What Do We Know About Rumen Development?

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Introduction

Synchronized microbial, morphological, and metabolic developments of the rumen are 3 vital processes that must occur for pre-ruminants to become ruminants. Little is currently known about how each of the 3 processes occurs and how they may synergize to affect calf growth and nutrient utilization, 2 processes important to comprehend for future productivity.

The dairy calf is born with an immature gastrointestinal tract **(GIT)** and begins life as a functional monogastric. The transition from a functional monogastric to a ruminant centers on the ability of the rumen to support fermentation. The capacity for ruminal fermentation is minimal at birth and is dependent on 5 key elements: microbial establishment in the rumen, substrate availability, presence of liquid, absorptive ability of rumen tissue, and outflow of material from the rumen to the lower GIT. Despite our knowledge of these requirements for rumen development, we still do not completely understand how the rumen undergoes metabolic changes at the molecular level to support fermentation; how, when, and under what circumstances various classes of rumen microbes populate the rumen; or how rumen microbial populations change in response to the diet that is fed as calves transition from the pre-weaning phase of life. All of these processes contribute to the growth and capability of the rumen and may affect lifetime profitability, either positively or negatively.

All 4 chambers of the ruminant stomach are present at birth, but not all are functional at birth. The rumen in mature ruminants is essentially a large anaerobic fermentation chamber where plant-degrading rumen microbiota (bacteria, protozoa, archaea, and fungi) ferment otherwise non-digestible plant-based feedstuffs into primarily the volatile fatty acids **(VFA)** acetate, propionate, and butyrate. In mature ruminants, VFA and microbial protein combine to meet the animal's energy demands for survival, growth, and production. The luminal surface of the rumen in mature ruminants is lined with numerous papillae. Ruminal papillae are epithelial structures comprised of multiple cell layers; the main functions of papillae are to increase the absorptive surface area of the rumen and to absorb VFA, leaving microbial protein to be digested in lower regions of the GIT. Ruminal papillae absorb VFA by passive- and facilitated-diffusion and transfer them to the animal's bloodstream. Acetate and propionate are mostly transferred to the animal's portal circulation intact, whereas as much as 85 to 90% of ruminal butyrate is oxidized to ketone-form prior to entering the portal circulation. Ruminal butyrate is primarily oxidized to β-hydroxybutyrate **(BHBA)**, and to a lesser extent, to acetoacetate. Because of this change in form of butyrate, it is commonly viewed as an energy substrate for ruminal epithelial cells and is also implicated in ruminal papillae growth (discussed later).

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Microbial Colonization of the Rumen

The mature rumen harbors a complex microbiota, with bacteria being dominant (Brulc et al., 2009), but newborn calves have sterile rumens. However, within 1 to 2 days after birth, the rumen starts to be colonized with numerous microbes (Anderson et al., 1987). Microbial colonization of the neonatal rumen initiates a cascade of growth and developmental changes within the host animal that ultimately allow the animal to function as a true ruminant. The colonization process of the rumen has been investigated in early cultivation-based studies that found broad classes of rumen microbes (e.g., amylolytic, cellulolytic, proteolytic, and lactate-utilizing), changes with age (Anderson et al., 1987), and changes with diet (Pounden and Hibbs, 1949; Anderson et al., 1987) in young dairy calves. Species-level identification of rumen microbes was not readily available until recently (reviewed in Morgavi et al., 2012), and to our knowledge, only one study has examined the succession of microbiota in dairy calves (Li et al., 2012). The work of Li et al. (2012) represents the first attempt at documenting the temporal sequence of microbial establishment in the rumen with modern metagenomic tools.

The earlier dry feed is introduced into the calf's rumen, the earlier microbial development occurs, resulting in higher ruminal metabolic activity and increased total VFA concentrations of rumen contents (Anderson et al., 1987). This is reflective of a nutrient substrate requirement for rumen microbiota. In young calves, this can be sloughed ruminal epithelial cells, milk or milk replacer **(CMR)**, or dry feed. The passage of feedstuffs into the rumen in young calves is a regulated process. When calves drink milk or CMR, either from a teat, bottle, or bucket, reflexive closure of the esophageal groove occurs. This shunts milk past the reticulo-rumen into the abomasum and keeps the consumed milk or CMR from being fermented in the rumen. Dry feed consumption and "spillage" of milk or CMR are the only means for foodstuffs to enter the rumen and to be subjected to ruminal fermentation. Development of ruminal papillae is also affected by dry feed consumption. Heinrichs (2005) showed that milk- or CMR-only fed calves had under-developed rumen papillae and musculature. Thus, the growth promoting agents for ruminal papillae are not the ruminal microbiota alone or dry feed alone. Rather, it is the fermentation endproduct butyrate that is responsible for the growth of ruminal papillae (Sander et al., 1959). This is substantiated by direct administration of butyrate either into the oral cavity or into the rumens of cannulated animals (Flatt et al., 1958; Sander et al., 1959; Mentschel et al., 2001) and also by inclusion of butyrate in calf feed (Gilliland et al., 1962; Górka et al., 2009; Górka et al., 2011; Kato et al., 2011). However, these practices are not common and ignore the potentially important role of native ruminal microbiota in producing butyrate and contributing to the balance of the ruminal ecosystem.

Morphological Development of the Rumen

Morphological development of the rumen mainly refers to papillae characteristics, muscle thickness, and organ size (Van Soest, 1994). Ruminal papillae are present at birth on the luminal surface of the entire rumen; they can be visualized macroscopically. Papillae length, width, and area increase with age and are responsive to diet and butyrate, as mentioned above. Rumen morphology can also be studied at the microscopic level. The rumen is composed of stratified epithelium with an outer keratin layer. Morphologically, from the lumen surface, 4 distinct layers can be visualized: stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Figure 1). Feeding animals highly fermentable diets (Bull

et al., 1965) or pelleted diets (Jensen et al., 1958; Bull et al., 1965; Hinders and Owen, 1965), or the infusion of butyrate (Tamate et al., 1962) can cause morphological changes to ruminal papillae. Butyrate stimulates rumen papillae growth through unknown means; many theories exist, but a mechanism remains to be elucidated.

Metabolic Development of the Rumen

Metabolic development of the rumen centers on the capacity of ruminal epithelial cells to produce ketones from absorbed fermentation endproducts, VFA. Existing data suggest that VFA entry into ruminal epithelial cells can occur via facilitated transport and passive diffusion (Aschenbach et al., 2011) and likely is dependent on the cell layer within the rumen epithelium. Entry via facilitated transport requires membrane transporters. In dairy cattle research, commonly studied VFA transporters include members of the solute carrier **(SLC)** family. These include down-regulated-in-adenoma (**DRA;** *SLC26A3*), putative anion transporter 1 (**PAT1;** *SLC26A6*), and monocarboxylate transporters 1, 2, and 4 (**MCT-1**, *SLC16A1*; **MCT-2**, *SLC16A7*; **MCT-4**, SLC16A3) [Connor et al., 2010; Laarmen et al., 2012; Naeem et al., 2012; Schlau et al., 2012; Steele et al., 2012].

Passive diffusion of butyrate and other VFA (undissociated form) into ruminal epithelial cells is enhanced at low rumen pH (Dijkstra et al., 1993). Once undissociated forms of VFA enter ruminal epithelial cells, intracellular dissociation can occur which can increase intracellular H^+ concentration. This, coupled with the loss of intracellular HCO_3^- in exchange for VFA^- due to activity of DRA, can further lower rumen pH. To regulate intracellular pH, ruminal epithelial cells have Na^+/H^+ exchangers **(NHE)**. Graham and Simmons (2005) identified 3 ruminal isoforms of NHE; these are known as NHE1, NHE2, and NHE3. Collectively, the expression and localization patterns of ruminal VFA transporters (DRA, PAT1, MCT-1, MCT-2, and MCT-4) and transporters that aid in VFA uptake (NHE1, NHE2, and NHE3) are not well characterized in young calves, and very little is known about the impact of age or diet on their abundance. This is a gap in our understanding because it appears that they play important roles in VFA uptake and intracellular pH regulation.

The rumens of neonatal ruminants, such as dairy calves, are non-ketogenic. This means that rumen tissue is unable to oxidize butyrate (the primary ketogenic substrate for ruminal epithelial cells) into the ketones BHBA or acetoacetate at birth. As a result, blood concentrations of these metabolites are low. In milk-fed-only lambs, it was noted that BHBA production is minimal before 42 days of age (Lane et al., 2000). After that, BHBA production rate from isolated rumen epithelial cells from 42-day-old milk-only fed lambs was equivalent to BHBA production rates of isolated rumen epithelial cells from 56–day-old lambs fed milk and dry feed (Lane et al., 2000). Ruminal ketogenesis is a hallmark of mature ruminants. The capacity of rumen tissue to support ketogenesis is apparently age-dependent and is not affected by solid feed intake or intraruminal VFA concentration, which are both recognized stimulators of morphological development of the rumen (Lane et al, 2000; Lane et al., 2002). Lane et al. (2002) showed that mRNA abundance of 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase) increased in parallel with ruminal ketogenesis before 49 d aysof age in lambs and suggested that HMG-CoA synthase is the rate-limiting enzyme in ruminal ketogenesis. Two isoforms of HMG-CoA synthase are known to exist: **HMGCS1,** which is cytoplasmic, and **HMGCS2,** which is mitochondrial (Hegardt, 1999). Naeem et al. (2012) suggested that intramitochondrial ketogenesis is the primary pathway for generating

BHBA in ruminal epithelial cells of young calves. The promoter region for the HMGCS2 gene contains a peroxisome proliferator-activated receptor response element, and its mRNA is transcriptionally regulated by peroxisome proliferator-activated receptor-α (**PPAR-α**) (Meertens et al., 1998). PPAR-α are nuclear receptors; known ligands for PPAR-α include fatty acids, presumably butyrate. In the absence of ruminal ketogenesis, a developing idea is that intraruminal butyrate, even in small quantities, appears to stimulate transcription of select genes, which ultimately regulate metabolic maturation of the rumen. According to Penner et al. (2011), the enzymes of ruminal ketogenesis have not yet been localized within the ruminal epithelium using immunohistochemistry, but the cells responsible for this process are thought to be located in the stratum basale, where the most mitochondria are present. Thus, in dairy calves, there is opportunity to pinpoint when ruminal ketogenesis begins and where these enzymes are located.

Conclusions

Microbial, morphological, and metabolic development of the rumen are still not well understood in young dairy calves. Scientists considering study in this area would be wise to consider both calf age and diet effects when planning their investigations. While not all-inclusive, the rumen microbiome could be studied with metagenoomic tools. Rumen morphology could be examined using gross measurements and molecular and histological techniques. Studies on the metabolic development of the rumen could center on evaluation of abundance and localization of ruminal VFA transporters and enzymes involved in ketogenesis. Continued research in these interrelated areas should provide novel information about rumen development in peri-ruminant calves.

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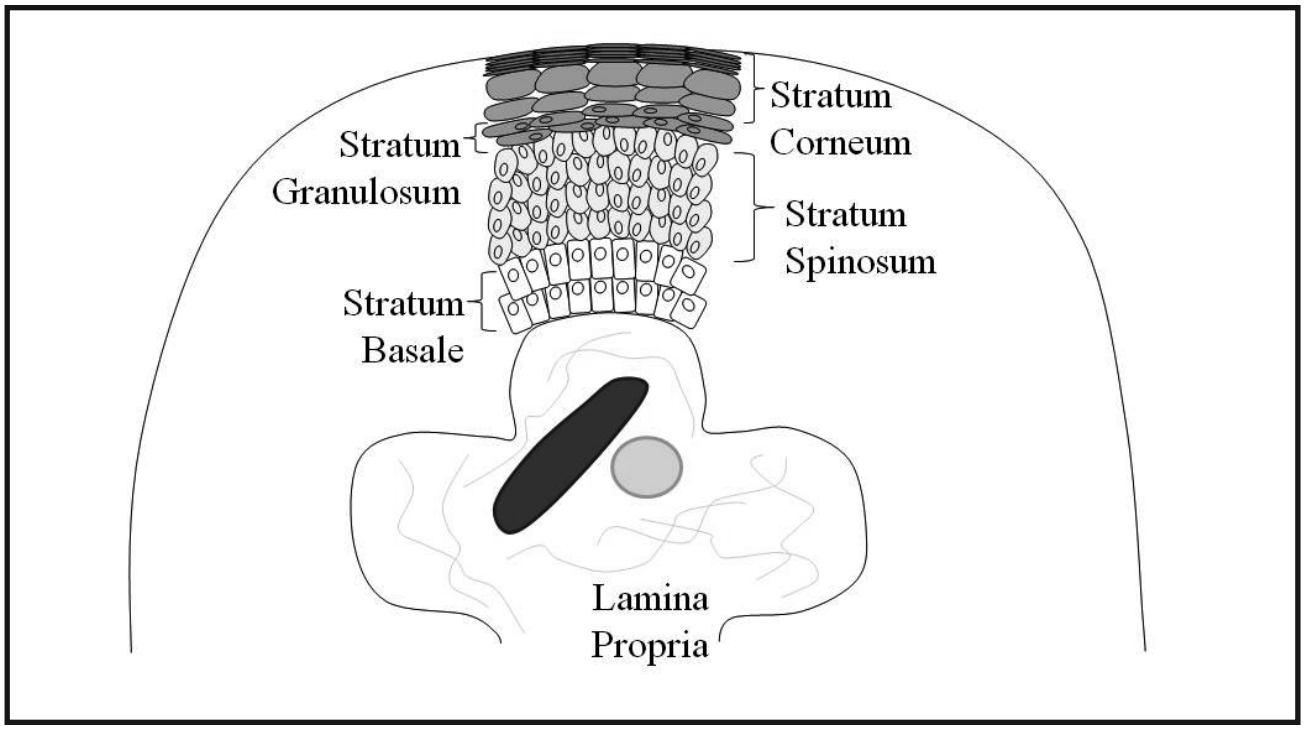

Figure 1. Illustration of the organization of the rumen epithelia on a single papilla tip. A layer of keratin exists above the stratum corneum (not labeled).