Recent Research on Rumen Development in Dairy Calves

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Abstract

Our understanding of rumen development has evolved over the years with initial research targeting dietary stimuli to current research identifying relationships between the rumen epithelial transcriptome, rumen-specific microRNA, and rumen microbiome. While we have come a long way in our appreciation of how we can influence rumen development, there are still improvements to be made in regard to translating scientific data to help producers efficiently raise calves. Throughout this proceedings article, I will discuss both classic and modern research findings on the topic of rumen development to showcase what still holds true and how new insights are directing future research. The main topics discussed include the general areas of dietary and microbial effects on rumen development, but also some lesser studied areas are examined including: weaning, rumen pH, and volatile fatty acid (**VFA**) absorption in calves. Ultimately, my goal is to leave the reader with a better understanding of the history and current understanding of rumen development with the hope that some practical knowledge surrounding diet and management (e.g., weaning strategy) may be of use when making on-farm decisions surrounding calf nutrition.

Introduction

A quick distinction must be made before you read further to prevent any potential confusion; the term "rumen development" is used throughout even though in other works you may see a separation between rumen growth (i.e., physical growth of the tissue) and rumen development (i.e., maturation of metabolic processes). It becomes unnecessarily tedious to separate these terms out, especially when some results within the same study pertain to just growth or development, but in general, I have chosen to use rumen development as an all-encompassing term to make it easier on myself and you.

One of the earliest reports on rumen development dates back to 1897 when Davenport noted in a University of Illinois Agricultural Experiment Station bulletin the importance of fiber compared to milk for developing the rumen (Davenport, 1897). Since then, we have learned a considerable amount on the topic of rumen development, branching out to multiple areas of focus including nutrient transporter regulation, microbial population shifts in response to diet, and measuring actual VFA absorption rates in preweaning calves. At the time of writing this, one of the most recent studies published that investigated rumen development (Guo et al., 2021) utilized rumen cannulated calves undergoing weaning and high-throughput

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sequencing to take temporal rumen epithelial samples to assess rumen epimural (adhering to the epithelium) microbial populations over time and how these changes affected growth of the rumen tissue. New studies such as this not only showcase how advanced our techniques have become, but that our approach to studying rumen development is evolving as well where microbial populations are not just viewed in relation to substrates created, but also their direct influence on tissue growth and development. Overall, it is important to understand how the actual function of the rumen is developing, though, which is an area that recent research has addressed looking at VFA absorption in preweaning calves with either a developed or an undeveloped rumen (Yohe et al., 2019b) that will be discussed later. While there are still gaps in our knowledge regarding rumen development, we have come a long way and will continue to find important areas of research that are both novel and may be of practical use to producers growing calves.

Dietary Factors Influencing Rumen Development

It is well established that calves fed a diet consisting of only milk/milk replacer (**MR**) will have an undeveloped rumen (e.g., smaller overall size, less musculature, smaller papillae size) compared to calves consuming dry feed (Tamate et al., 1962; Heinrichs, 2005; Yohe et al., 2019a). The 1950-60s brought a flood of research focusing on the effects of feeding forage compared to concentrate/starter, with both leading to increased muscle/overall size of the rumen and epithelial growth (i.e., papillae size), respectively (Conrad and Hibbs, 1956; Warner et al., 1956; Sutton et al., 1963b). Further evidence narrowed down the importance of the microbial fermentation end-products, VFA (mainly butyrate and propionate), on rumen papillae growth (Tamate et al., 1962; Sutton et al., 1963b). Thus, recommendations for the past few decades have focused on increasing dry feed consumption and subsequently VFA production in the rumen, which has demonstrated to be vital for development of the rumen and will ultimately lead the calf through a successful transition from a non-ruminant to a ruminant.

Recent research focusing on the effects of milk/MR fed to the calf on rumen development has been conducted, with Koch et al. (2019) comparing calves fed MR via automated feeder either ad libitum or restricted (6 L/day) and at 80 days of age noting an increase in weight of the empty rumen and width of papillae in the atrium ruminis in restricted (2.30 ± 0.25 kg and 1.35 ± 0.03 mm, respectively) compared to ad libitum (1.80 ± 0.25 kg and 1.29 ± 0.03 mm, respectively) fed calves. Schäff et al. (2018) compared MR fed via automated feeder either ad libitum for 5 weeks and then reduced to 6 L/day until day 60 or restricted (6 L/day the entire study) and then took rumen measurements on day 60 and found no differences in overall rumen size, papillae length, papillae width, or papillae surface, but did measure increased papillae density in 2 regions of the rumen in restricted (atrium ruminis: 88.9 ± 2.4 papillae/cm² and ventral sac: 104.8 ± 2.4 papillae/cm²) compared to ad libitum (atrium ruminis: 82.9 ± 2.4 papillae/cm² and ventral sac: 98.8 ± 2.4 papillae/cm²) fed calves. The differences between rumen results presented in these 2 studies is most likely due to concentrate intake where restricted and ad libitum fed calves in Schäff et al. (2018) had no differences throughout the study compared to restricted having higher intakes than ad libitum fed calves from week 4 to 11 in Koch et al. (2019). Looking at how the diet influences the growth of the rumen at the cellular level, Yohe et al. (2019a) found a trending increase in number of proliferating cells (i.e. positively stained cells for Ki67 protein) in the rumen epithelium (basale layer) of calves at 6 weeks of age fed MR and pelleted starter compared

to just MR (Figure 1). The results of Yohe et al. (2019a) demonstrated the importance of concentrate/starter on rumen epithelial growth and corroborated that of Goodlad (1981) who saw an increase in rumen epithelial cell turnover rate in sheep fed concentrate (10.9 ± 2.0 days) compared to forage (16.5 ± 0.7 days). Taken together, there is a body of evidence to support the importance of concentrate/starter intake on growth of the rumen, which is promoting hypertrophy of the tissue, but the mechanisms behind this proliferation are still being explored.

Some potential mechanisms behind rumen epithelial proliferation have been proposed in the past, with recent data shedding more light on the subject. Studies have suggested a role of insulin and insulin-like growth factor (**IGF-1**) in the proliferating rumen epithelium (Sakata et al., 1980; Baldwin, 1999). Novak et al. (2019) measured genes relevant to rumen epithelial growth in Jersey steers either fed a diet transitioning from forage to concentrate or concentrate to forage and found an increase in IGF-binding protein 5 (**IGFBP5**), which further supports a role for IGF-1 in proliferation of rumen epithelial cells in response to highly fermentable diets. Other potential mechanisms involved in rumen epithelial growth include transcription growth factor-β1 (**TGFβ1**), which had increased levels of mRNA and protein in calves fed starter compared to just hay after weaning (Connor et al., 2014) and was upregulated in calves fed hay and starter compared to just starter after weaning (Kim et al., 2016), as well as estrogen-related receptor-α (**ESRRA**), which had increased gene expression as calves aged and consumed dry feed with a link to increased hay intake at weaning (Connor et al., 2014). Taken together, these studies show that there are underlying mechanisms involved in promoting the growth of the rumen epithelium that responds to dietary stimuli, but further evidence is needed to elucidate these (and any other potential) pathways.

Staying at the molecular level, there have been recent studies exploring the impact of microRNAs (**miRNA**) on rumen development. MiRNAs are endogenous, non-coding, single-stranded RNA molecules that act as post-transcriptional regulators of mRNA molecules, which displays a unique relationship between mRNA and miRNAs that further convolutes our understanding of how gene expression relates to function. Xue et al. (2019) compared diets differing in the ratio of nonfiber carbohydrate (**NFC**):NDF fed to calves up to 105 days of age and found an increase in expression of bta-miR-128 (*bos taurus* miRNA-128) in the rumen of calves fed a lower ratio (0.80) compared to a higher ratio (1.35). After looking into functional pathway enrichment, it was found that bta-miR-128 is associated with monocarboxylate transporter 1 (**MCT1**), which is a VFA transporter shown to have increased mRNA in calves fed MR, starter, and hay compared to MR and hay only (Laarman et al., 2012) and demonstrates that miRNAs may play a role in the relationship between gene expression and the function of VFA absorption in the developing rumen. Do et al. (2019) characterized miRNA levels in the rumen tissue of calves during the preweaning period (calves fed up to 9 L/day of MR with access to starter; rumen samples taken on day 33) or during the postweaning period (calves underwent a gradual weaning process from days 43 to 53 and had access to starter and hay thereafter; rumen samples taken on day 96) and found multiple differentially expressed miRNAs between the groups. Bta-miR-493 was upregulated in calves at 96 compared to 33 days of age (and further confirmed with quantitative real-time PCR (**qPCR**) analysis) and has been linked to the gene coding for IGF-1 receptor (**IGF1R**), which further connects the potential importance of IGF-1 to rumen development. On a macro level, use of gene ontology analysis by Do et al. (2019) revealed miRNAs relevant for the biological processes

of digestive tract development, digestive tract morphogenesis, and digestive system processes to be enhanced in calves at 96 compared to 33 days of age. It is worth noting that age and diet are both confounding factors in this study, but considering the lack of studies investigating miRNAs in rumen development, this is still good data to help guide future research into the role of this novel area on the effects of miRNA on rumen tissue development.

Another novel area of rumen epithelial homeostasis was investigated in Yohe et al. (2016) and Yohe et al. (2019a) where the concept of putative rumen epithelial stem and progenitor cells was explored in calves fed either MR only or MR and starter. Yohe et al. (2016) was able to detect expression of a few proposed stem cell markers from the intestine (leucine-rich repeat containing G protein-coupled receptor 5 (**LGR5**) and musashi RNA-binding protein 1 (**MSI1**)) and skin (tumor protein p63 (**TP63**), keratin 14 (**KRT14**), integrin, β 1 (**ITGB1**), and notch 1 (**NOTCH1**)) in the rumen epithelium of post-weaned 62 day old calves. The topic was further investigated in Yohe et al. (2019a) where the thymidine analog 5-bromo-2'-deoxyuridine (**BrdU**) was used to identify differences in putative rumen epithelial stem cell abundance in calves fed only MR or MR and starter. Interestingly, there was an increase in rumen epithelial basale cells that retained the BrdU label in MR only fed calves at 6 weeks of age (Figure 2), which suggests an increase in abundance of putative stem cells in the rumen of calves that have not started to increase proliferation in response to starter in the rumen (Yohe et al., 2019a). From the same rumen samples, we also attempted to colocalize BrdU presence with other potential stem cell markers (ITGB1, NOTCH1, and FK506-binding protein 51 (**FKBP51**)) in order to provide more support for the existence of rumen stem cells, but colocalization of the proteins did not occur. Future research into the area of rumen stem and progenitor cells should investigate: other potential markers to help identify stem cells in future research, differences in BrdU (and Ki67) stained cells between calves fed starter and forage, and the effects of weaning on any potential long-term effects on putative rumen stem and progenitor cell populations.

Other researchers have focused on less studied areas involving dietary impacts on rumen development, including utilizing microencapsulated sodium butyrate (**MSB**) with alternate protein sources used in starter, branched chain VFA (**BCVFA**), utilizing enzymes in feed, and length of fiber used when feeding forage. Burakowska et al. (2021) investigated the addition of MSB to diets either containing soybean meal (commonly used in starters) or canola meal (not commonly used in starters) as the main protein source and overall found no effect of protein source, but MSB addition decreased rumen epithelial surface area and dry rumen epithelial mass as well as a tendency to decrease papillae length. The use of BCVFA and fibrolytic enzyme effects (cellulase and xylanase) on rumen development was investigated by Liu et al. (2020) who found addition of both to the preweaning and postweaning diet (calves were weaned on day 60 with rumen samples taken on day 90) led to improved feed conversion, increased rumen VFA concentration, increased rumen papillae length and width, increased mRNA of growth hormone receptor (**GHR**) and IGF1R, and increased GH and IGF-1 in the blood. The effect of BCVFA and fibrolytic enzymes on the GH/IGF-1 axis is another example of how dietary components can affect mechanisms involved in development of the rumen tissue. Yang et al. (2018) performed a study feeding lambs starter with or without available chopped alfalfa and found the genus *Butyrivibrio*, which is known primarily as a butyrate producer (Diez-Gonzalez et al., 1999),

was positively correlated with papillae length. This connection of butyrate to rumen epithelial proliferation is well known and adds further connection between diet, rumen microbes, and rumen development.

Microbial Impacts on Rumen Development

While dietary impacts on rumen development are evident, the substrates that are absorbed and subsequently metabolized by the rumen are mainly derived from the rumen microbial population, which has also been an important area of study in recent years. Research performed in young ruminants (calves and lambs) by Anderson et al. (1987a) and Fonty et al. (1987) demonstrated the presence of amylolytic, cellulolytic, fibrolytic, and proteolytic bacterial populations within the first week and as early as 2 days of age. Earlier than the 1980s, Bryant et al. (1958) characterized some initial findings on culturing bacteria able to survive anaerobically in specific media. It is safe to say that since 1958, methods of detection have improved immensely with current studies utilizing metagenomic and transcriptomic approaches to examine rumen microbial populations.

One cogent study involving both rumen microbial and tissue development was completed by Malmuthuge et al. (2019) who investigated microbial populations in the rumen utilizing both RNA- and DNA-based quantification approaches, along with metagenomic analyses of both rumen contents and tissue. One interesting takeaway from the study by Malmuthuge et al. (2019) included the importance of butyrate (created by rumen microbes) in rumen development due its involvement in inhibiting epigenetic modification of the rumen epithelial transcriptome (via histone deacetylase coding genes, **HDAC**), which subsequently upregulates peroxisome proliferator activated receptor gamma (**PPARG**) expression and has been shown to promote epithelial proliferation. Overall, Malmuthuge et al. (2019) explored the effect of the rumen microbiome on both the rumen transcriptome and microRNAome and found that 26.3% of genes and 46.4% miRNAs were associated with VFA (i.e. microbial fermentation end-products), respectively, which demonstrates the importance of microbial populations having an indirect effect on development of the rumen compared to just effects over time.

Both Dill-McFarland et al. (2017) and Guo et al. (2021) used high-throughput sequencing techniques to look at microbial populations present in the rumen over time and noted some interesting findings, not just regarding establishment of microbial populations, but how long these populations persist. Takeaways from Dill-McFarland et al. (2017) relevant to the rumen were that specific bacterial communities change from weaning to adulthood (e.g. even genomic sequences within the *Prevotella* spp. were different at these time points) and fungal communities were undetectable prior to weaning, but during and shortly after weaning, these communities start to become established and some genera even persist into adulthood (e.g. *Caecomyces* spp.); furthermore, fungal populations were only found to positively correlate with growth as these animals aged. Guo et al. (2021) did not follow calves into adulthood but compared rumen epimural bacterial populations between preweaning and postweaning and found genera that decreased (*Bacteroides* spp.) and increased (*Prevotalla* 9 spp.) in abundance, respectively. These differences (or lack thereof) found over time will help direct future research to elucidate the importance of these microbial communities, but the obvious next step is looking closer at these population differences and seeing how they affect both the gastrointestinal and gut-associated tissues in the calf.

Examining further the relationship between the rumen microbiome and other tissues in the calf, Guo et al. (2021) and Li et al. (2019) examined rumen epimural bacterial populations. Guo et al. (2021) assessed population changes throughout weaning and found interesting correlations between rumen tissue measurements over time and bacterial populations, such as a positive relationship between papillae surface area and the *Pyramidobacter* spp. ($R \sim 0.64$; $P < 0.05$) or a negative relationship between rumen epithelial thickness and *Bacteroides* spp. ($R = -0.59$; $P < 0.05$). Li et al. (2019) went further than looking at just the rumen and examined how rumen epimural bacteria and the liver transcriptome is affected by lowering the rumen pH via starter diet. There were no differences mentioned between the control (texturized (DM-basis): starch = 35.3%, NDF = 25.3%) and treatment (pelleted (DM-basis): starch = 42.7%, NDF = 15.1%) fed to calves in regards to correlations between liver transcriptome and rumen epimural bacteria, but it was found that calves fed the treatment diet had unique expression of high density lipoprotein (**HDL**)-encoding genes compared to control diet calves. This expression of HDL-encoding genes suggests an alteration in lipoprotein metabolism of the liver in response to low rumen pH and potential abnormalities in the liver *(i.e.*, development of an ulcer, an abscess, or sepsis). While the results from Li et al. (2019) are not directly measuring rumen development, they are important in the context of rumen development via diet having unintended consequences on calf health. Further investigation into how epimural bacterial populations alter both rumen development, as well as whole animal health and development, are warranted to help get a holistic picture of the calf during this period.

Weaning and pH Effects on Rumen Development

While all areas involving rumen development are interconnected, there are some topics that we are able to isolate (i.e., dietary and microbial effects) due to their ubiquitous nature. Other areas, such as weaning and rumen pH, have become important areas of research that help fill in gaps surrounding our understanding of rumen development and thus are crucial to include in its discussion.

The preweaning diet of the calf mainly consists of liquid feed (i.e., MR/milk), but as the calf undergoes the weaning process, their consumption of solid feed will dramatically increase. This increase in solid feed intake, which typically is in the form of a small-particle sized feed (e.g., pellets or grains), is where the main issues arise from weaning, stemming from a large amount of readily fermentable feed now present in the rumen for rapid digestion. It should be noted, though, that solid feed consumption is low within the first 3 weeks of lif, despite amount of liquid diet fed (Quigley et al., 2018) and that at 30 days of age for each 100 g of milk/MR DMI, there is a decrease of 13 g/day of starter DMI (this increases to ~93 g/day of starter DMI at 60 days of age) (Silva et al., 2019). While altering feeding and management practices to help calves through this weaning period is useful, a major change that is not easily controlled is the drastic change in form and composition of diet. This transformation in diet will require a general change in feeding behavior by the calf (Overvest et al., 2018; Xiao et al., 2018), a shift in the overall metabolism in the gastrointestinal tract (Baldwin et al., 2004), and an alteration of the microbial population in the gut (Meale et al., 2016) in order to promote a positive transition postweaning.

There are negative effects associated with weaning (e.g., impaired gut barrier function and body growth slump due to reduced intake; Wood et al., 2015; Steele et al., 2017), which may be related to rumen pH, but strategies such as a step-down weaning procedure (Steele et al., 2017; Klopp et al., 2019) or pair-housing of calves (Bolt et al., 2017; Overvest et al., 2018) have shown promising results via improvements in feed intake, weight gain, and behavioral observations. While rumen development was not assessed directly in these studies, Klopp et al. (2019) demonstrated the influence of the rumen on intake and growth by showing gradually weaned calves (2 step-down weaning protocol over 3 weeks) had increased starter intake and enhanced ADF digestibility compared to calves weaned only using a one week step-down. This is just one example of a successful weaning strategy amongst many that could be utilized. Another concept that promotes comparable growth via starter intake (and thus lower MR intake) is weaning based on individual solid feed intake, which has become much more realistic with the use of automated milk feeders (Benetton et al., 2019).

Despite the weaning protocol utilized, the increase in starter consumption will lead to the same result. As the feed enters the rumen and is exposed to the now proliferating microbial population (Anderson et al., 1987b), the resultant VFA and lactate (mainly) cause a decrease in ruminal pH (Bergman, 1990). Adult ruminants are often plagued by metabolic issues stemming from subacute rumen acidosis (pH < 5.6; or even acute rumen acidosis), but calves do not seem to be adversely affected by this low rumen pH as evidenced by a lack of health responses in calves (Li et al., 2012; van Niekerk et al., 2021). Interestingly, it appears that diets containing highly fermentable substrates often stimulate development of the rumen epithelium (Tamate et al., 1962; Stobo et al., 1966). However, a low rumen pH has been identified as a causative agent of ruminal parakeratosis/lesions (Suárez et al., 2006; Gelsinger et al., 2020), but no clear implications on the calf's overall health have been observed. Wood et al. (2015) demonstrated a negative effect of rumen pH, where a longer duration of time for rumen pH below 5.5 in the weaning and postweaning periods led to an observed decrease in barrier function (i.e. increase in permeability) in the rumen. Also, Gelsinger et al. (2020) noted that calves fed a diet that induced ruminal acidosis experienced negative outcomes (e.g. decreased intake and growth), but there were no signs of systemic acidosis or impaired general overall health. That being said, rumen pH should always be considered, even if negative effects are not apparent in young ruminants, and future research should investigate the relationship of mucosal immunity and rumen pH in calves to further understand the immune cost.

Rumen Development and VFA Absorption

So far, I have not acknowledged (one of) the main reasons we study rumen development, which is to assess how well this organ will be able to perform its main function of absorbing VFA. Measuring VFA absorption is not an easy task, but too often research stops at the molecular level before measuring actual function in vivo, which is the actual reason why we perform this research; for practical, real life application. While there is a plethora of data on rumen VFA absorption in adult cows, the topic has been scarcely touched in calves, but a recent study of mine did attempt to add to our collective knowledge about rumen VFA absorption in young calves.

Previous studies performed by Khouri (1969) and Sutton et al. (1963a) had opposing results in regard to VFA absorption between calves fed only milk or milk and solid feed

(starter and/or hay). Yohe et al. (2019b) supported the results of Khouri (1969) in that preweaning calves had similar absorption of VFA whether they consumed starter or not, which is showcased in Table 1 where VFA absorption rate and total VFA absorbed over the 6 hour experimental period were the same between treatments for acetate, propionate, and butyrate. This was an interesting finding considering we generally accept the concept of promoting rumen development via feeding solid feed (mainly starter) as correct, but if it is the case that a calf fed only MR can still absorb just as much VFA as a calf fed MR and starter, then how is that happening? We also looked at protein abundance of VFA transporters (MCT1 and MCT4) located in the rumen epithelium of these calves and found no difference (Figure 3), which led us to think of the possibility that passive diffusion was aiding in absorbing VFA that were not involved in any protein-mediated transport. It is known that a lower pH enhances passive diffusion of VFA (Penner et al., 2011). It should be noted that in our study, we utilized a washed rumen technique and provided a buffer that contained a fixed amount of VFA at a supraphysiological concentration to test the ability of the rumen to absorb acetate, propionate, and butyrate. The calves that were fed MR and starter still had a larger rumen overall with increased surface area (i.e., larger papillae) compared to calves only fed MR so there is a possibility that under normal circumstances, calves consuming MR and starter would have an increase in total VFA absorbed due to larger capacity for starter consumption compared to calves fed only MR that have smaller rumens and thus a smaller capacity. Although both treatments would have similar rumen epithelial function considering the importance of age rather than diet on ketogenic development (Lane et al., 2000; Lane et al., 2002; Yohe et al., 2019b). Another important aspect to consider is that age is believed to be a critical factor for development of ketogenesis in the rumen epithelium, which shows lack of dietary effect on rumen function as well. The experimental conditions used in Yohe et al. (2019b) were different from a typical scenario (i.e., starter being eaten and subsequently fermented in the rumen and supplying the rumen environment with VFA), but important nonetheless to recognize that even a rumen that appears undeveloped is still able to absorb VFA effectively.

Conclusions

With shifts in dietary trends, different feed ingredients becoming available, and the need to adjust on-farm management of calves, we can expect to see more data on rumen development in the coming years with the hope of more functional data (i.e., VFA absorption) being generated. Much of what was discussed in this proceedings article consisted of molecular biology and basic science, but some practical considerations can be taken away, such as utilizing a weaning protocol that allows both adequate growth preweaning while simultaneously allowing appropriate starter intake for rumen development (e.g., 2 step-down weaning protocol). Also, it is important to understand the delicate balance between feeding starter with the goal of promoting rumen development but acknowledging the potential negative effects on barrier function and epithelial lesions in the rumen. Lastly, an important takeaway is even an undeveloped rumen has the ability to absorb VFA, but the important consideration is how much starter has been consumed to allow for VFA creation and subsequent absorption to meet the energy needs of the calf.

References

Anderson, K.L., T.G. Nagaraja, J.L. Morrill, T.B. Avery, S.J. Galitzer, and J.E. Boyer. 1987a. Ruminal microbial development in conventionally or early-weaned calves. J. Anim. Sci. 64:1215-1226.

Anderson, K.L., T.G. Nagaraja, and J.L. Morrill. 1987b. Ruminal metabolic development in calves weaned conventionally or early. J. Dairy Sci. 70:1000-1005.

Baldwin, R.L. 1999. The proliferative actions of insulin, insulin-like growth factor-1, epidermal growth factor, butyrate and propionate on ruminal epithelial cells in vitro. Small Ruminant Res. 32:261-268.

Baldwin, R.L.,VI, K.R. McLeod, J.L. Klotz, and R.N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87(E.Suppl.):E55–E65.

Benetton, J.B., H.W. Neave, J.H.C. Costa, M. A.G. von Keyserlingk, and D.M. Weary. 2019. Automatic weaning based on individual solid feed intake: Effects on behavior and performance of dairy calves. J. Dairy Sci. 102:5475-5491.

Bergman, E.N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70:567-590.

Bolt, S.L., N.K. Boyland, D.T. Mlynski, R. James, and D.P. Croft. 2017. Pair housing of dairy calves and age at pairing: Effects on weaning stress, health, production and social networks. PLoS ONE 12:e0166926.

Bryant, M.P., N. Small, C. Bouma, and I. Robinson. 1958. Studies on the composition of the ruminal flora and fauna of young calves. J. Dairy Sci. 41:1747-1767.

Burakowska, K., G.B. Penner, J. Flaga, M. Przybyło, J. Barć, J. Wojciechowska-Puchałka, D. Wojtysiak, Z.M. Kowalski, and P. Górka. 2021. Canola meal or soybean meal as protein source and the effect of microencapsulated butyrate supplementation in calf starter mixture. II. Development of the gastrointestinal tract. J. Dairy Sci. 104 (In press).

Connor, E.E., R.L.T. Baldwin, M.P. Walker, S.E. Ellis, C. Li, S. Kahl, H. Chung, and R.W. Li. 2014. Transcriptional regulators transforming growth factor-beta1 and estrogen-related receptor-alpha identified as putative mediators of calf rumen epithelial tissue development and function during weaning. J. Dairy Sci. 97:4193-4207.

Conrad, H.R. and J.W. Hibbs. 1956. A high roughage system for raising calves based on the early development of rumen function.7. Utilization of grass silage, pasture, and pelleted alfalfa meal. J. Dairy Sci. 39:1170-1179.

Davenport, E. 1897. On the importance of the physiological requirements of the animal body; Results of an attempt to grow cattle without coarse feed. Pages 363-371 in Ill. Agr. Expt. Sta. Bull. Vol. 46. A. E. S. University of Illinois, ed.

Diez-Gonzalez, F., D.R. Bond, E. Jennings, and J.B. Russell. 1999. Alternative schemes of butyrate production in *Butyrivibrio fibrisolvens* and their relationship to acetate utilization, lactate production, and phylogeny. Arch. Microbiol. 171:324-330.

Dill-McFarland, K.A., J.D. Breaker, and G. Suen. 2017. Microbial succession in the gastrointestinal tract of dairy cows from 2 weeks to first lactation. Sci. Rep. 7:40864.

Do, D.N., P.L. Dudernaine, B.E. Fomenky, and E.M. Ibeagha-Awemu. 2019. Integration of mirna weighted gene co-expression network and mirna-mrna co-expression analyses reveals potential regulatory functions of mirnas in calf rumen development. Genomics 111:849-859.

Fonty, G., P. Gouet, J.P. Jouany, and J. Senaud. 1987. Establishment of the microflora and anaerobic fungi in the rumen of lambs. J. Gen. Microbiol. 133:1835-1843.

Gelsinger, S.L., W.K. Coblentz, G.I. Zanton, R.K. Ogden, and M.S. Akins. 2020. Physiological effects of starter-induced ruminal acidosis in calves before, during, and after weaning. J. Dairy Sci. 103:2762-2772.

Goodlad, R.A. 1981. Some effects of diet on the mitotic index and the cell cycle of the ruminal epithelium of sheep. Q. J. Exp. Physiol. 66:487-499.

Guo, W., J.K. van Niekerk, M. Zhou, M.A. Steele, and L.L. Guan. 2021. Longitudinal assessment revealed the shifts in rumen and colon mucosal-attached mirobiota of dairy calves during weaning transition. J. Dairy Sci. 104 (In press).

Heinrichs, A.J. 2005. Rumen development in the dairy calf. Adv. Dairy Technol. 17:179-187.

Khouri, R. H. 1969. Absorption of steam volatile fatty acids from reticulo-rumen of anaesthetised calves. N. Z. J. Agric. Res. 12:299-312.

Kim, Y.H., N. Toji, K. Kizaki, S. Kushibiki, T. Ichijo, and S. Sato. 2016. Effects of dietary forage and calf starter on ruminal pH and transcriptomic adaptation of the rumen epithelium in Holstein calves during the weaning transition. Physiol. Genomics 48:803-809.

Klopp, R.N., F.X. Suarez-Mena, T.S. Dennis, T.M. Hill, R.L. Schlotterbeck, and G.J. Lascano. 2019. Effects of feeding different amounts of milk replacer on growth performance and nutrient digestibility in Holstein calves to 2 months of age using different weaning strategies. J. Dairy Sci. 102:11040-11050.

Koch, C., C. Gerbert, D. Frieten, G. Dusel, K. Eder, R. Zitnan, and H.M. Hammon. 2019. Effects of ad libitum milk replacer feeding and butyrate supplementation on the epithelial growth and development of the gastrointestinal tract in Holstein calves. J. Dairy Sci. 102:8513-8526.

Laarman, A.H., A.L. Ruiz-Sanchez, T. Sugino, L.L. Guan, and M. Oba. 2012. Effects of feeding a calf starter on molecular adaptations in the ruminal epithelium and liver of Holstein dairy calves. J. Dairy Sci. 95:2585-2594.

Lane, M.A., R.L. Baldwin IV, and B.W. Jesse. 2000. Sheep rumen metabolic development in response to age and dietary treatments. J. Anim. Sci. 78:1990-1996.

Lane, M.A., R.L. Baldwin IV, and B.W. Jesse. 2002. Developmental changes in ketogenic enzyme gene expression during sheep rumen development. J. Anim. Sci. 80:1538-1544.

Li, R. W., E.E. Connor, C. Li, V.I. Baldwin, L. Ransom and M.E. Sparks. 2012. Characterization of the rumen microbiota of pre-ruminant calves using metagenomic tools. Environ. Microbiol. 14:129-139.

Li, W.L., S. Gelsinger, A. Edwards, C. Riehle, and D. Koch. 2019. Changes in meta-transcriptome of rumen epimural microbial community and liver transcriptome in young calves with feed induced acidosis. Sci. Rep. 9.

Liu, Y.R., C. Wang, Q. Liu, G. Guo, W.J. Huo, Y.L. Zhang, C.X. Pei, and S.L. Zhang. 2020. Effects of branched-chain volatile fatty acids and fibrolytic enzyme on rumen development in pre- and post-weaned Holstein dairy calves. Anim. Biotechnol. 31:512-519.

Malmuthuge, N., G.X. Liang, and L.L. Guan. 2019. Regulation of rumen development in neonatal ruminants through microbial metagenomes and host transcriptomes. Genome Biol. 20:172.

Meale, S.J., S. Li, P. Azevedo, H. Derakhshani, J.C. Plaizier, E. Khafipour, and M.A. Steele. 2016. Development of ruminal and fecal microbiomes are affected by weaning but not weaning strategy in dairy calves. Front. Microbiol. 7:582.

Novak, T.E., S.L. Rodriguez-Zas, B.R. Southey, J.D. Starkey, R.M. Stockler, G.F. Alfaro, and S.J. Mois. 2019. Jersey steer ruminal papillae histology and nutrigenomics with diet changes. J. Anim. Physiol. 103:1694-1707.

Overvest, M.A., R.E. Crossley, E.K. Miller-Cushon, and T.J. DeVries. 2018. Social housing influences the behavior and feed intake of dairy calves during weaning. J. Dairy Sci. 101:8123-8134.

Penner, G.B., M.A. Steele, J.R. Aschenbach, and B.W. McBride. 2011. Ruminant nutrition symposium: Molecular adaptation of ruminal epithelia to highly fermentable diets. J. Anim. Sci. 89:1108-1119.

Quigley, J.D., T.M. Hill, T.S. Dennis, and R.L. Schlotterbeck. 2018. Effects of feeding milk replacer at 2 rates with pelleted, low-starch or texturized, high-starch starters on calf performance and digestion. J. Dairy Sci. 101:5937–5948.

Sakata, T., K. Hikosaka, Y. Shiomura, and H. Tamate. 1980. Stimulatory effect of insulin on ruminal epithelium cell mitosis in adult sheep. Br. J. Nutr. 44:325-331.

Schäff, C.T., J. Gruse, J. Maciej, R. Pfuhl, R. Zitnan, M. Rajsky, and H.M. Hammon. 2018. Effects of feeding unlimited amounts of milk replacer for the first 5 weeks of age on rumen and small intestinal growth and development in dairy calves. J. Dairy Sci. 101:783-793.

Silva, A.L., T.J. DeVries, L.O. Tedeschi, and M.I. Marcondes. 2019. Development of equations, based on milk intake, to predict starter feed intake of preweaned dairy calves. Animal 13:83-89.

Steele, M.A., J.H. Doelman, L.N. Leal, F. Soberon, M. Carson, and J.A. Metcalf. 2017. Abrupt weaning reduces postweaning growth and is associated with alterations in gastrointestinal markers of development in dairy calves fed an elevated plane of nutrition during the preweaning period. J. Dairy Sci. 100:5390-5399.

Stobo, I.J., J.H. Roy and H.J. Gaston. 1966. Rumen development in the calf. 1. The effect of diets containing different proportions of concentrates to hay on rumen development. Br. J. Nutr. 20:171-188.

Suárez, B.J., C.G. Van Reenen, W.J.J. Gerrits, N. Stockhofe, A.M. van Vuuren, and J. Dijkstra. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. J. Dairy Sci. 89:4376–4386.

Sutton, J.D., A.D. Mcgilliard, and N.L. Jacobson. 1963a. Functional development of rumen mucosa. 1. Absorptive ability. J. Dairy Sci. 46:426-436.

Sutton, J.D., M. Richard, A.D. Mcgilliard, and N.L. Jacobson. 1963b. Functional development of rumen mucosa. 2. Metabolic activity. J. Dairy Sci. 46:530-537.

Tamate, H., R. Getty, A.D. Mcgilliard, and N.L. Jacobson. 1962. Effect of various dietaries on anatomical development of stomach in calf. J. Dairy Sci. 45:408-420.

van Niekerk, J.K., M. Middeldorp, L.L. Guan, and M.A. Steele. 2021. Preweaning to postweaning rumen papillae structural growth, ruminal fermentation characteristics, and acute-phase proteins in calves. J. Dairy Sci. 104:3632-3645.

Warner, R.G., W.P. Flatt, and J.K. Loosli. 1956. Dietary factors influencing the development of the ruminant stomach. J. Agr. Food Chem. 4:788-792.

Wood, K.M., S.I. Palmer, M.A. Steele, J.A. Metcalf, and G.B. Penner. 2015. The influence of age and weaning on permeability of the gastrointestinal tract in Holstein bull calves. J. Dairy Sci. 98:7226–7237.

Xiao, J.X., L.Y. Guo, G.M. Alugongo, Y.J. Wang, Z.J. Cao, and S.L. Li. 2018. Effects of different feed type exposure in early life on performance, rumen fermentation, and feed preference of dairy calves. J. Dairy Sci. 101:8169-8181.

Xue, M.M., K.J. Wang, A.S. Wang, R.T. Li, Y. D. Wang, S.J. Sun, D. Yan, G.H. Song, H.F. Xu, G.R. Sun, and M. Li. 2019. Microrna sequencing reveals the effect of different levels of non-fibrous carbohydrate/neutral detergent fiber on rumen development in calves. Animals-Basel. 9.

Yang, B., J.Q. Le, P. Wu, J.X. Liu, L. Guan, and J.K. Wang. 2018. Alfalfa intervention alters rumen microbial community development in Hu lambs during early life. Front. Microbiol. 9:574.

Yohe, T.T., H.L. Tucker, C.L. Parsons, A.J. Geiger, R.M. Akers, and K.M. Daniels. 2016. Short communication: Initial evidence supporting existence of potential rumen epidermal stem and progenitor cells. J. Dairy Sci. 99:7654-7660.

Yohe, T.T., H. Schramm, C.L.M. Parsons, H.L. M. Tucker, B.D. Enger, N.R. Hardy, and K.M. Daniels. 2019a. Form of calf diet and the rumen. I: Impact on growth and development. J. Dairy Sci. 102:8486-8501.

Yohe, T.T., H. Schramm, R.R. White, M.D. Hanigan, C.L.M. Parsons, H.L.M. Tucker, B.D. Enger, N.R. Hardy, and K.M. Daniels. 2019b. Form of calf diet and the rumen. II: Impact on volatile fatty acid absorption. J. Dairy Sci. 102:8502-8512.

Table 1. Fluid exit, VFA absorption rate, and total 6 hour absorption of VFA after being exposed to a defined concentration of VFA in calves fed 2 differing forms of diet for 6 weeks of age.

Item	Treatment[1]		SEM	Test of Fixed Effects, *P*-value
	MRO	MRS		Treatment
Fluid exit (%/hour)	15.7	10.7	2.9	0.14
VFA absorption (%/hour)				
Acetate	20.9	35.3	7.7	0.20
Propionate	27.4	33.7	7.9	0.57
Butyrate	41.1	42.0	10.5	0.95
Total 6 hour VFA flux (mmol)				
Acetate	176.7	165.7	22.8	0.73
Propionate	74.8	70.5	10.3	0.76
Butyrate	78.8	75.9	11.8	0.83

[1]Isocaloric and isonitrogenous dietary treatments: MRO = MR only, MRS = MR with starter.
Adapted from Yohe et al. (2019b).

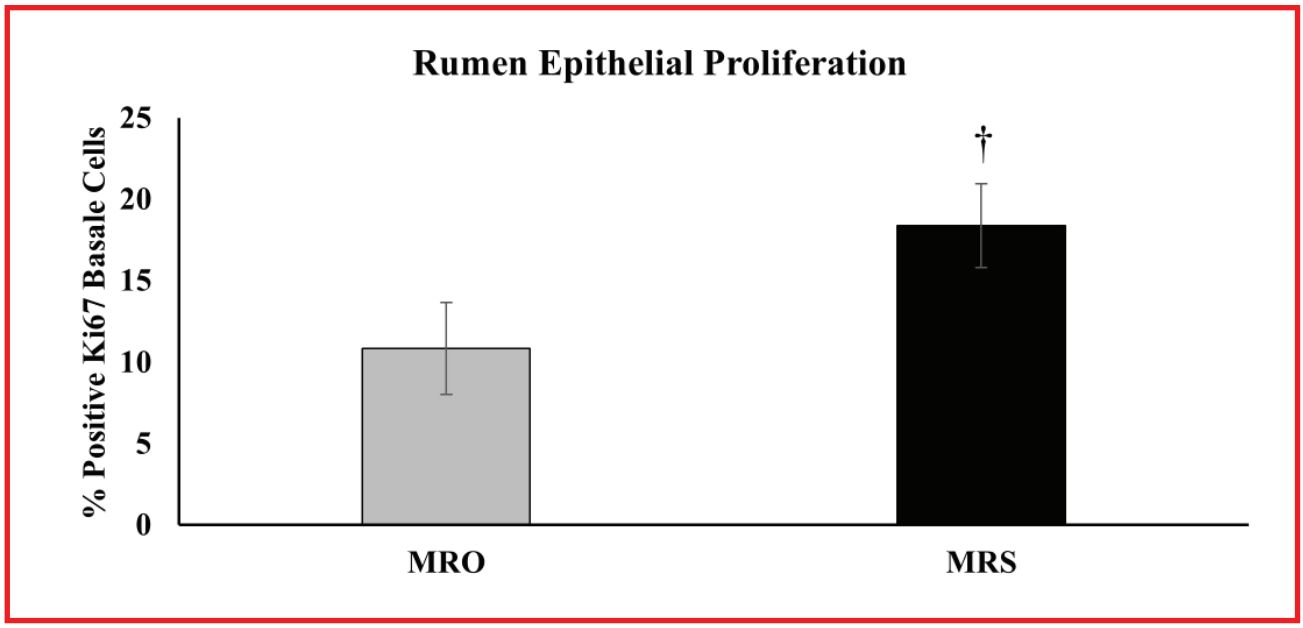

Figure 1. Immunohistochemistry measurements of Ki67 positive basale cells in the rumen epithelium of calves fed 2 dietary treatments (MRO: milk replacer only; MRS: milk replacer and starter) during the 6 week trial. There was a tendency for an increase in the proportion of Ki67-labeled basale cells (i.e. actively proliferating cells) in MRS compared with MRO calves ($P = 0.08$). †Tendency between treatments, $0.10 \geq P \leq 0.05$. Adapted from Yohe et al. (2019a).

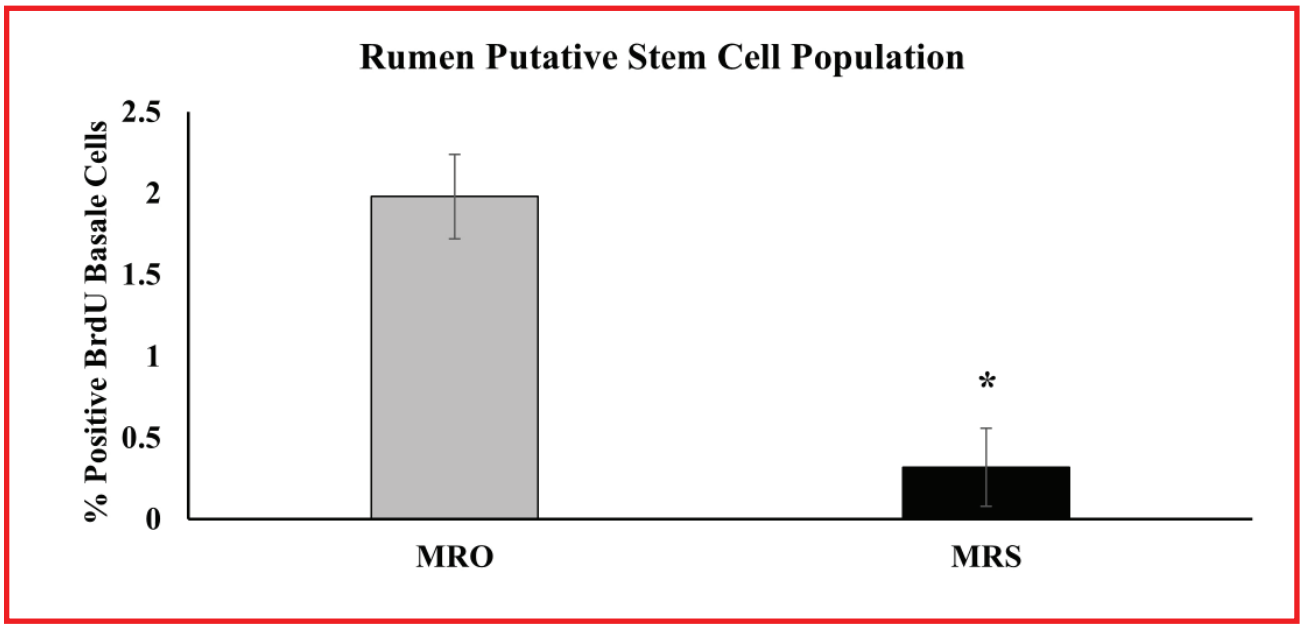

Figure 2. Immunohistochemistry measurements of BrdU positive basale cells in the rumen epithelium of calves fed 2 dietary treatments (MRO: milk replacer only; MRS: milk replacer and starter) during the 6 week trial. There was an increase in proportion of BrdU-labeled basale cells in MRO compared with MRS calves ($P = 0.001$). *Significant difference between treatments, $P \leq 0.05$. Adapted from Yohe et al. (2019a).

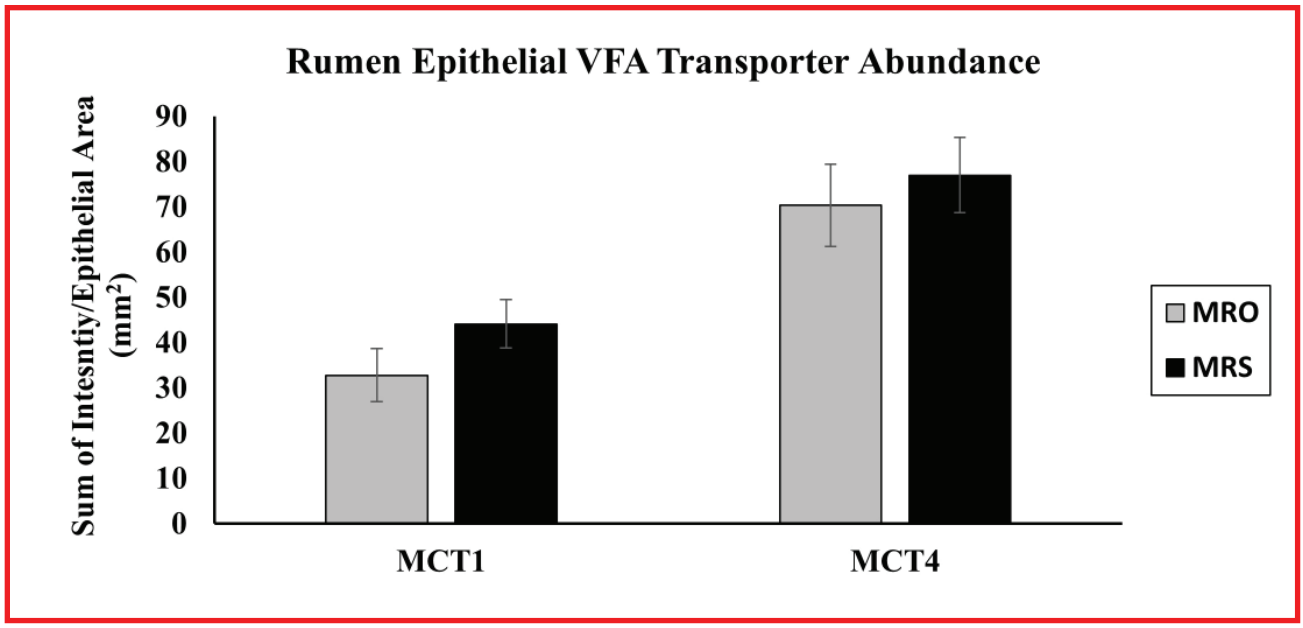

Figure 3. Immunohistochemistry measurements of intensity/epithelial area for VFA transporter proteins MCT1 (monocarboxylate transporter 1) and MCT4 (monocarboxylate transporter 4) protein in the rumen of MRO calves (milk replacer only) and MRS calves (milk replacer and starter) during the 6 week trial. There were no differences present for either MCT1 ($P = 0.19$) or MCT4 ($P = 0.60$) abundance. Adapted from Yohe et al. (2019b).