Research Advances on the Effects of Subacute Ruminal Acidosis on Rumen Epithelium and Internal Environment in Ruminants: Postprint

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Abstract

Subacute ruminal acidosis (SARA) is the most common nutritional metabolic disease in modern intensive ruminant production. SARA is a chronic condition that occurs due to increased concentrate feeding and altered dietary structure, which leads to excessive accumulation of volatile fatty acids in the rumen, decreased ruminal pH, and consequent alterations in the microbial flora. This paper primarily addresses two aspects: the effects of SARA on rumen epithelium and the ruminal internal environment, detailing the alterations in rumen epithelial structure, cell junctions, permeability, and internal environment, thereby providing a theoretical reference for further research on SARA.

Full Text

Research Progress on the Effects of Subacute Ruminal Acidosis on Rumen Epithelium and Internal Environment in Ruminants

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Abstract: Subacute ruminal acidosis (SARA) is the most common nutritional metabolic disease in modern intensive ruminant production systems. SARA develops as a chronic condition resulting from increased dietary concentrate levels that alter feed structure, leading to excessive accumulation of volatile fatty acids in the rumen, decreased ruminal pH, and consequent shifts in microbial flora. This review focuses on two primary aspects: the effects of SARA on

rumen epithelium and on the rumen internal environment, detailing changes in epithelial structure, cellular junctions, permeability, and internal environment to provide a theoretical foundation for further SARA research.

Keywords: subacute ruminal acidosis; ruminants; rumen epithelium; cell junctions; permeability; internal environment

In recent years, rising consumption of dairy and meat products has driven China' s dairy, beef, and mutton industries toward large-scale, intensive production models. To maximize productivity, producers have continuously increased concentrate feeding to meet animals' energy demands and enhance performance. However, this practice has substantially elevated the risk of nutritional metabolic diseases, with incidence rates remaining persistently high for conditions including subacute ruminal acidosis (SARA), ketosis, laminitis, and fatty liver syndrome [1-2]. Among these, SARA is particularly prevalent and represents one of the most damaging, common, and economically costly diseases in modern ruminant production [3]. Nevertheless, SARA prevention, definition, and diagnosis remain challenging due to its subtle clinical symptoms and complex etiology, causing significant losses to the livestock industry [4]. Therefore, investigating SARA' s pathogenesis, pathological changes, and effective prevention and control strategies is crucial for safeguarding ruminant health and improving production performance.

1 Overview of SARA

SARA is a widespread subclinical disease in fattening cattle and high-yielding dairy production, characterized by herd-level outbreaks and high morbidity [5], with typical features of low ruminal pH and high volatile fatty acid (VFA) concentrations. High-producing and periparturient dairy cows are particularly susceptible populations [6].

As modern dairy, beef, and mutton production systems pursue greater efficiency, producers have increased high-concentrate diet feeding. Compared to North America and Europe, China lacks high-quality forage; while large farms may purchase imported alfalfa and hay, most regions still rely on crop residues as the primary roughage source. Consequently, production systems must feed large quantities of starch-rich concentrates to meet nutritional requirements, thereby increasing the incidence of nutritional metabolic diseases such as SARA. In Europe, up to 26% of mid-lactation and 19% of early-lactation dairy cows suffer from SARA [7], while the North American dairy industry suffers annual economic losses of $500 million to $1 billion from SARA [8]. In China, losses are even more severe, primarily due to reduced milk yield, decreased product quality, increased culling rates, and higher mortality [2,4].

Extensive research has advanced SARA diagnosis, treatment, and pathogenesis understanding. Proposed toxic mechanisms include endotoxin (lipopolysaccharide) and histamine (HIS) toxicity [3], lactic acidosis, and organic acidosis. However, due to technological limitations, most dietary SARA studies have focused

on single rumen metabolites (e.g., lactate, VFA, endotoxin), while the integrated metabolic characteristics and interconnections among different metabolites under varying dietary conditions remain poorly understood. Thus, the pathogenesis and regulatory mechanisms of SARA in ruminants represent a critical scientific question.

2.1 Effects of SARA on Rumen Epithelial Structure

The rumen epithelium is a stratified squamous epithelium (SSE) consisting of four layers from mucosa to serosa: stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS), and stratum basale (SB) [9]. In adult ruminants, stratum corneum cells continuously shed through mechanical friction with feed and bacterial attachment, undergoing regular renewal [10]. Tight junctions (TJ) between granulosum cells constitute a critical structure for maintaining mucosal barrier integrity [9]. The basale layer connects to the muscular layer and contains fully functional mitochondria, representing the primary metabolic site of the rumen.

Researchers induced SARA in dairy goats by gradually increasing dietary non-fibrous carbohydrate (NFC)/neutral detergent fiber (NDF) ratios and observed significant desquamation and damage to the rumen epithelial stratum corneum, with reduced papilla length, width, and corneum thickness compared to controls [11]. Consistent results from Yang [12] and Liu [13] demonstrated that SARA decreased spinosum layer and total epithelial thickness, significantly reduced granulosum thickness, and diminished papilla length in dairy goats. Building on these findings, Cheng [14] examined rumen papillae under 40× light microscopy, revealing that SARA disrupted epithelial morphological integrity and caused severe keratinization (Fig. 2 [Figure 2: see original paper]). Additional studies have confirmed severe structural damage under high-concentrate diets. Steele et al. [15] reported that compared to high-forage diets, high-concentrate feeding caused severe stratum corneum desquamation, gradually disappeared deep fissures, and reduced basale, spinosum, granulosum, and total epithelial thickness. Weng et al. [16] fed single straw with high concentrate levels and observed slight corneum degeneration, accelerated cell migration in underlying layers, and degraded cellular connections in the granulosum.

2.2 Effects of SARA on Rumen Epithelial Cell Junctions

Rumen epithelial cell junctions in ruminants, from apical to basal membrane, consist of tight junctions, desmosome junctions (DJ), adhesion junctions (AJ, also called anchoring junctions), and gap junctions (GJ, also called slit junctions). These junctions work cooperatively to ensure rumen epithelial barrier function.

2.2.1 Effects of SARA on Rumen Epithelial Tight Junctions

Tight junctions are essential components of the apical junctional complex in epithelial cells, with structure shown in Fig. 3 [Figure 3: see original paper][17]. In rumen epithelial cells, tight junctions primarily form ring-like structures at the apical side of the basolateral membrane [17-18]. Tight junctions serve three functions: barrier function—preventing mixing of basolateral membrane proteins with apical domains and blocking harmful substance invasion; gating function—controlling paracellular flux of ions and other solutes; and intercellular communication—transmitting intercellular signals and maintaining physiological homeostasis [19].

Epithelial cells regulate paracellular barrier function by controlling solute and fluid transport. As shown in Fig. 4 [Figure 4: see original paper][20], transmission electron microscopy revealed significant ultrastructural changes in SARA goats compared to healthy goats (Fig. 4-A vs. 4-B), including markedly reduced tight junction numbers, blurred structures, enlarged intercellular spaces, mitochondrial degradation in the spinosum layer, and compromised epithelial integrity. Tight junctions are composed of associated proteins including Claudin family members, Occludin, and Zonula occludens (ZO) family proteins [21-23]. Steel et al. [24] first determined the complete coding sequences of tight junction proteins Claudin-1, Claudin-4, and Claudin-7 in sheep rumen epithelium. Subsequently, Aschenbach et al. [25] reported expression and distribution patterns of Claudin-1, Claudin-4, Claudin-7, Occludin, and ZO-1. Researchers using high-concentrate diets to induce SARA in goats measured increased Claudin-4 gene expression [20], providing mechanistic insight into rumen epithelial permeability changes.

2.2.2 Effects of SARA on Rumen Epithelial Desmosome Junctions

Desmosome junction structure is shown in Fig. 5 [Figure 5: see original paper][26]. These button-like point structures connect adjacent cells, while hemidesmosomes at the epithelial cell-extracellular matrix interface similarly form point structures anchoring the basal membrane to the basement layer, reinforcing cellular connections. Rumen epithelial desmosome junctions located between spinosum and granulosum layers enhance tissue toughness, providing critical support against pressure and tension while maintaining structural organization.

Desmosomal plaques are dense structures at desmosome junction sites that connect cells. Composed of anchoring proteins linked to intracellular intermediate filaments, desmosome junctions connect intermediate filaments between cells, forming tight intercellular networks [27]. Confirmed desmosome proteins in ruminant rumen epithelium include desmoglein 1 and desmocollin 1 (DSG1). Feeding dairy cows high-concentrate diets (concentrate-to-forage ratio 70:30) significantly downregulated desmoglein 1 gene expression [26]. Goats fed high-concentrate diets (60:40 ratio) also showed decreased desmoglein 1 expression, in-

dicating reduced rumen epithelial cell junction function under high-concentrate feeding. Subsequent SARA studies feeding goats diets with NFC/NDF of 3.23 detected significantly lower desmoglein 1 expression in SARA groups versus controls, suggesting that progressive increases in dietary concentrate-to-forage ratio gradually alter rumen epithelial structure, with SARA causing significant damage to desmosome junctions [14], triggering inflammatory responses.

2.2.3 Effects of SARA on Rumen Epithelial Adhesion Junctions

Adhesion junctions utilize the cytoskeletal system to form ordered, robust cell communities between cells and matrix or cell-cell interfaces, providing strong adhesive resistance to mechanical tension. These junctions are widely distributed in epithelial tissues.

Adhesion junctions primarily exist in the stratum granulosum and consist of two types: microfilament-associated adhesion junctions including adhesion belts and adhesion plaques [28], collectively termed adherens junctions, which form sheet-like transcellular networks through intracellular adhesion proteins and transmembrane adhesion proteins; and intermediate filament-associated adhesion junctions including desmosomes and hemidesmosomes, which participate in nutrient transport. The ruminant rumen undergoes continuous peristalsis under vagal control with persistent mechanical stretching, which adhesion junctions must withstand by maintaining plasma membrane adhesion between adjacent cells. During ruminal acidosis, adhesion junction protein gene expression downregulates, enlarging intercellular spaces, though specific regulatory mechanisms require further investigation [14].

2.2.4 Effects of SARA on Rumen Epithelial Gap Junctions

Gap junctions represent the only known membrane channel structure enabling direct intercellular material exchange [29]. These specialized channels facilitate intercellular electrical and chemical signal communication, enabling coordinated cellular responses. Gap junctions are widely present between granulosum and spinosum cells in vertebrates, forming channels that are not continuously open. Cells regulate channel opening to control substance transport and signal transduction, achieving intercellular electrochemical and metabolic coupling. Beyond information and material transfer, gap junction coupling plays roles in embryonic development and cancer therapy. While 21 connexin proteins have been identified in humans and 20 in mice, only Connexin 43 has been confirmed in ruminant rumen epithelium. Studies show that increasing dietary concentrate reduces ruminal pH and downregulates Connexin 43 expression, impairing rumen barrier function [23]. Cheng [14] validated these findings, reporting significantly lower Connexin 43 expression in SARA groups. In vitro rumen epithelial cell culture studies are also widely applied; Wang [26] cultured primary rumen epithelial cells to examine pH and VFA effects on Connexin 43, finding that low pH significantly increased gene expression while VFAs had opposite effects. This discrepancy may arise because in vitro dual-factor studies cannot fully replicate

the complex in vivo environment and metabolic state, with additional influencing factors including goat breed, tissue sampling location, and experimental design.

2.3 Effects of SARA on Rumen Epithelial Electrophysiology

Increased rumen epithelial permeability is a critical marker of early barrier damage. Danish scholar Ussing first used the Ussing chamber system to study epithelial ion transport [30], and this technique has become a hotspot for investigating gastrointestinal epithelial permeability. Electrophysiological parameters in Ussing chambers reflect rumen epithelial permeability: short-circuit current (Isc) indicates ion transport capacity across epithelium (increased Isc means enhanced transport); tissue conductance (Gt) reflects ion permeability and epithelial barrier integrity (increased Gt indicates compromised integrity and greater permeability); and potential difference (PD) demonstrates tissue viability. These parameters collectively characterize epithelial barrier function [31].

Using isotopic or molecular markers to measure transepithelial passage in Ussing chambers has become a common and important method [32], with common markers including horseradish peroxidase (HRP), fluorescein isothiocyanate (FITC), and ^{3}H-mannitol [33]. Through continuous application and improvement, this technique has become the "gold standard" for evaluating gastrointestinal barrier function [14].

Klevenhusen et al. [33] used Ussing chambers to study rumen epithelial permeability, finding that high-concentrate diets significantly increased Isc and Gt while enhancing flux of both large-molecule marker HRP and small-molecule marker FITC, indicating significantly increased permeability. Yang [12] investigated SARA effects on rumen epithelial electrophysiology, reporting that SARA and recovery groups had significantly higher Isc, Gt, and HRP flux but lower PD compared to controls. Our research group used HRP and FITC markers and found that SARA increased Isc and Gt while decreasing PD in dairy goats both short- and long-term, also significantly elevating HRP and FITC flux across rumen epithelium [34]. These results align with Klevenhusen et al. [33] and Yang [12], demonstrating that SARA compromises rumen epithelial integrity, increases permeability to molecular markers, and chronically weakens barrier function.

3.2 Volatile Fatty Acids (VFA)

Rumen anaerobic microbes degrade carbohydrates into VFAs, which rumen epithelium absorbs efficiently, primarily through passive transport, providing 60%–80% of the animal' s energy requirements. VFAs are short-chain fatty acids (C2–C6), with acetate, propionate, and butyrate comprising approximately 95% of total VFA (TVFA) [39]. Propionate serves as an effective gluconeogenic precursor providing metabolic glucose. Ruminants secrete saliva through chewing and

rumination; high-producing dairy cows generate approximately 79.8–90 mol/d of VFA in the rumen, with saliva neutralizing about 30%–40% of H to maintain acid-base homeostasis [40].

Khafipour et al. [41] induced SARA with concentrate diets and observed decreased acetate but increased propionate and butyrate concentrations, reducing the acetate/propionate ratio from 3.0:1.0 to 2.1:1.0. Wu [15] induced SARA in dairy goats and found that increasing dietary NFC/NDF elevated TVFA concentrations, with upward trends in acetate, propionate, and butyrate, though lactate remained consistently low. Costa et al. [42] also reported increased VFA concentrations after high-concentrate feeding. Other studies show that increasing NFC/NDF decreases acetate concentration and acetate/propionate ratio while increasing TVFA, with significant reductions in acetate and acetate/propionate and significant TVFA increases when NFC/NDF reached 2.58 at SARA induction, indicating close association between elevated butyrate and SARA [43].

Weng [44] revealed molecular-level changes in VFA absorption under high-concentrate feeding, showing that compared to a 62.9% concentrate plus single corn straw diet, a 41.4% concentrate plus mixed forage diet downregulated expression of VFA transporter genes Na /H exchanger (NHE) 1, NHE3, and NHE4 while significantly upregulating monocarboxylate transporter-1 (MCT-1) and downregulating acyl-CoA synthetase short-chain family member 1 (ACSS-1) in rumen papillae.

3.3 Abnormal Rumen Metabolites

Endotoxin, a Gram-negative bacterial cell wall component with permeability barrier function [45], is released into the rumen when Gram-negative bacteria lyse during SARA, damaging rumen epithelial cells and compromising barrier function [46]. Endotoxin subsequently translocates across the rumen barrier into blood [47], increasing plasma concentrations and triggering systemic inflammatory responses and immune activation [48]. Accumulation to critical levels causes endotoxemia. Our previous studies showed that increasing dietary NFC/NDF elevated plasma endotoxin concentrations and induced endotoxemia in dairy goats [49]. Literature also reports increased endotoxin in plasma or rumen fluid during high-concentrate induced SARA [37]. Our earlier research found that increasing NFC/NDF from 1.40 to 3.23 progressively increased plasma endotoxin from 15.76×10^3 EU/mL to 85.55×10^3 EU/mL, consistent with previous reports [50]. Chin et al. [51] demonstrated that endotoxin increased nitric oxide (NO) production in intestinal epithelial cells, disrupting tight junction protein ZO-1 structure and function. Zhang [52] investigated effects of different dietary patterns on plasma endotoxin and metabolites, showing that plasma endotoxin concentration was primarily affected by dietary concentrate-to-forage ratio, while plasma metabolites closely correlated with immune and production performance changes [53].

Histamine (HIS) is an important biologically active substance and key mediator

in type I hypersensitivity reactions, participating in allergic responses, vasoconstriction/dilation, and serving as a critical inflammatory and immune damage mediator. During SARA, rumen environment disruption and chronic low pH promote histidine decarboxylation to HIS, increasing abnormal metabolites [53] that damage rumen mucosa and reduce barrier function. Abnormal HIS metabolites then enter circulation through compromised mucosa, triggering systemic inflammation [1]. Guo et al. [54] reported increasing plasma HIS concentrations with higher NFC/NDF ratios. Aschenbach et al. [25] demonstrated that HIS induces apoptosis, increases cell shedding, and interferes with nuclear division and cell maturation, suggesting that during SARA, abnormal HIS metabolites disrupt epithelial cell regeneration, causing cellular damage and inflammation. Reports also indicate that when dairy cows develop SARA at NFC/NDF of 2.58, rumen fluid HIS concentration rises to 116.74 ng/mL while plasma HIS shows initial elevation followed by decline [1]. Elevated HIS correlates with pathological changes, triggering inflammatory responses and representing an important SARA pathogenic factor that exacerbates disease progression.

3.4 Rumen Microflora

The rumen harbors large populations of fungi, bacteria, and protozoa that form a stable symbiotic fermentation system. Excessive consumption of readily fermentable carbohydrates dramatically increases microbial growth rates, accelerating fermentation, producing excess organic acids, and reducing ruminal pH to induce SARA. SARA alters microbial structure and populations: protozoa and fiber-degrading bacteria decline while Gram-negative bacteria lyse massively [55]. Han et al. [56] induced SARA in dairy goats and found maximal protozoal numbers at NFC/NDF of 1.24, with dramatic declines as NFC/NDF increased, reaching minimum values during SARA. Guo et al. [54] similarly found that starch-degrading bacteria showed the most pronounced changes, increasing with NFC/NDF. As ruminal pH further declines, microbial balance is disrupted, acid-tolerant bacteria proliferate, producing harmful substances like lactate that worsen SARA. Simultaneously, *Fusobacterium necrophorum* populations surge to more than ten times normal levels. *Prevotella ruminicola* exhibits broad pH buffering capacity, and while most fiber-degrading bacteria become inactive at pH 6.0, genetically engineered *P. ruminicola* B.4 strains can survive at pH 5.5, making genetic regulation of acid-resistant fiber-degrading bacteria one of the most effective approaches for rumen microbial manipulation [40].

3.5 Other Factors

SARA exerts insidious effects on ruminant health, impairing immune function, promoting cell-mediated immunity, and increasing inflammatory cell secretion. Reduced feed intake and milk yield cause substantial economic losses. As SARA progresses, animals develop diarrhea, intestinal mucosal damage, laminitis, liver abscesses, and other inflammatory responses [57].

4 Summary

SARA induces functional and structural changes in rumen epithelium, disrupts rumen fermentation, alters microbial community structure, increases abnormal metabolites, and compromises mucosal integrity, damaging the rumen epithelial barrier. While most research has focused on SARA prevention, diagnosis, treatment, and pathology, the precise mechanisms and pathways of rumen epithelial damage remain unclear. Therefore, in-depth studies exploring SARA' s effects on rumen epithelial barrier function and molecular regulatory mechanisms are necessary. Meanwhile, the era of big data has brought abundant sequencing analyses, with rumen microbial metagenomics emerging as a powerful tool for understanding SARA-related functional gene regulatory networks and microecological environments, identifying key signaling pathways, and providing theoretical foundations for developing nutritional strategies that improve feed efficiency and maintain rumen health under high-concentrate feeding conditions.

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