Early Rumen Microbiota Development and Its Regulation Postprint

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Abstract

A vast array of microorganisms resides in the rumen of ruminants, providing the energy and nutrients required for animal maintenance and production. Regulating the rumen microbiota can enhance feed utilization efficiency in the rumen and reduce the emission of metabolic by-products, thereby ultimately improving the overall economic efficiency of ruminant farming while mitigating the environmental pollution associated with livestock production. Recent studies have demonstrated that rumen microbiota plays a critical role in nutrient metabolism and growth and development of young animals. This review aims to summarize the development and regulatory effects of early-life rumen microbiota in ruminants, providing a reference for the application of early rumen microbiota manipulation in practical production.

Full Text

Development and Manipulation of Early Rumen Microbiota

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Abstract: The rumen harbors a vast diversity of microorganisms that provide energy and nutrients essential for ruminant maintenance and production. Manipulating rumen microbiota can enhance feed utilization efficiency and reduce metabolic by-product emissions, thereby improving the overall economic benefits of ruminant production while mitigating environmental pollution. Recent studies have demonstrated that rumen microbiota play a critical role in nutritional metabolism and growth development of young ruminants. This review synthesizes current knowledge on the development and manipulation of early-life rumen microbiota to provide a reference for practical applications in ruminant production.

Keywords: young ruminants; rumen; microbiota; manipulation

With advances in science and technology, research on the ruminant rumen has expanded beyond physiological and biochemical parameters to deeper investigations of how rumen microorganisms affect the animal as a whole. Rumen microorganisms are primarily composed of bacteria, archaea, and eukaryotes (fungi and protozoa) [1], with bacterial populations alone far exceeding the number of host animal cells [2]. These microbes convert dietary nutrients into absorbable substances for the ruminant host, serving as the primary agents of nutrient transformation [3] while also playing vital roles in animal metabolism and health.

Early rumen microbiota represents an important research frontier. Growing evidence indicates that common issues in young animals, such as diarrhea and weaning stress, are associated with changes and disturbances in early rumen microbiota, prompting numerous studies on potential solutions. This review focuses on the development of early rumen microbiota in cattle and sheep, examining influencing factors and manipulation strategies to provide practical guidance for improving health and growth performance in young livestock.

1.1 Early Rumen Microbiota

The term "early rumen" refers to the underdeveloped rumen of young ruminants, which lacks the full functionality and microbial diversity of mature rumen. While some studies define mature rumen microbiota as established after 3-4 weeks of age [4-6], when the microbial community becomes largely stable and resistant to change (except with major dietary or health alterations), other research suggests significant shifts may occur up to 2 years of age [7-8]. Despite controversy over the exact timing of full rumen development, rumen microbiota eventually achieves a dynamic equilibrium adapted to diet and environment. Therefore, early rumen should be considered the period before microbiota stabilization [9].

The rumen of newborn ruminants has long been considered sterile [10-11], with microbial colonization beginning through milk intake and environmental contact. Although the rumen is an anaerobic environment populated exclusively by anaerobic bacteria in mature animals, early rumen microbiota is not entirely anaerobic. Traditional cultivation methods previously identified aerobic and facultative anaerobic bacteria in young ruminants, which were later completely replaced by anaerobes as the animal matured [12]. Recent pyrosequencing studies of 1-3 day-old calves have confirmed these findings [8]. However, the transient presence of these bacteria, combined with limitations in modern sequencing technologies (such as inability to reconstruct fragmented codon sequences) [13-14], has prevented detailed investigation of their functional roles or impacts on growth performance.

In mature ruminants, the dominant bacterial phyla and their relative abundances are Firmicutes (56%), Bacteroidetes (31%), and Proteobacteria (4%) [15-16]. While these three phyla are also detectable in young ruminants, their proportions differ markedly from mature patterns. Unlike the relatively stable microbiota of mature rumen, early rumen microbiota undergoes significant quantitative and qualitative changes with age. For instance, 8-10 month-old calves harbor 2,095 bacterial OTUs across 24 phyla [17], whereas 7-63 day-old calves have only 1,588 OTUs representing 23 phyla [18]. At the species level, Jami et al. [8] used real-time PCR to show that Proteobacteria dominated the rumen of newborn calves during days 1-3, exceeding even Firmicutes and Bacteroidetes, but its proportion declined over time. Conversely, Rey et al. [5] found Bacteroidetes became the most abundant phylum by day 3, reaching up to 62.1% before day 83. Although both studies confirm eventual convergence toward mature phyla proportions, they underscore the profound influence of age on early rumen microbiota.

Diet represents a critical confounding factor when comparing microbiota across ages [19]. To isolate age effects, comparisons should be made under identical dietary conditions, ideally with milk-only feeding to minimize dietary variation. Based on experimental results [5,8,20], we summarized age-dependent changes in rumen bacterial composition under exclusive milk feeding .

Notably, recent research in goats revealed that between 80-100 days of age, Synergistetes (not Bacteroidetes) became the third most abundant phylum (exceeding 30%), only to be replaced by Bacteroidetes after day 110 [21]. This unprecedented observation suggests that early rumen microbiota may follow distinct patterns during specific developmental windows, necessitating more precise temporal resolution in future studies.

1.2 Rumen Epithelium and Rumen Microbiota

Rumen epithelium, comprising rumen papillae and mucosa, directly interacts with microbial fermentation products and exhibits reciprocal relationships with microbial composition and abundance [19,22]. When studying early rumen microbiota development, clear differentiation of rumen epithelial developmental stages is essential because the early rumen lacks digestive capacity, functioning like a monogastric stomach where only the abomasum performs digestion and absorption. Following this monogastric phase, rumen papillae and mucosal tissues develop in response to microbial fermentation products, with dietary composition influencing microbial proportions [23-24].

Importantly, microbiota composition differs between rumen mucosa and digesta. Mao et al. [25] found that in adult cattle, mucosal OTUs totaled 6,327 versus 5,573 in digesta, with Firmicutes and Bacteroidetes being significantly more abundant in digesta, while Proteobacteria dominated mucosal surfaces. In contrast, Malmuthuge et al. [26] observed 6,051 mucosal OTUs and 7,374 digesta OTUs in pre-weaned calves (7 days old), with Bacteroidetes being most abundant in both compartments.

2 Factors Influencing Early Rumen Microbiota

Rumen microbiota development represents a colonization process from sterile to diverse communities, with microbes adapting their composition and abundance to confer host adaptability to dietary and environmental changes. Early rumen microbiota development is shaped by multiple influences that ultimately establish a stable, beneficial state adapted to the host’ s conditions.

2.1 Maternal Influence Microorganisms acquired from the dam at birth represent the earliest colonizers of the rumen, transmitted through two primary routes: direct contact during birth and colostrum ingestion. From birth to day 20, calves harbor microbes identical to those found in the dam’ s vagina and colostrum [27]. Protozoa, present in saliva, are reportedly transmitted through inter-animal licking during the first two weeks of life, as isolation at birth prevents protozoal detection during this period [9]. Abecia et al. [6] compared two rearing systems: lambs reared with their dams showed significantly increased bacterial richness on days 3, 5, 7, and 14, most notably rapid protozoal proliferation. A subsequent study using the same model found significantly higher Spirochaetes and consistently greater Bacteroides in dam-reared lambs [28].

Remarkably, even after sanitizing the dam’s birth canal, anus, tail, and legs with sterile water before delivery and using sterile cloths during birth, methanogens (*Methanobrevibacter mobile*, *Methanobrevibacter votae*, and *Methanobrevibacter* spp.), fibrolytic bacteria (*Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Prevotella ruminicola*), and *Geobacter* spp. were detectable in calf rumen within 20 minutes postpartum [4]. Additionally, 16S rRNA sequencing of half-sibling calves suggests potential vertical transmission of rumen microbiota [29]. These findings collectively indicate that early rumen microbes may be acquired not only through postnatal contact and colostrum but also directly from the dam during fetal development.

2.2 Starter Feed Starter feed is commonly used in young ruminant production, typically combined with milk or milk replacer, and represents a critical factor for successful weaning. In lambs, *Prevotellaceae* abundance increased from 31% to 48% after starter feeding, becoming the dominant bacterial family [5]. Feeding starter to 10-day-old lambs significantly increased Synergistetes abundance in rumen epithelium by day 56, while decreasing Proteobacteria, Tenericutes, and candidate phyla, and altering abundances of unclassified bacteria at the genus level [30]. In calves fed milk versus milk plus concentrate, Actinobacteria proportions began declining from day 28 in milk-fed calves [20]. Another study found 47 bacterial genera in concentrate-fed calves compared to 72 genera in concentrate-plus-forage-fed calves, with significantly increased *Prevotella* in the latter group [31].

2.3 Weaning Weaning induces pronounced shifts in rumen microbiota [16,22]. Meale et al. [32] used 16S rRNA sequencing to demonstrate reduced OTUs and diversity (Chao1 and Shannon indices) during weaning, with Bacteroidetes abundance decreasing significantly from 66.1% pre-weaning to 42.2% post-weaning. Concurrently, Actinobacteria and Verrucomicrobia decreased, while Proteobacteria and Firmicutes increased significantly. A subsequent study showed weaning reversed the dominance hierarchy, reducing Bacteroidetes and elevating Firmicutes to become the most abundant phylum [33]. However, weaning timing (early vs. delayed) did not affect the final proportions of the three dominant phyla [32–33]. Although weaning consistently induces substantial microbiota changes, no studies have successfully altered the dominant bacterial groups through weaning strategies. Nevertheless, delayed weaning moderates microbiota transitions, potentially reducing stress from abrupt early-weaning changes [34].

2.4 Animal Species and Breed Mammalian gastrointestinal microbiota varies by species [2]. For example, ruminants such as cattle and sheep exhibit distinct microbiota profiles. In lambs (6–9 months), *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, and *Propionibacterium* dominate rumen epithelium, whereas in calves (9–11 months), *Clostridium* replaces *Bifidobacterium* among the most abundant genera, and total bacterial load is higher in lamb mucosa [35]. Breed differences also exist within species; Holstein cows show significantly greater bacterial richness (OTU and Chao1 indices) than Jersey cows [36]. Geographic variation affects early microbiota composition: 3-day-old Holstein calves in France had Bacteroidetes as the dominant phylum [5], while those in Israel [8] and the USA [27] showed Proteobacteria dominance. Even within the same breed and region, individual calves exhibit substantial variation in methanogen species [37].

3 Manipulation of Early Rumen Microbiota

Manipulating early rumen microbiota can reduce the incidence of diarrhea and mortality in young ruminants [38], decreasing economic losses while improving growth performance. Given the vast diversity and functional heterogeneity of rumen microbes and current methodological limitations, our understanding remains incomplete, but accumulating research continues to advance manipulation strategies.

Probiotic administration (primarily lactic acid bacteria and bifidobacteria) directly modulates fermentation parameters while promoting beneficial bacteria [39]. Qadis et al. [40] observed increased *Bacteroides* abundance, elevated rumen pH, and increased ammonia nitrogen in Holstein calves [(12±3) weeks old] fed lactic acid bacteria before morning feeding. Foditsch et al. [38] administered *Faecalibacterium prausnitzii* to 2-day-old calves 1 hour after milk feeding, which increased fecal *F. prausnitzii* counts, significantly reduced diarrhea incidence and mortality, and improved average daily gain during the weaning period.

Prebiotics, primarily carbohydrates and yeast cultures (commonly mannan, cello-oligosaccharides, and yeast culture), show limited efficacy. Reports indicate that prebiotic supplementation during the weaning transition may marginally improve calf growth performance but does not significantly alter rumen microbiota, with minimal effects in healthy calves [39,41].

Ionophores such as monensin are commonly used feed additives. Monensin supplementation in calf diets significantly reduces Firmicutes and Spirochaetes abundance while increasing rumen pH and decreasing methane production [42].

Rumen fluid inoculation represents another manipulation approach. Inoculating lambs with ewe rumen fluid significantly increased microbial abundance and diversity, improved average daily gain, and enhanced digestibility of dry matter, neutral detergent fiber, and acid detergent fiber without affecting health or feed intake, though effects diminished over time [43-44].

Bromochloromethane (BCM) supplementation from birth to 3 months of age in lambs significantly reduced methanogen abundance and methane production by 6 months. When BCM was also fed to dams from lambing through the 2-month suckling period, lamb average daily gain increased significantly [45]. Similarly, linseed oil supplementation from 2-6 weeks of age persistently reduced *Methanobrevibacter* and *Methanosphaera* abundance at 16 weeks without affecting growth, feed intake, or health [46].

4 Conclusion

Early rumen microbiota is simpler yet distinct from that of adult animals, characterized by several unique transitional phases: a monogastric-like period where only the abomasum functions, a transition from aerobic/facultative anaerobic to strictly anaerobic bacteria, and distinct pre- and post-weaning periods. Development is influenced by numerous factors, and manipulation can mitigate diarrhea and mortality while improving growth performance and reducing economic losses. However, the immense diversity and functional complexity of rumen microbes, coupled with methodological limitations, mean current understanding remains fragmentary. Continued research will undoubtedly elevate our knowledge and manipulation capabilities to new levels.

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