Effects of Roughage on Rumen Development in Young Ruminants and Its Mechanisms: Postprint

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Abstract

The rumen of young ruminants is characterized by underdeveloped structure and function and an immature microbial community. During the first few months after birth, the rumen structure and function undergo tremendous changes, and the developmental degree of rumen structure and function during the juvenile stage directly affects the production performance of adult ruminants. Research has found that feeding only concentrate diets can easily cause symptoms such as decreased rumen fluid pH, rumen papillae clumping, and parakeratosis in young animals, whereas supplementing with roughage significantly increases rumen fluid pH, promotes the development of the rumen muscular layer, and maintains rumen wall health. This paper reviews recent research on the effects of roughage source, level, and particle size on rumen tissue morphology, microbial community, fermentation parameters, and rumen epithelial nutrient absorption and transport function in young ruminants, and elaborates on the possible mechanisms, aiming to provide a reference for research on the application of roughage in diets for young ruminants.

Full Text

Effects of Forage on Rumen Development in Young Ruminants and Its Mechanisms

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Abstract: The rumen of young ruminants is characterized by incomplete structural and functional development and an immature microbiota. During the first

few months after birth, the rumen undergoes dramatic structural and functional changes, and the degree of rumen development during this juvenile stage directly affects the production performance of adult ruminants. Previous research has shown that feeding only concentrate diets can cause reduced rumen fluid pH, papillae clumping, and parakeratosis in young animals, whereas forage supplementation significantly increases rumen fluid pH, promotes muscular layer development, and maintains rumen wall health. This review synthesizes recent findings on how forage source, level, and particle size affect rumen tissue morphology, microbial communities, fermentation parameters, and epithelial nutrient absorption and transport functions in young ruminants, and elaborates on the underlying mechanisms to provide references for research on forage application in diets for young ruminants.

Keywords: young ruminant; forage; rumen; regulation; mechanism

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The digestive system of young ruminants undergoes tremendous physiological changes in structure and function from birth to adulthood, with rumen development being particularly critical. At birth, young animals primarily obtain energy through glucose absorption in the abomasum and small intestine, whereas adults mainly rely on volatile fatty acids (VFAs) absorbed from the rumen [1-2]. This dramatic shift in physiological function is closely related to rumen development, including its microbial community, fermentation capacity, and tissue morphology. Rumen development in young animals is influenced by many factors [3-5], among which the intake and characteristics of solid feed play an important role [6]. Solid feed sources can generally be divided into two categories: concentrates and forage. Concentrates are generally considered to contain large amounts of readily fermentable carbohydrates that produce substantial propionate and butyrate, promoting rapid rumen epithelial development [7]. In contrast, forage is characterized by low energy density and slow fermentation and passage rates in the rumen [8]. Consequently, the NRC and some researchers [9] do not recommend providing forage to pre-weaned ruminants. However, feeding pre-weaned young animals concentrate-only diets may cause rapid accumulation of fermentation products, leading to decreased rumen fluid pH, papillary parakeratosis, and mucosal clumping [10], ultimately impairing nutrient absorption by the rumen epithelium [11]. Recent studies have found that forage can increase rumen fluid pH, expand rumen volume, promote muscular layer development, maintain epithelial integrity [12], and even improve production performance [6,13-15]. Therefore, this review focuses on recent research regarding the effects of forage on rumen development in young ruminants and its mechanisms, aiming to provide a theoretical basis for fiber nutrition in young ruminants.

1. Effects of Forage on Production Performance of Young Ruminants

Current research on the effects of pre-weaning forage supplementation on production performance in young ruminants remains controversial, though most studies indicate that forage supplementation improves performance. Castells et al. [13] found that supplementing calves with oat hay, barley straw, and rye silage increased starter intake, average daily gain (ADG), and final body weight, whereas alfalfa supplementation did not produce similar results. Similarly, Terré et al. [16] reported that oat hay supplementation increased post-weaning starter intake, ADG, and final body weight. Furthermore, Nemati et al. [6] added alfalfa hay at different levels and particle sizes to calf starters and found that when dietary alfalfa hay level was 25%, long alfalfa hay resulted in higher ADG than short alfalfa, but at 12.5% alfalfa level, particle size showed no effect on performance. More recently, Ebnali et al. [17] compared feeding alfalfa in total mixed ration (TMR) versus pelleted form and found that alfalfa supplementation increased pre- and post-weaning dry matter intake (DMI) compared with concentrate-only diets, but TMR feeding increased post-weaning DMI more than pelleted feeding. These studies collectively demonstrate that pre-weaning forage supplementation can improve performance, though effects vary depending on forage source, level, physical form, and feeding method. In contrast, Hill et al. [8] found that dietary inclusion of soybean hulls or hay reduced post-weaning DMI and ADG in calves, with both parameters decreasing linearly as forage level increased. Jahani-Moghaddam et al. [18] investigated the effects of different forage forms under high liquid feeding regimens and found no significant differences in performance among calves fed long alfalfa, pelleted alfalfa, or concentrate-only diets.

Forage supplementation is generally believed to increase starter intake by improving the rumen environment. When young animals are fed concentrate-only diets, large amounts of readily fermentable carbohydrates (especially starch) are rapidly fermented, producing high concentrations of VFAs. Combined with incomplete rumen development, this leads to decreased rumen fluid pH [10], with some researchers suggesting it may cause rumen acidosis [19], ultimately suppressing feed intake. Conversely, forage consumption helps maintain a suitable rumen environment [20], thereby increasing intake and ADG. Imani et al. [19] used meta-analysis to examine the effects of forage supplementation on calf performance with different starter types and found that feeding finely ground or pelleted starters increased feed intake compared with coarsely ground starters. This may be because finely ground and pelleted starters increase the surface area available for microbial attachment, accelerating fermentation rate. Additionally, starch gelatinization during pelleting facilitates its degradation in the rumen, ultimately causing reduced rumen fluid pH. Under these conditions, forage supplementation increases rumen fluid pH and maintains it at appropriate levels, thereby improving intake. Conversely, coarsely ground starters have slower starch degradation rates, reducing the need for forage. In such cases, for-

age supplementation may cause relative gut fill due to low energy density and slow passage rate, suppressing intake and hindering growth. Hill et al. [8] used coarsely ground starters in their experiment, which may explain the reduced performance.

In summary, the effects of forage on young animal performance are regulated not only by forage source, level, and physical characteristics but also by feeding method and liquid feeding strategy.

2. Effects of Forage on Rumen Tissue Morphology of Young Ruminants

From birth to adulthood, young ruminants experience dramatic changes in rumen weight and volume, which are closely related to diet composition. The physical fill effect of solid feed, particularly forage, is one factor contributing to rumen volume development [21]. Pre-weaned young ruminants have low digestive capacity, and forage occupies substantial space due to slow passage rate, with this physical fill effect promoting rumen volume expansion [22]. Increased volume, in turn, accommodates more feed, creating a synergistic relationship between rumen capacity and feed fill. Physical stimulation from forage promotes rumen muscle development, which is another factor increasing rumen volume [23] and contributes to weight gain. While forage affects rumen volume and weight, concentrate also plays an important role. Chemical stimulation from VFAs produced through concentrate fermentation promotes rumen epithelial cell proliferation, which underlies cellular-level changes in rumen volume and weight.

Rumen tissue morphology development directly affects feed intake and digestive capacity in adulthood, with well-developed morphology enabling ruminants to achieve optimal production performance and feed efficiency. Morphological development indicators include epithelial cell growth, papillae morphology, stratum corneum thickness, and rumen wall and muscle layer thickness. Rumen papillae are small protrusions of the mucosal epithelium, and their length, width, and density directly determine the surface area available for contact between epithelium and digesta. While VFA production from concentrate fermentation is generally considered the primary chemical stimulus for papillae development—with butyrate having the strongest effect, followed by propionate and acetate [24]—Suárez et al. [10] found that feeding calves concentrate-only diets caused severe papillae clumping (adhesion of digesta, hair, and cellular debris) and mucosal dysplasia. Subsequent research on forage source and concentrate-to-forage ratio revealed that forage reduced papillae clumping and mucosal dysplasia [12], indicating that forage helps maintain normal papillae morphology and ensures nutrient absorption. Additionally, Yang et al. [14] found that alfalfa supplementation increased papillae length in lambs. Collectively, these findings demonstrate that papillae development requires not only chemical stimulation from concentrate-derived VFAs but also forage for normal development. However, the underlying mechanisms remain unclear and warrant further investigation.

The rumen epithelium consists of the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale, with the stratum corneum providing protective function. The number of cell layers in the stratum corneum reflects the degree of keratinization and is related to diet structure. Rumen epithelium continuously renews as cells are sloughed off through friction with digesta. When dietary abrasion on the stratum corneum decreases, squamous epithelial cells produce a hard keratin layer, resulting in parakeratosis [11]. Excessive stratum corneum thickness reduces VFA absorption, rumen epithelial blood flow, and causes papillae degeneration and sloughing [22]. Research shows that high-concentrate diets, while promoting epithelial development, readily cause parakeratosis in calves and lambs [25], primarily due to low physical abrasiveness (small particle size, low grinding value) and rapid VFA production with low rumen fluid pH. Conversely, forage has large particle size and high fiber content, providing greater roughness and abrasiveness that removes keratin or dead epithelial cells through continuous contact, maintaining appropriate keratinization and epithelial integrity. Furthermore, Mirzaei et al. [26] demonstrated that at low alfalfa levels (8%), increasing particle size reduced rumen wall stratum corneum thickness, but this effect was absent at high alfalfa levels (16%), indicating that particle size effects depend on alfalfa level. Additionally, hay promotes muscular layer thickening in young animals [27], which serves two purposes: providing structural support for accommodating more digesta and generating powerful contractions for thorough mixing of digesta with microbes [28].

3. Effects of Forage on Rumen Microbiota of Young Ruminants

The rumen harbors symbiotic microbial communities comprising bacteria, anaerobic fungi, and protozoa. The rumen provides a suitable environment for microbial colonization and proliferation, while microbes meet host nutritional needs by producing VFAs, microbial protein, and vitamins through feed degradation. Young ruminants exhibit distinct microbial colonization sequences after birth [29], with anaerobic, facultative anaerobic, and aerobic bacteria predominating shortly after birth, followed by strict anaerobes. Jiao et al. [30] noted that rumen microbiota establishment precedes rumen metabolic and structural development, suggesting that microbial colonization may be the core element of rumen development in young ruminants.

Research indicates that fiber-degrading bacteria can be detected in lamb rumen at 2–4 days of age, reaching levels similar to adult animals by approximately 10 days [31-32]. Colonization of fiber-degrading bacteria before starter consumption provides the basic conditions for fiber utilization. Currently, very few studies have reported on forage effects on rumen microbiota in young ruminants. Yáñez-Ruiz et al. [33] found that feeding concentrate plus forage increased total bacterial numbers and decreased *Fibrobacter succinogenes* and methanogen populations compared with forage-only diets in pre-weaned lambs, demonstrat-

ing that microbiota structure and composition are diet-related. Yang [34] reported that in calves with identical DMI, populations of *Ruminococcus albus*, *Butyrivibrio fibrisolvens*, *Ruminobacter amylophilus*, and *Lactobacillus* increased significantly with increasing forage levels in pelleted feed. High-throughput sequencing-based metagenomics and other technologies provide scientific tools for studying forage regulation of rumen microbiota, yielding extensive microbial information. In adult goats, Liu [35] found that high-grain diets reduced microbiota diversity indices (Chao1, Ace, and Shannon) and simplified community composition. At the phylum level, high-grain diets decreased relative abundances of Actinobacteria, Fibrobacteres, Proteobacteria, and Tenericutes, while at the genus level, they increased *Selenomonas* and *Ruminococcus* but decreased *Butyrivibrio*. High-grain diets inhibit fiber-degrading bacteria while promoting starch-degrading bacteria, primarily due to differences in diet composition that ultimately affect rumen fluid pH. Currently, no studies have reported on the mechanisms and persistence of effects of forage level and source on rumen microbiota in early-weaned young animals. Therefore, modern molecular biotechnology should be employed to further investigate these mechanisms and provide theoretical foundations for understanding how young animals adapt to forage.

4. Effects of Forage on Rumen Fermentation Parameters of Young Ruminants

Microbial colonization and substrate availability form the basis of rumen fermentation. Studies show that VFAs can be detected in calf rumen at 2 weeks of age [22], reaching adult levels by 8 weeks [9]. However, carbohydrate structure, source, and level affect VFA production and microbial populations, altering fermentation patterns and influencing rumen development. The effects of forage on total VFA (TVFA) concentration vary among studies. Castells et al. [36] reported that forage addition decreased rumen fluid TVFA concentration, whereas Beiranvand et al. [21] found that forage increased TVFA concentration in a level-dependent manner. Conversely, Nemati et al. [15] observed no effect of forage on TVFA concentration. Since forage effects on solid feed intake are related to both forage level [15] and particle size [6], and intake directly affects rumen development [37], examining these three studies reveals that forage did not affect intake in the first experiment but promoted intake in the latter two. These inconsistent results regarding TVFA concentration likely stem from differences in forage level and particle size among experiments, leading to varying degrees of rumen development and ultimately different TVFA concentrations [6,26].

Generally, forage supplementation increases acetate concentration while decreasing propionate concentration and increasing the acetate-to-propionate ratio compared with concentrate-only diets [14,28]. This shift in fermentation pattern occurs because non-structural carbohydrates in forage favor fiber-degrading bacteria that produce large amounts of acetate [38], and the increased rumen fluid pH from forage feeding also promotes fiber-degrading bacterial proliferation [39],

thereby increasing the acetate-to-propionate ratio. Suárez et al. [12] found that at a concentrate-to-forage ratio of 7:3, straw increased rumen fluid acetate concentration compared with hay and corn silage, while propionate remained unchanged. Conversely, a 4:6 ratio (corn silage) increased propionate but not acetate compared with a 7:3 ratio. Additionally, Nemati et al. [6] reported that increasing alfalfa length increased the acetate-to-propionate ratio in calves. These results demonstrate that forage source and particle size also affect acetate and propionate concentrations at different forage levels. Coverdale et al. [40] found that supplementing different alfalfa levels significantly decreased rumen fluid butyrate concentration compared with concentrate-only diets, possibly due to high structural carbohydrate content in alfalfa producing less butyrate, or because forage feeding increased rumen epithelial butyrate metabolism, reducing rumen fluid butyrate concentration [15]. Furthermore, high alfalfa level diets increased serum -hydroxybutyrate (BHBA) concentration in calves [6], and BHBA can serve as an indicator of rumen wall metabolic function, further suggesting that forage may promote butyrate metabolism in rumen epithelium. The molecular mechanisms underlying forage effects on rumen fluid butyrate and blood BHBA concentrations require further investigation.

Appropriate rumen fluid pH is crucial for rumen development, microbial fermentation, and animal health in young ruminants [20,38,41]. Feeding concentrate-only diets reduces rumen fluid pH [10] because readily fermentable carbohydrates are rapidly fermented, increasing TVFA concentration [41]. Additionally, underdeveloped rumen epithelium in young animals cannot absorb VFAs quickly enough, contributing to pH reduction [1]. Conversely, forage supplementation significantly increases rumen fluid pH [20,14] because forage ferments slowly, and fiber stimulates rumination, promoting saliva secretion that buffers rumen fluid. Particle size also plays an important role in maintaining appropriate pH. Nemati et al. [6] found that long alfalfa increased rumen fluid pH, possibly due to fermentation characteristics and the high abrasiveness of large particles, which helps maintain appropriate stratum corneum thickness and enhances VFA absorption capacity. However, Mirzaei et al. [26] found no effect of alfalfa particle size on pH, likely due to differences in experimental factors. Terré et al. [16] reported that forage feeding increased rumen fluid pH, whereas increasing dietary NDF level through soybean hulls did not affect pH [36], because soybean hulls have higher fiber and pectin content and lower lignin content than forage, resulting in faster fermentation and reduced chewing activity and saliva secretion. This indicates that pH is affected by fiber source. In summary, forage increases and helps stabilize rumen fluid pH, though the effect depends on forage level, source, and particle size.

5. Effects of Forage on VFA Absorption and Transport Function in Young Ruminants

VFAs produced by rumen microbes provide 60–80% of the energy required by ruminants [42]. Two primary mechanisms for VFA absorption across rumen

epithelium have been identified: (1) lipophilic diffusion of protonated VFAs, and (2) carrier-mediated absorption of VFA anions in exchange for bicarbonate (HCO^-). VFA efflux at the basolateral membrane is primarily mediated by monocarboxylate transporters (MCTs). Three VFA transporters have been identified in rumen epithelium: VFA $^-$/HCO^- exchangers (DRA, PAT1, and anion exchanger 2 [AE2]), MCTs (MCT1 and MCT4), and anion channels.

Studies in adult ruminants show that increasing dietary forage proportion decreases mRNA expression of VFA transporters DRA and MCT1 in rumen epithelium [43]. Yan et al. [44] found that increasing dietary forage from 65% to 90% significantly decreased mRNA expression of VFA $^-$/HCO^- exchangers (DRA, PAT1, and AE2) and MCTs in goat rumen epithelium, suggesting that high-forage diets may reduce VFA absorption capacity. Additionally, Weng [45] reported that feeding high-quality forage increased MCT1 mRNA expression compared with low-quality corn stover, indicating that VFA transport may also be affected by forage source. Sodium-hydrogen exchangers (NHEs) are membrane ion transporters that regulate intracellular pH by exchanging intracellular H^+ for extracellular Na^+. Forage affects not only VFA transporter mRNA expression but also pH-regulating protein expression in rumen epithelium. Yan et al. [44] found that high-forage diets decreased mRNA expression of intracellular pH-regulating proteins (NHE1, NHE2, and NHE3) in goat rumen epithelial cells. The concurrent upregulation of VFA transporters and pH-regulating proteins in low-forage diets has important physiological significance, as VFA absorption increases intracellular H^+ concentration and decreases pH, requiring pH-regulating proteins to export H^+ and maintain cellular homeostasis. Currently, very few studies have examined forage effects on VFA absorption and transport gene expression in young ruminants. Castells et al. [36] found that forage supplementation in pre-weaned calves significantly increased MCT-1 mRNA expression compared with concentrate-only diets. MCT-1, located at the basolateral membrane, transports lactate, acetate, and H^+ from rumen epithelium to blood. The decreased rumen fluid TVFA concentration observed with forage supplementation may be related to increased MCT-1 expression, which reduces intracellular pH by enhancing H^+ efflux, potentially increasing lipophilic diffusion or VFA $^-$/HCO^- exchange and ultimately reducing rumen TVFA concentration. Additionally, Castells et al. [36] found that alfalfa supplementation increased NHE1 mRNA expression compared with oat hay, suggesting that pH-regulating protein expression is affected by forage source. Research on molecular mechanisms of forage effects in young animals is limited. Given that young ruminants have incomplete rumen structure and function that is highly susceptible to dietary influences, future studies should investigate molecular mechanisms of forage effects on rumen epithelium to better understand rumen mucosal development, epithelial cell differentiation, and intracellular metabolic mechanisms.

6. Conclusions

Forage is important for production performance and rumen development in young ruminants. Forage supplementation improves performance by promoting rumen weight and size, maintaining papillae morphology and appropriate stratum corneum thickness, and positively affecting rumen function through altered microbiota, increased rumen fluid pH, and enhanced expression of VFA absorption and transport protein mRNA. Although forage has been partially investigated in young ruminant diets, and rumen development is known to be affected by forage source, level, and particle size, mechanistic understanding remains limited. Future research should investigate mechanisms of rumen development in early-weaned young animals by examining interactions among forage level, particle size, and their combined effects, using modern molecular techniques such as metagenomics, metatranscriptomics, and metabolomics to explore nutrient absorption and transport, metabolic mechanisms, and microbiota in rumen epithelium. This will provide theoretical foundations for understanding interactions among rumen morphology, VFA absorption and metabolism, and microbiota.

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