Thiamine Synthesis in the Rumen and Its Application in the Prevention of Subacute Rumen Acidosis Postprint

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Abstract

Subacute ruminal acidosis is a common nutritional metabolic disorder in modern intensive ruminant production; elucidating its pathogenesis and preventive measures constitutes an important scientific issue in livestock production. This paper, by reviewing the application effects of thiamine in ruminant production, factors influencing thiamine synthesis, the relationship between thiamine and subacute ruminal acidosis, and the possible mechanisms through which thiamine regulates subacute ruminal acidosis, further elucidates the pathogenic mechanism of subacute ruminal acidosis and the physiological role of thiamine, and provides theoretical reference for ruminant production.

Full Text

Preamble

Thiamine Synthesis in the Rumen and Its Application in Preventing Subacute Ruminal Acidosis

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Abstract: Subacute ruminal acidosis (SARA) is a common nutritional metabolic disorder in modern intensive ruminant production. Elucidating the mechanisms of SARA occurrence and its prevention measures represents an important scientific challenge in animal agriculture. This paper reviews the application effects of thiamine in ruminant production, factors influencing thiamine synthesis, the relationship between thiamine and SARA, and the potential regulatory pathways through which thiamine alleviates SARA. These

insights further reveal the pathogenesis of SARA and the physiological functions of thiamine, providing a theoretical reference for ruminant production.

Keywords: ruminant; thiamine; subacute ruminal acidosis; regulation pathway

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In current Chinese ruminant production, insufficient high-quality forage resources such as alfalfa have led to substantially increased concentrate proportions in diets to meet the energy demands of high-yielding dairy cows and intensive fattening of beef cattle and sheep. However, this high-concentrate feeding pattern increases the incidence of nutritional metabolic diseases like ruminal acidosis. Plaizier et al. [1] reported that approximately 20% of high-yielding dairy cows suffer from subacute ruminal acidosis (SARA), with the North American dairy industry losing $0.5–1.0 billion annually due to ruminal acidosis [2]. If dairy cows remain in a subacute ruminal acidosis state for extended periods, it may lead to hepatic edema, diarrhea, laminitis, and other diseases. SARA has become a critical factor restricting the development of China's animal husbandry industry, making the search for prevention and control measures a research priority in recent years. Recent studies have found that high-concentrate diets may affect ruminal microbial thiamine synthesis and flow, and appropriate thiamine supplementation can alleviate SARA by increasing ruminal fluid pH, improving volatile fatty acid composition, and reducing lactic acid and endotoxin accumulation [3-4]. However, these studies have primarily focused on the effects of thiamine on single ruminal metabolites, while the reasons why SARA affects thiamine synthesis and the pathways through which thiamine alleviates SARA remain unclear. Therefore, this review synthesizes research on thiamine application in ruminant production and the relationship between thiamine and SARA, aiming to further understand thiamine' s nutritional role and provide technical references for effective SARA prevention and regulation in production.

1.1 Application of Thiamine in Ruminant Production

Previous research suggested that ruminal microorganisms could synthesize sufficient thiamine to meet ruminant nutritional requirements without dietary supplementation [5-6]. However, under current intensive feeding systems, increased dietary carbohydrate levels have raised thiamine requirements for carbohydrate metabolism, and microbial thiamine synthesis may no longer meet animal needs. Consequently, thiamine application in ruminant production has regained attention in recent years. Neville et al. [7] found that supplementing growing lambs with 100 mg/(head · d) thiamine increased feed intake and reduced feed-to-gain ratio. Shaver et al. [8] reported that adding 300 mg/d thiamine to high-concentrate diets of high-yielding dairy cows improved milk production and milk protein percentage. Kholif et al. [9] also demonstrated that 340 mg/d thiamine supplementation increased milk yield by 12.28% and significantly improved milk fat and protein yield, though milk fat percentage, lactose percentage, and milk protein percentage showed no significant differences. Solouma et al. [10] found

that adding 40 mg/d thiamine to ewe diets significantly increased blood precursors for milk synthesis (albumin, globulin, glucose) and milk yield. These improvements in milk production, milk fat percentage, and milk protein percentage may be related to thiamine' s role in promoting carbohydrate metabolism. Thiamine, as thiamine pyrophosphate, participates in decarboxylation reactions as a coenzyme for pyruvate dehydrogenase and -ketoglutarate dehydrogenase [11]. Increased concentrate-to-carbohydrate ratios raise thiamine requirements, but insufficient microbial thiamine synthesis causes glucose to be metabolized through alternative pathways such as the polyol, hexosamine, and protein kinase C pathways [12]. Impaired pyruvate decarboxylation leads to its conversion to lactic acid, reducing the supply of milk component precursors like acetate and propionate. Exogenous thiamine supplementation may increase milk component precursor synthesis by promoting pyruvate decarboxylation into the tricarboxylic acid (TCA) cycle, thereby improving lactation performance.

However, the effects of thiamine supplementation in ruminant production have been inconsistent. Rowghani et al. [13] investigated different thiamine supplementation levels (0, 4, 6 mg/kg DM) in lambs fed high-concentrate diets and found that, except for reducing blood urea nitrogen levels, thiamine had no significant effects on other parameters. Silzell et al. [14] found that adding 140 mg/kg DM thiamine to steer diets did not significantly affect average daily gain, average daily feed intake, or feed conversion efficiency. The discrepancies in these studies may be attributed to differences in experimental animals, thiamine supplementation levels, and dietary nutritional levels affecting microbial thiamine synthesis in the rumen. Additionally, since feed intake, dietary nutritional level, and digestibility are primary factors influencing animal performance, variations in diets and experimental conditions across studies can also lead to differences in thiamine application effects.

1.2 Thiamine Synthesis in the Rumen and Influencing Factors

Thiamine is primarily present in bacterial cells within the solid phase of rumen contents, with lower concentrations in the liquid phase [15]. Thiamine mainly originates from ruminal microbial synthesis, with partial release from feed ingredients through microbial degradation. However, reports on the amount of thiamine synthesized by ruminal microorganisms have been inconsistent. Schwab et al. [16] found apparent thiamine synthesis in the rumen of 50.6 mg/d, similar to the 51.7 mg/d reported by Breves et al. [17] but 6.1 mg/d higher than reported by Santschi et al. [18]. Steinberg et al. [19] demonstrated that thiamine synthesis varied from 23–50 mg/d (average 32 mg/d), while NRC (2001) [20] overestimated ruminal thiamine synthesis at 127 mg/d. These discrepancies partly result from inconsistent experimental conditions across studies. Additionally, thiamine synthesis is influenced by multiple factors, which are discussed below.

1.2.1 Dietary Nitrogen Source

Buziassy et al. [21] showed that ruminal thiamine content decreased significantly when dietary protein levels were reduced, but remained unaffected when dietary protein was completely replaced by urea. Breves et al. [17] also found that reducing dietary nitrogen content by 26% significantly decreased net thiamine synthesis in the rumen and thiamine flow to the duodenum. Thiamine synthesis in the rumen was positively correlated with microbial protein nitrogen flow to the duodenum ($r = 0.76$), further demonstrating that ruminal microbial metabolic activity affects thiamine synthesis.

1.2.2 Dietary Structure and Carbohydrate Levels

Hunt et al. [22] found that adding starch to hay- and alfalfa-based diets increased B-vitamin synthesis in fistulated cattle. Schwab et al. [16] observed that increasing non-fibrous carbohydrate (NFC) levels in dairy cows fed 60% forage diets enhanced vitamin B1 synthesis, but decreased apparent ruminal thiamine synthesis when cows were fed 35% forage diets. Tafaj et al. [23] demonstrated a negative quadratic relationship between thiamine and concentrate levels, with the highest ruminal fluid thiamine content at 70% high-concentrate conditions, lower content at 60% and 50% concentrate, and intermediate content at 40% and 25% concentrate. Miller et al. [5] reported that beef cattle fed high-grain diets (90% corn) had significantly lower ruminal thiamine content than those fed low-grain diets (30% corn). These findings indicate that appropriately increasing dietary carbohydrate levels and adding readily digestible carbohydrates can promote thiamine synthesis, but excessive carbohydrates are detrimental to synthesis, suggesting that thiamine synthesis is primarily influenced by energy intake and digestible organic matter intake [23]. This may be because ruminal propionate-producing bacteria can synthesize thiamine or its intermediates [24]. Moderately increasing dietary carbohydrate levels enhances energy supply for bacterial metabolism, promoting proliferation of propionate-producing bacteria such as *Megasphaera elsdenii* and *Selenomonas ruminantium* [25], thereby increasing thiamine synthesis. However, excessive proportions of readily fermentable carbohydrates like corn can induce acidosis, causing accumulation of volatile fatty acids and lactic acid and decreasing pH, which inhibits growth of propionate-producing bacteria like *M. elsdenii* and *S. ruminantium* [25] and affects thiamine synthesis. Additionally, decreased ruminal fluid pH promotes release of bacterial thiaminase, which further degrades thiamine [26].

1.2.3 Rumen Environment and Metabolites

Ruminal fluid pH and metabolites affect thiamine synthesis. Tufaj et al. [27] showed that when total mixed rations (TMR) contained the same thiamine content but differed in forage (grass silage) particle size, thiamine content in high-yielding dairy cows was negatively correlated with pH (R^{2} = 0.21–0.26). Tafaj et al. [23] investigated the relationship between thiamine content and fermentation patterns by gradually increasing dietary concentrate levels, finding a

linear negative correlation between ruminal thiamine content and pH (R^{2} = 0.07–0.09), with strong correlations at sampling times of 16:00 and 19:00 (R^{2} = 0.70–0.77). Ruminal thiamine content was also linearly positively correlated with short-chain fatty acid (SCFA) concentration, with propionate concentration showing higher correlation with thiamine content than other SCFAs [23]. The possible reasons for these effects of ruminal pH and SCFA concentration on thiamine content are that under high-concentrate conditions, rapid fermentation increases carbohydrate intake, causing elevated propionate concentration and decreased pH [4], while increased dietary energy levels enhance ruminal microbial thiamine synthesis efficiency [17]. However, if ruminal fluid pH drops too low and SARA or acute acidosis occurs, changes in ruminal microbial composition, increased thiaminase activity from acidotic ruminal bacteria, impaired thiamine absorption, and increased metabolic requirements may cause thiamine deficiency [26].

2.1 Mechanisms of Subacute Ruminal Acidosis and Its Impacts

When ruminants consume excessive high-concentrate diets, rapid carbohydrate degradation leads to accumulation of acidic substances such as volatile fatty acids and lactic acid, while reduced physically effective neutral detergent fiber content decreases saliva secretion, impairing ruminal buffering capacity and causing rapid pH decline. SARA occurs when ruminal fluid pH remains below 5.6 for longer than 3 h/d [28]. Current research suggests that SARA development involves mechanisms of lactic acidosis, organic acidosis, and endotoxin and histamine actions [4]. SARA induces changes in ruminal microbial populations and metabolites, primarily manifested in three aspects: First, alterations in ruminal microbial community structure. Organic acid accumulation gradually decreases ruminal fluid pH during SARA. Since different bacteria have varying pH tolerances, pH decline affects bacterial growth and proliferation, causing community imbalance. For example, *Streptococcus bovis*, *Selenomonas ruminantium*, and *M. elsdenii* have pH tolerances of 5.6, 4.8, and 5.4, respectively. When pH drops below 6.0, major lactic acid-producing bacteria (e.g., *S. bovis* and *Lactobacillus*) increase significantly, while major lactic acid-utilizing bacteria (e.g., *M. elsdenii* and *S. ruminantium*) are inhibited. Additionally, fiber-degrading bacteria decrease substantially, and protozoa die in large numbers, causing ruminal microbial dysbiosis [29-30]. Second, changes in ruminal microbial metabolites. Prolonged acidic conditions cause massive lysis of Gram-negative bacteria, releasing abnormal metabolites such as lipopolysaccharide (LPS) and histamine [1,31-32]. Altered ruminal acidity causes metabolic pathways and products of bacteria like *S. bovis* to shift from acetate-propionate fermentation to lactate fermentation, increasing lactate accumulation. Third, alterations in ruminal epithelial structure and permeability. While appropriately increasing dietary concentrate proportions and nutritional levels can enhance papilla length and density, excessive concentrate induces SARA. High VFA concentrations cause incomplete keratinization and abnormal morphology of ruminal papillae. SARA

reduces the thickness of ruminal stratified squamous epithelium, weakening adhesion between granular layers, increasing ruminal epithelial cell permeability, and compromising the barrier protective function of ruminal epithelium [33]. Additionally, LPS can alter tight junction protein ZO-1 structure and function by increasing nitric oxide (NO) production [34] and induce tumor necrosis factor- (TNF-). Interleukin (IL)-3 and IL-4 promote apoptosis and disrupt tight junction proteins, thereby increasing ruminal mucosal permeability [35].

2.2 Subacute Ruminal Acidosis and Thiamine Deficiency

The erythrocyte transketolase activity coefficient, or thiamine pyrophosphate (TPP) effect value, reflects thiamine status. Thiamine deficiency reduces transketolase activity and increases TPP levels in red blood cells. A TPP effect value exceeding 45% indicates potential thiamine deficiency [36]. Studies have found that when cattle and sheep exhibit clinical acute acidosis symptoms, TPP effect values are 46.7% and 109.3%, respectively [26], while sheep with SARA show a TPP effect value of 59.4% [37]. Karapinar et al. [38] investigated the effects of a high-concentrate diet containing 75% oats on thiamine status in 1–2-year-old fattening cattle and found that the high-concentrate group had a mean blood TPP effect value of 47.2%, significantly higher than the control group (19.53%). These results demonstrate that despite increased dietary thiamine content with higher grain levels, animals still experience thiamine deficiency under high-concentrate feeding conditions. Thiamine deficiency can elevate blood pyruvate and lactate concentrations and reduce transketolase and pyruvate dehydrogenase activities [39], leading to accumulation of lactate and volatile fatty acid metabolites that may induce acidosis. Possible causes of thiamine deficiency include: TPP serves as a coenzyme for pyruvate dehydrogenase and -ketoglutarate dehydrogenase, participating in -keto acid oxidative decarboxylation during carbohydrate metabolism. Increased carbohydrate levels in high-concentrate diets raise thiamine requirements. Additionally, when high-concentrate diets induce ruminal acidosis, decreased ruminal fluid pH and lactate accumulation inhibit microbial growth, suppressing synthesis of B-vitamin-producing microorganisms and reducing thiamine synthesis. Furthermore, increased bacterial thiaminase activity after acidosis enhances thiamine degradation [26].

3.1 Research Progress on Thiamine Alleviating Subacute Ruminal Acidosis

Thiamine deficiency impairs carbohydrate metabolism, causing accumulation of intermediate metabolites pyruvate and lactate, thereby inducing ruminal acidosis. Current research indicates that dietary thiamine supplementation can alleviate SARA. Dong et al. [3] demonstrated that when goats experienced SARA, supplementing the diet with 240 mg/kg thiamine increased ruminal fluid pH, significantly decreased ruminal lactate and acetate concentrations and acetate/propionate ratio, while significantly increasing propionate and butyrate

concentrations, thereby alleviating SARA in goats. Wang et al. [40] showed that adding 180 mg/kg thiamine to high-concentrate diets of lactating dairy cows reduced populations of lactate-producing bacteria (*S. bovis* and *Lactobacillus*) and increased populations of lactate-utilizing bacteria (*M. elsdenii*), thereby inhibiting lactate accumulation and increasing ruminal fluid pH. Hölter-shinker et al. [41] demonstrated that thiamine protected protozoa under SARA conditions. Additionally, thiamine may alleviate SARA by promoting ruminal epithelial tissue growth and function. Wang [42] found that adding 120 mg/kg thiamine to high-concentrate diets of calves promoted ruminal epithelial development. Calves with SARA showed cystic papillae and abnormal stratum corneum shedding, while thiamine-supplemented calves had relatively normal papillary development without microabscesses at papilla tips and with smooth, intact stratum corneum. However, the mechanisms by which thiamine promotes ruminal epithelial development remain unclear.

3.2 Potential Mechanisms of Thiamine Alleviating Subacute Ruminal Acidosis

The fundamental cause of SARA is unreasonable concentrate-to-forage ratios that disrupt ruminal microbial metabolism, leading to organic acid accumulation and release of abnormal metabolites from microbial lysis, while ruminal epithelial tissue damage from prolonged low-pH environments causes absorption disorders that worsen systemic acidosis. Therefore, investigating thiamine' s mechanisms for alleviating SARA should consider the overall changes in the ruminal microbial system (including protozoa, fungi, and bacteria) and their metabolites, as well as their interactions with ruminal epithelial function and animal metabolism.

3.2.1 Systematically Regulating Ruminal Carbohydrate Metabolism and Microecological Balance

Current research on thiamine' s alleviating effects has focused on ruminal fermentation parameters and single metabolites (e.g., volatile fatty acids, lactate, LPS, histamine), with limited investigation of the relationship between ruminal metabolism and animal metabolism or the intrinsic connections among different metabolites. Beyond causing changes in lactate and SCFA concentrations, Brownlee [12] demonstrated that thiamine deficiency inhibits transketolase in the pentose phosphate pathway and pyruvate dehydrogenase and -ketoglutarate dehydrogenase in the TCA cycle, causing glucose to be metabolized through the polyol, hexosamine, and protein kinase C pathways. Bubber et al. [11] found that thiamine deficiency also significantly reduced activities of non-thiamine-dependent enzymes in the TCA cycle, including succinate dehydrogenase, succinate thiokinase, and malic enzyme, with reductions greater than those of pyruvate dehydrogenase and -ketoglutarate dehydrogenase. This would alter concentrations of all TCA cycle intermediates, demonstrating that thiamine affects multiple carbohydrate metabolic pathways. Moreover, thiamine' s effects

on ruminal microorganisms have been limited to lactate-metabolizing bacteria such as *S. bovis*, *Butyrivibrio fibrisolvens*, *S. ruminantium*, *Lactobacillus*, and *M. elsdenii* [40], while complex symbiotic and competitive relationships exist among ruminal microorganisms. Changes in a few bacterial species cannot reflect the overall microbial response to thiamine. Therefore, current single-angle studies cannot accurately reveal thiamine's effects. Future research must systematically elucidate thiamine' s mechanisms for alleviating SARA from holistic perspectives of ruminal microbial digestion and metabolism, metabolite composition balance, and clearance. Current high-throughput sequencing and metabolomics technologies provide technical means for systematically studying overall changes in ruminal microorganisms and their metabolites [43-44], which will help reveal the comprehensive effects of thiamine and SARA on ruminal microbial communities and their metabolites.

3.2.2 Regulating Ruminal Epithelial Barrier Function

Thiamine deficiency may promote SARA development through impaired tissue barrier function. Current research on thiamine' s effects on tissue barriers has primarily focused on the human blood-brain barrier, with no studies on ruminal epithelial function. On one hand, thiamine deficiency induces epithelial cell apoptosis and increases permeability. Studies by Gioda et al. [45] and Hazell et al. [46] showed that thiamine deficiency enhances endothelial nitric oxide synthase (eNOS) activity, increasing NO production. Excessive NO combines with superoxide anion O_{2}^{-} to form peroxynitrate (ONOO^{-}), which exerts toxic effects and causes cell apoptosis. Thiamine deficiency also reduces protein expression of occludin, tight junction protein ZO-1, and ZO-2, increasing permeability of brain vascular endothelial cells [47]. On the other hand, thiamine deficiency induces pro-inflammatory cytokine release and inflammatory responses [48]. Vemuganti et al. [49] used microarray analysis to show upregulated expression of inflammation-related genes in brain-sensitive regions following thiamine deficiency, including pro-inflammatory cytokines [IL-6, IL-18, TNF- , allograft inflammatory factor 1 (AIF1), and osteopontin], chemokines [monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1 , MIP-1, and growth-related oncogene-1 (Gro1)], interferon (IFN), and IFN-induced proteins. Karuppagounder et al. [50] also found that thiamine deficiency significantly increased immunoreactivity of CD11b, glial fibrillary acidic protein (GFAP), and intercellular adhesion molecule-1 (ICAM-1) in the thalamic paraventricular nucleus, along with markedly elevated mRNA expression of eNOS (4-fold), IL-1 (43-fold), IL-6 (44-fold), and TNF- (64-fold), and increased cortical TNF- mRNA expression by 22-fold. These findings demonstrate that thiamine deficiency increases cerebrovascular endothelial permeability and triggers inflammatory responses that disrupt blood-brain barrier function, a condition clinically treated with intravenous thiamine administration. However, few reports exist on thiamine deficiency' s effects on ruminal epithelial permeability and tissue integrity. The potential mechanisms by which thiamine supplementation alleviates SARA and promotes ruminal epithelial development may involve

enhanced barrier function and reduced inflammatory responses, which require further verification.

3.2.3 Promoting Ruminal Epithelial Proliferation and Renewal

Dietary nutritional levels regulate ruminal epithelial growth and structure through the insulin-like growth factor (IGF) system [51], which includes IGF-I, IGF-II, IGF receptors, IGF-binding proteins (IGFBP), and IGFBP enzymes. Thiamine deficiency impairs tissue growth and proliferation. Molina et al. [52] demonstrated that thiamine-deficient mice exhibited 28–60% reductions in plasma IGF-I content and 20–60% reductions in renal IGF-I content, along with decreased IGFBP-3 and IGFBP-1/2 levels. Therefore, thiamine may influence ruminal epithelial proliferation and renewal by regulating the IGF system, though this requires further verification.

Conclusion

Thiamine synthesis is influenced by dietary nitrogen source, carbohydrate levels, and diet structure. Under high-concentrate feeding conditions, increased carbohydrate metabolism raises thiamine requirements, while carbohydrate decomposition produces large amounts of lactate and volatile fatty acids that induce ruminal acidosis, causing rapid pH decline and ruminal microbial dysbiosis and functional impairment. This suppresses growth of B-vitamin-synthesizing microorganisms and reduces thiamine synthesis. Additionally, increased bacterial thiaminase activity after acidosis enhances thiamine degradation. Thus, SARA development is closely related to thiamine deficiency. Thiamine supplementation under high-concentrate conditions improves animal performance and alleviates SARA, potentially through systematic regulation of ruminal microbial and carbohydrate metabolism, modulation of ruminal epithelial barrier function, and promotion of ruminal epithelial proliferation and renewal. Therefore, to further understand SARA pathogenesis and thiamine' s alleviating mechanisms, future research should strengthen systematic investigations of thiamine' s effects on ruminal carbohydrate and microbial metabolism and its impacts on ruminal epithelial function. This will provide new scientific approaches for SARA diagnosis and prevention and reduce economic losses in ruminant production.

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