# Postprint: Elucidation of Rumen Adaptive Mechanisms in Ruminants Under High-Concentrate Diet Conditions

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## Abstract

Feeding high-energy, high-starch diets is a common strategy to enhance production performance in ruminants within intensive production systems; however, high-concentrate diets readily induce a series of nutritional and metabolic disorders, among which ruminal acidosis is the most common. The rumen of ruminants not only serves functions in digestion and nutrient absorption, but the rumen epithelium also constitutes an important immune barrier; therefore, rumen health is crucial for ruminants. This review primarily elaborates on changes in three aspects when ruminants consume high-concentrate diets: rumen tissue morphology, molecular mechanisms of rumen epithelial adaptation, and rumen microbial community, with the aim of providing a reference for research on rumen adaptation mechanisms under high-concentrate dietary conditions.

## Full Text

## Analysis of Rumen Adaptive Mechanisms under High-Concentrate Diets

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**Abstract:** Feeding diets high in energy and starch is a common strategy in intensive production systems to enhance ruminant performance, but high-concentrate diets frequently induce various nutritional metabolic disorders, with ruminal acidosis being the most prevalent. The rumen not only functions in digestion and nutrient absorption but also serves as a critical immune barrier;

thus, rumen health is essential for ruminant well-being. This review primarily addresses three aspects of rumen adaptation to high-concentrate feeding: morphological changes in rumen tissue, molecular adaptive mechanisms of the rumen epithelium, and alterations in rumen microbial flora, aiming to provide insights for understanding rumen adaptation mechanisms under high-concentrate conditions.

**Keywords:** ruminant; high-concentrate diet; ruminal acidosis; epithelial transport; microbiota

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In production systems, feeding high-energy, high-starch diets—commonly referred to as high-concentrate diets—is routinely employed to improve ruminant performance and economic returns. However, excessive concentrate intake, particularly grains rich in starch but low in physically effective neutral detergent fiber (peNDF), leads to accumulation of volatile fatty acids (VFA) in the rumen, causing pH decline and increasing the risk of ruminal acidosis [1-3]. High-concentrate feeding strategies also trigger additional complications, including reduced feed intake, laminitis, liver abscesses, and decreased milk fat percentage [3-4]. Consequently, some researchers have termed subacute ruminal acidosis (SARA) as "high-concentrate syndrome" [5]. The rumen plays a vital role in digestion and metabolism in adult ruminants. Research indicates that excessive high-concentrate consumption damages rumen epithelium and alters the structure, diversity, and abundance of rumen microbial communities, ultimately impairing rumen absorption function [6-7]. Conversely, moderate increases in dietary concentrate can promote rumen development in young animals. Therefore, understanding the patterns of rumen epithelial structural and functional changes and microbial flora shifts under high-concentrate conditions will provide novel approaches for diagnosing, preventing, and treating digestive metabolic diseases, thereby reducing economic losses in production.

## Adaptive Mechanisms of the Rumen under High-Concentrate Feeding

### 1.1 Morphological Adaptation of Rumen Tissue

High-concentrate diets stimulate rumen papillae development, increasing papillae density per unit area as well as papillae length and width, thereby enhancing VFA absorption capacity [8-11]. This effect is attributed to the higher propionate and butyrate production from grain fermentation. One study found that adding 780 g of concentrate to hay significantly increased papillae length, width, and density in the rumen mucosa of German dairy goats [11]. The rumen epithelium comprises four layers: stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. While the stratum corneum provides protective function, animals with SARA exhibit parakeratosis of rumen papillae [12-13].

Research on dairy goats with SARA demonstrated significantly increased stratum corneum thickness, reduced intercellular tight junctions, decreased stratum granulosum thickness, but no significant change in stratum spinosum thickness [14]. When dietary grain content reached 65%, goat rumen epithelium showed not only thickened stratum corneum, thinned stratum granulosum, and parakeratosis, but also necrosis across all cell layers and reduced cellular cohesion [15]. In periparturient dairy cows abruptly transitioned from low- to high-energy diets, severe delamination occurred between basal, spinous, granular, and cornified cells in rumen papillae stratified squamous epithelium. Over time, papillae surfaces became increasingly rigid, with loose connections among outermost keratinized cells [16]. These findings indicate differential adaptation among rumen epithelial cell layers to high-concentrate diets and suggest that excessive concentrate proportions increase epithelial permeability and compromise barrier function, potentially allowing microbes or toxic substances to enter circulation and elevate immune stress risk [17].

Rumen morphological structure in young ruminants is also influenced by dietary concentrate levels. Studies show that weaned lambs fed high-concentrate diets exhibited no significant differences in papillae height and width in the dorsal sac compared to pasture-fed lambs, though papillae surface area tended to increase. Additionally, papillae color differed between feeding regimens [18]. Papillae color serves as an indicator of epithelial keratinization, with darker coloration signifying greater keratinization [13]. Research demonstrated that papillae area was larger and color darker in alfalfa-fed fattening lambs compared to those receiving total mixed rations with high concentrate [19]. In summary, appropriate VFA concentrations stimulate papillae development [12], but VFA accumulation beyond the absorptive capacity of rumen epithelium causes epithelial damage [20].

### 1.2.1 Absorption and Metabolism of VFA and H+

Rumen pH decline is accompanied by epithelial morphological changes and corresponding adaptive alterations in gene expression related to H+ transport, VFA absorption, and metabolism [12]. VFA exist in both free and non-free forms in the rumen, predominantly as free VFA [21]. Ruminal H+ originates from VFA dissociation, with epithelial absorption accounting for 53% of H+ removal pathways [22]. Studies in calves fed starter rations showed significantly increased expression of monocarboxylate transporter-1 (MCT-1) and decreased expression of Na+/H+ exchanger-3 (NHE-3) in rumen epithelium [23]. Since MCT-1 transports monocarboxylates along with H+, its upregulation facilitates H+ removal. Maintaining normal absorptive function requires not only appropriate ruminal H+ concentrations but also intracellular ion balance [24]. NHE-3 mediates Na+ uptake and H+ extrusion; reduced NHE-3 expression helps stabilize intracellular pH in rumen epithelial cells [25].

When dietary concentrate increased from 10% to 35%, goats showed significantly elevated expression of genes encoding monocarboxylate transporters [MCT-1, MCT-4], H+ transporters [NHE-1, NHE-2, NHE-3], VFA transporters [DRA, putative anion transporter 1 (PAT1), anion exchanger (AE2)], and Na+/K+ ATPase [26]. Na+/K+ ATPase extrudes intracellular Na+ while absorbing extracellular K+; when H+ transporter expression increases, intracellular Na+ concentration rises, prompting increased Na+/K+ ATPase expression to maintain Na+/K+ homeostasis. Research indicates that mRNA expression of genes involved in short-chain fatty acid (SCFA) absorption in rumen epithelium is jointly regulated by rumen pH and SCFA concentration [26]. Although numerous genes related to H+ absorption have been identified in rumen epithelium [27], the specific expression sites of some genes remain uncertain, and whether their protein products exhibit transport specificity or rate differences requires further investigation [12].

Approximately 80% of ruminants' metabolic energy is derived from VFA, underscoring their critical role in energy supply [28]. Butyrate is absorbed by rumen epithelium and synthesized into -hydroxybutyrate (BHBA), with excess butyrate entering the liver for ketone body formation [29]. 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), a mitochondrial enzyme, is rate-limiting in rumen epithelial ketogenesis [12,30]. However, studies in lactating dairy cows and lambs fed high-concentrate diets showed no significant change in HMGCS2 expression [31-32], suggesting that HMGCS2 mRNA expression is not directly related to ketogenic capacity under high-concentrate conditions, possibly due to increased substrate availability for BHBA synthesis [32].

Current research on VFA absorption primarily focuses on free VFA. However, high-concentrate feeding reduces rumen pH, increasing non-free VFA concentrations. Non-free VFA are absorbed primarily via free diffusion, a process dependent on lipid composition—including cholesterol and phospholipids—of the rumen epithelial cell membrane. While studies have investigated genes related to cholesterol synthesis in rumen epithelium [31,33], the mechanisms of phospholipid synthesis remain unreported and warrant further exploration.

### 1.2.2 Utilization of Urea Nitrogen

Urea synthesized by the liver can be secreted into the rumen via rumen epithelium and parotid glands, serving as an essential nitrogen source for rumen microbial growth. Urea transporters (UT) facilitate urea movement across rumen epithelium [34]. Both in vivo and in vitro studies demonstrate that low pH and high VFA concentrations increase mRNA expression and protein abundance of urea transporter-B (UT-B) in rumen epithelium, accompanied by elevated expression of G protein-coupled receptors (GPR) 41 and GPR4 [35]. These findings suggest that UT-B expression is regulated by GPR41 and GPR4 under different dietary conditions [35]. Additionally, GPR41 and GPR43 may influence VFA-mediated regulation of rumen development and insulin/glucagon secretion [36], indicating potential interconnections among VFA, pH, GPRs, and UT-B. However, UT-B is not the sole urea transporter, as urea transport persists even when UT-B is inhibited [37]. Aquaporins (AQP) can permit urea passage, though whether rumen epithelial AQPs function in urea transport remains unclear [37]. When diets are abruptly switched to readily fermentable carbohydrates, expression of AQP-3 and UT-B in rumen epithelium increases linearly and significantly exceeds that in high-forage groups [37], suggesting AQP-3 may also contribute to urea transport. Furthermore, butyrate reportedly regulates urea synthesis and nitrogen redistribution in sheep [38], though the underlying mechanisms remain unclear and warrant investigation to understand rumen adaptation to high-concentrate feeding and energy-nitrogen balance.

### 1.2.3 Proliferation and Differentiation of Rumen Epithelial Cells

Maintenance of rumen epithelial structure and function requires continuous cell proliferation and differentiation, processes regulated by epidermal growth-related genes [8] such as insulin-like growth factor binding protein-5 (IGFBP-5), IGFBP-3, and IGFBP-6. Butyrate is considered a key regulator of rumen epithelial cell proliferation and differentiation. High-concentrate feeding elevates ruminal butyrate concentration, which may affect the insulin-like growth factor axis by promoting secretion of cell differentiation hormones while reducing pro-apoptotic hormone secretion [39]. Studies in lactating dairy cows adapting to high-concentrate diets showed significantly increased IGFBP-5 expression and decreased expression of IGFBP-3, IGFBP-6, and desmoglein-1 (DSG-1) in rumen epithelium. This expression pattern reduces thickness of stratified squamous epithelium and intercellular adhesion in the stratum granulosum, increasing epithelial permeability and ultimately causing epithelial damage [8]. Other research reported that postpartum cows exhibited significantly elevated expression of DSG-1, connective tissue growth factor (CTGF), corneodesmosin (CDSN), IGFBP-2, and epiregulin (EREG) genes, while EGFR and IGFBP-3 expression decreased significantly [16]. These findings suggest that at low pH, rumen epithelium upregulates expression of proliferation- and differentiation-related genes to enhance epithelial integrity and reduce damage.

Cholesterol is a crucial component of mammalian cell membranes, but excessive cholesterol triggers inflammation, oxidative stress, cell proliferation, and migration while altering membrane permeability, ultimately causing tissue damage [33,40]. 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1), a cytoplasmic enzyme, participates in cholesterol synthesis. Studies show that HMGCS1 expression decreases in dairy cows with ruminal acidosis, regardless of lactation status [31,33], and is significantly reduced in lambs fed high-concentrate diets [32]. This reduction may maintain cholesterol homeostasis in rumen epithelium when substrate concentrations (e.g., propionate, butyrate) increase during high-concentrate feeding, preventing cholesterol-induced epithelial damage.

### 1.3 Changes in Rumen Microbial Flora

Rumen microbes are vital for ruminants, fermenting carbohydrates to produce VFA and providing microbial protein to the host [41]. When rumen pH drops below 6.0, fibrolytic bacteria growth is inhibited while amylolytic bacteria proliferate [41-42]. Numerous studies demonstrate that high-concentrate feeding affects microbial density, diversity, and community structure in dairy cows [43-45]. Under high-concentrate conditions, microbial composition differs between solid and liquid phases of rumen contents: the solid phase shows reduced microbial density with Firmicutes dominance, while *Fibrobacter succinogenes* abundance decreases with increasing concentrate intake [43,45]. Research shows that increasing dietary grain content elevates protozoa of the order Entodiniomorphida; at 60% grain content, *Prevotella albensis* and *Fibrobacter succinogenes* decline while *Lactobacillus* increases [46]. *Lactobacillus* fermentation primarily produces lactate, a key factor in ruminal acidosis. Rumen epithelial microbial flora also changes significantly before and after high-concentrate feeding [47]. In beef cattle, epithelial colonization by *Treponema* sp., *Ruminobacter* sp., and *Lachnospiraceae* sp. occurs only when dietary grain content increases from 3% to 75% or 92% [48]. Both SARA severity and dietary composition influence rumen microbial community structure. In severe SARA induced by grains, *Streptococcus bovis* and *Escherichia coli* dominate; in moderate grain-induced SARA, *Megasphaera elsdenii* predominates; while alfalfa pellet-induced SARA features *Prevotella* as the dominant genus [49]. Thus, rumen microbes are influenced by both pH and dietary composition, as substrate sources and concentrations for microbial fermentation vary with diet. At low rumen pH, *Selenomonas ruminantium* proliferation is suppressed, and *Streptococcus bovis* maintains a low proportion, suggesting other microbes may contribute to acidosis development [50]. Overall, as rumen pH decreases, acid-tolerant microbes such as *Prevotella* and *Lactobacillus* proliferate while fiber-degrading bacteria like *Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens* decline, reducing forage digestibility and affecting animal health. Elucidating microbial succession in the digestive tract and host-microbe interactions under high-concentrate feeding will provide new strategies for addressing associated metabolic disorders.

In summary, ruminants possess adaptive mechanisms to high-concentrate diets that involve coordinated responses of rumen structure, function, and microbial flora. However, these mechanisms have limitations and are constrained by rumen pH and dietary composition; excessive intake of readily fermentable carbohydrates still disrupts rumen digestive and absorptive functions. Further systematic investigation of interactions among rumen morphology, VFA absorption, and microbial flora under high-concentrate conditions will provide theoretical foundations for optimizing rumen function.

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