figure 2 [Figure 2: see original paper]) and protein synthesis, dietary RPM' s regulation of liver function and nutrient metabolism during the transition period has become a research focus. Dietary RPM supplementation during the transition period upregulates hepatic expression of peroxisome proliferator-activated receptor (PPAR), which subsequently increases expression of pyruvate carboxylase (PC), microsomal triglyceride transfer protein (MTTP), and phosphoenolpyruvate carboxykinase (PEPCK), indicating that RPM promotes lipoprotein assembly and enhances gluconeogenesis in hepatocytes [18]. Further research suggests this may be related to the one-carbon cycle and promoter methylation of certain genes [18-19]. Li et al. [4] found that dietary RPM supplementation increased blood VLDL content while reducing hepatic TG content (4.70% vs. 3.40% on a wet weight basis), demonstrating that RPM promotes TG transport and reduces hepatic lipid deposition. Concurrently, blood NEFA content decreased, suggesting reduced body fat mobilization, possibly due to alleviated NEB. RPM can regulate lipid and energy metabolism and conversion, reducing lipid deposition in transition dairy cows, though the signaling networks, endocrine mechanisms, and other potential pathways require further elucidation. The roles and mechanisms of key pathways and regulatory factors such as AMP-activated protein kinase (AMPK), PPAR , and sterol regulatory element binding protein 1c (SREBP-1c) in the regulation of transition cow liver metabolism and health by dietary methyl nutrients (Met, choline, betaine, and folate) remain to be clarified.

2.2 Effects of Met on Overall Health of Transition Dairy Cows

Through the one-carbon cycle, RPM synthesizes antioxidant substances that reduce oxidative damage from free radicals, enhances immunity, and decreases the incidence of metabolic and other diseases. Studies show that dietary RPM supplementation promotes de novo synthesis of antioxidants such as glutathione, improves plasma free radical scavenging capacity, reduces oxidative stress and hepatic inflammatory responses, and enhances immune function [5-6]. Met supply is closely related to immune responses, and RPM affects immune function through multiple mechanisms: promoting T lymphocyte proliferation [21], enhancing neutrophil function [22], reducing oxidative stress-induced damage to immune cells [12], and altering peripheral blood T lymphocyte subset ratios (CD4+/CD8+) [3,11]. Additionally, using primary bovine hepatocyte culture techniques, high levels of NEFA and -hydroxybutyric acid (BHBA) have been shown to cause hepatocellular oxidative stress, trigger inflammatory responses, impair cellular function, and induce apoptosis [3,23-26]. It can be hypothesized that RPM may indirectly improve immune function during the transition period by alleviating NEB, reducing body fat mobilization, enhancing liver function, and decreasing blood NEFA and BHBA concentrations, thereby mitigating their damaging effects on immune cells—a hypothesis partially confirmed by existing research [27-29]. In summary, Met plays a vital role in maintaining redox balance and immune function in transition dairy cows.

Dietary RPM supplementation during the transition period increases hepatic gene expression of betaine-homocysteine S-methyltransferase (BHMT), phosphatidylethanolamine N-methyltransferase (PEMT), methionine adenosyltransferase 1A (MAT1A), S-adenosylhomocysteine hydrolase (SAHH), and cystathionine -synthase (CBS), while decreasing activity of 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR). These findings indicate that RPM influences hepatic one-carbon metabolism, promoting synthesis of phosphatidylcholine and antioxidants (taurine and glutathione), which may represent important mechanisms through which RPM benefits liver health, overall health, and productive performance [7,19-20,30]. Furthermore, RPM has been found to affect genome-wide methylation and methylation at specific sites in the PPAR promoter region, upregulating expression of PPAR and other genes related to energy and lipid metabolism, thereby promoting hepatocellular lipid metabolism and transport, reducing TG deposition, and enhancing carbohydrate metabolism and conversion [18].

2.3 Regulatory Effects of Met on Calf Physiology

After digestion and absorption in the small intestine, Met from RPM can be converted to SAM, providing free methyl groups that cause methylation of key gene promoter regions in the liver and other organs, thereby upregulating or downregulating expression of certain genes and regulatory factors to modulate metabolism. Some of these changes may be heritable to offspring [7].

Supplementing RPM during the prepartum period increases hepatic expression of DNA (cytosine-5)-methyltransferase 1 (DNMT1) in newborn calves [7]. However, this does not necessarily prove transgenerational inheritance of epigenetic changes from the maternal liver; it may also result from increased blood Met concentration in the dam, which crosses the placental circulation to the fetus, increasing Met or other methyl donors available to the embryo and inducing these changes directly. In primary hepatocyte culture experiments with newborn calves, increasing Met and choline chloride concentrations in the culture medium differentially affected methyl transfer, transsulfuration, and related processes, promoted VLDL synthesis, and reduced free radical accumulation [20]. Therefore, further research is needed to confirm the effects of maternal methyl donor supply during the prepartum period on calf liver metabolism and health, ultimately constructing a mechanistic network of RPM' s regulation of liver function, nutrient metabolism, and overall health during the transition period to provide a theoretical foundation for methyl donor nutrition in dairy cows.

3 Appropriate Supplementation Level of RPM for Transition Dairy Cows

As one of the most limiting amino acids for lactation, Met has been extensively studied in lactating dairy cows, primarily focusing on its regulation of protein synthesis in mammary epithelial cells and its mechanisms, often in combination with Lys [8,15,31-33]. However, research on transition dairy cows remains limited. When establishing Met requirements for lactating cows, the premise is to ensure adequate metabolizable protein (MP) in the diet, followed by consideration of amino acid composition and ratios within MP. The recommended Lys:Met ratio is approximately 3:1, with NRC (2001) recommending 7.2% and 2.4% of MP for Lys and Met, respectively. Therefore, RPM supplementation levels are typically calculated based on measured MP, Lys, and Met contents of basal diets according to these ratios. Current nutritional standards and research have not provided specific recommendations for RPM supplementation during the transition period. Based on our research group' s studies and comprehensive analysis of published results (Table 1), we recommend an RPM supplementation level of 10–20 g/d (expressed as Met) for transition dairy cows.

4 Summary

Nutrition and management during the transition period are critical for fetal development, cow health, lactation, and reproductive performance, even affecting the entire lactation cycle. RPM plays an important role in regulating liver health and nutrient metabolism during the transition period, though the physiological and molecular mechanisms are not fully understood, and standardized supplementation levels and forms remain to be established. Future research should focus on four main aspects: (1) Standardization of precise RPM supplementation levels and methods, considering dietary MP, energy-nitrogen balance, and amino acid ratios, while accounting for Met form, rumen protection efficiency, bioavailability, and efficacy to standardize supplementation protocols; (2) Elucidation of key signaling pathways through which RPM regulates liver function, metabolism, and health during the transition period, exploration of neuroendocrine and other physiological mechanisms, systematic analysis of regulatory mechanisms, and integration with choline-related research to construct a mechanistic and technical network of one-carbon metabolism regulation of liver metabolism; (3) Investigation of RPM' s regulation of antioxidant and immune function, focusing on pathways such as nuclear factor kappa B (NF- B), toll-like receptor 4 (TLR4), and nuclear factor erythroid 2-related factor 2 (Nrf2); and (4) Further exploration of maternal Met supply effects on embryonic development, metabolism, and calf health, including related physiological mechanisms, signal transduction, and potential epigenetic mechanisms.

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