

Influence of Microbial Ecology in the Rumen and Lower Gut on Production Efficiency of Dairy Cows

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Introduction

From a classical nutrition perspective, the ruminal microbes have been recognized as a critical component for the success of feeding programs for dairy cows. Generally, the relationship between dairy cattle and the microbial community inhabiting their gastrointestinal tract (**GIT**) has been referred to as a symbiotic relationship where both the cow and microbial community provide benefits for each other. The cow provides a regular supply of fermentable material, maintains an anaerobic environment, regulates osmolality and ruminal pH, and removes end products of fermentation that could be inhibitory to microbes, such as volatile fatty acids (**VFA**). In return, the microbes digest feed that would otherwise be indigestible, provide a source of energy as VFA, provide a source of vitamins, convert non-protein nitrogen into protein, and are the primary source of metabolizable protein. Despite the known importance of the microbiome, modeling their activity and outcomes on production remains a challenge.

Perhaps part of the challenge with modeling the activity of the microbial community is related to its complexity. It is currently estimated that over 5000 species inhabit the GIT (Henderson et al., 2015). In addition, the relationship between the cow and microbes is much more complex than described above.

In fact, recent research has demonstrated that the host animal and its microbial inhabitants communicate with each other (Thomas and Versalovic, 2010) and that specific microbes might be critical for the development of the host immune system (Chung et al., 2012). A challenge in the field of dairy nutrition and physiology is that past research has largely focused on the ruminal microbiome, thereby ignoring more distal regions. Obviously, this presents a challenge when trying to extrapolate results from monogastric species to applicable approaches for dairy cattle. Reasons to focus on the ruminal microbiome are numerous and include, among other things, the importance of the ruminal microbial community for fermentation of feed, large capacity and high diversity of the ruminal microbial community, and ease of access for sampling. However, as demonstrated for monogastric species, the microbial community structure of the intestinal tract may have critical roles in the health and productivity of cattle.

An Overview of Microbial Colonization of the GIT

While a detailed description of the microbial community structure across the GIT of cattle is out of scope for this paper, it is important to understand some of the characteristic changes that occur among regions and how the microbial communities are established. Firstly, our

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knowledge of the microbial community structure has increased dramatically with the advancement of culture-independent methods. Culture-independent methods allow researchers to use highly conserved regions of the bacterial genetic information (DNA and RNA) to evaluate what species are present, and with in-depth techniques, researchers can also get an understanding of the activity that those species may have. To identify species, researchers cluster the genetic sequence and assume that sequences with more than 97% similarity represent the same species. Rather than stating the number of species (as errors can occur), the term 'operational taxonomic unit' (OTU) is used (Khafipour et al., 2016). In a recent study (Henderson et al., 2015) comparing the ruminal microbial inhabitants from a broad group of ruminant and camelids with samples coming world-wide, they identified that *Prevotella*, *Butyrivibrio*, *Ruminococcus*, *Lachnospiraceae*, *Ruminococcaceae*, *Bacteroidales*, and *Clostridiales* were the dominant genus. Interestingly, only 35% of the OTU were named species or species awaiting a name, and most of these groups have not been cultured. The low proportion of identified species, let alone a lack of characterization of their activity, highlights the need for more research in this area.

Establishment of the GIT microflora begins with the onset of calving. While there is no clear consensus on the dominant species inhabiting the GIT of calves, it is evident that age-dependent changes occur along with differences in the dominant genera among regions of the GIT (Malmuthuge et al., 2014, 2015). Differences in dominant species among studies may be due to the environment, milk and solid feed composition, and genetics. In general, the abundant genera in the rumen were *Streptococcus*, *Bacteroidetes*, and *Prevotella* within 12 h of birth, with the abundance of *Firmicutes* and *Bacteroidetes* increasing while

Proteobacteria decreases with advancing age. Interestingly, in the small intestine, *Lactobacillus*, *Bifidobacterium*, *E. coli*, and *Streptococcus* were dominant, while the large intestine followed patterns reported for the rumen. The similarity for the major inhabitants of the forestomach and hind-gut regions of the GIT are perhaps not surprising given that microbes in both regions have extensive fermentation activity. Moreover, as this research was conducted initially with suckling calves, differences in the microbial community structure due to changes in nutrient supply are likely causative. Regardless of the individual species, all studies support the notion that the ruminal community adapts in a sequential approach to become more similar to a mature ruminant, but that the exact composition may affect on many factors (Li et al., 2012; Rey et al., 2014; Malmuthuge et al., 2015). In addition to the changes in composition, there is a reduction in the microbial density from the reticulo-rumen to the small intestine, with density increasing again in the large intestine (Mao et al., 2015). The change in the microbial community towards that found in mature ruminants questions whether accelerating this microbial colonization pattern would have beneficial effects on the host and provide the means necessary to ensure colonization with a stable and diverse microbial community structure is achieved.

In addition to age-related responses, dietary change induces alterations of the microbial community structure. Mohammed et al. (2012) evaluated changes in the microbial community structure as primiparous heifers transitioned from the far-off dry period into the close-up dry period, and finally into lactation. They noted that there was marked variability for the changes in the microbial community among cows where the microbial community structure was resistant for some cows and variable for others. They found that variability

in the microbial community structure was not associated with ruminal acidosis post-partum. The variability in the resilience of the microbial community to tolerate dietary changes is puzzling and poses a challenge when designing strategies to manipulate the microbial community structure.

Although not dairy focused, Petri et al. (2013a) evaluated how the microbial community structure as beef heifers were transitioned to a high-grain finishing diet. That study demonstrated a reduction in *Butyrivibrio* and an increase in *Prevotella*. Interestingly, there was a core group of microbes that were present, including the Bacteroidetes, Firmicutes, and Proteobacteria (Petri et al., 2013b). This core microbiome fits with that found in a much larger study by Henderson et al. (2015). The identification of a core microbiome common within an animal even with diets that differ in the forage-to-concentrate ratio (initial was 95% forage, final was 9% forage) suggests that part of the microbial community may be essential or perhaps are robust enough to adapt to differing dietary scenarios and can resist change.

Stability of the Microbiome: A Case for Host-Microbe Regulation

There is no doubt that diet can influence the microbial community structure within the gastrointestinal tract (Petri et al., 2013ab; Mohammed et al., 2012; Khafipour et al., 2016). However, there are a number of studies suggesting that the microbial community, at least the core community, is relatively stable within individual cattle. Initial evidence for a stable microbial community has been provided by Weimer et al. (2010). In that study, dairy cows fed the same diet were selected based on differences in the microbial community structure using automated ribosomal intergenic spacer analysis (**ARISA**). The ARISA allows

for a general identification of the microbial community structure. To test whether the microbial community structure was specific for each cow, ruminal digesta were manually evacuated from each cow and the digesta were swapped (i.e. digesta from 1 cow was placed in the rumen of another and vice versa). The microbial community structure was then evaluated over time to determine if change in the composition occurred within a 65-day period. The results of this study were interesting. Firstly, cows with differing microbial community structures also had differing ruminal conditions (pH and VFA concentration), even when fed the same diet. Following the introduction of ruminal digesta from the other cow, the microbial community and ruminal conditions were similar to the donor. However, after a period of 65-days, the microbial community profile and ruminal fermentation conditions again resembled that which occurred prior to the ruminal digesta swap. Other studies have also suggested that the microbial community structure is more similar for an individual cow measured over time and when fed diets that differed than between cows when fed the same diet (Li et al., 2010; Petri et al., 2013a,b). Collectively, these studies suggest that the rumen microbial community structure appears to be specific for each cow, although modest changes relative to the original community structure can occur when major perturbations are imposed. The data also provided some initial evidence in dairy cattle that there may be some form of communication between the cow and their resident microbes.

The existence of communication mechanisms between the host and the microbes is not a new concept; however, its application for production animals is novel. Mechanisms for communication are not fully elucidated but include luminal nutrient sensing and the direct impacts that byproducts of microbial fermentation have on the host. A good example of

this are free fatty acid receptors that detect VFA. Moreover, VFA (particularly butyrate) promotes proliferation of the ruminal epithelium and other GIT tissues, with changes in hormones likely mediating the response (Penner et al., 2011). In addition to luminal nutrient sensing, the GIT has receptors that can detect bacteria or fragments of bacteria, protozoa, and fungi (Ishii et al., 2008). When these receptors are stimulated, an immune response can be initiated. The intestinal regions of the GIT also secrete antimicrobial proteins and mucus that help to control the microbial community structure. It is not clear whether antimicrobial proteins are released from the rumen, although a recent report has suggested that there may be the release of immune-related compounds, such as tumor-necrosis factor alpha, interferon gamma, and leukocytes (Trevisi et al., 2014). Sensing and control of the microbial community is essential to limit the transfer of pathogenic organisms across the GIT, create tolerance for commensal microflora thereby reducing the risk for chronic inflammation, and helping to activate an immune response when needed. In addition, it is likely that a combination of luminal sensing and the secretion of antimicrobial proteins helps to explain why cows have differences and resilience to change for the microbial community structure.

Modifying the Microbial Community: Applicability to Dairy Production and Efficiency

Is there a need to manipulate the microbial community structure? The necessity or motivation to manipulate the microbial community structure assumes that there may be an ideal microbial community or at least part of a community that would be beneficial for the health or production efficiency. To date, there is no work that conclusively proves there is a beneficial microbial community structure; however, there are associations

between desirable production parameters and the microbial community structure. Jami et al. (2014) evaluated the microbial community profile in 15 primiparous heifers at the same physiological state. As with other studies, they demonstrated that although cows were fed similar diets, there was substantial variability in the microbial community structure among heifers. Then they compared indicators of the microbial community structure with production characteristics. The first variable they examined was the Firmicutes:Bacteroidetes ratio, and they reported a positive association with milk fat yield ($R^2 = 0.51$). Abundance of the phyla Actinobacteria was positively related to milk, fat, and lactose yields, and Bacteroidetes was negatively associated with residual feed intake and milk fat yield. While they were able to detect positive and negative associations with some genera, many of the correlations were weak. Regardless, this is the first study to report that the microbial community profile may be associated with production outcomes in dairy cattle. Research is needed to verify whether similar relationships can be detected in a broader population and whether attempts to manipulate the ruminal microbial community structure can improve production outcomes.

Altering early microbial community establishment

As mentioned above, once established it appears that, at least part, of the microbiome is stable (Weimer et al., 2010; Petri et al., 2013a,b). This presents a challenge when it may be desirable to manipulate the microbial community structure. Given that the microbial community structure in calves is being established, early postnatal exposure may be a practical time point when the microbial community of the GIT could be modified. It may be important to consider inoculation strategies that promote diversity of the microbial community as

diversity is considered an essential component and that diversity increases with advancing age (Oikonomou et al., 2013; Jami et al., 2014; Malmuthuge et al., 2015). Calves with less microbial diversity assessed in fecal samples were also associated with greater incidence rates for diarrhea and pneumonia (Oikonomou et al., 2013). These findings are possible as establishment of the GIT microflora also stimulates development of the immune system (Ishii et al., 2008) and may help calves face immune challenges beyond that localized to the GIT.

Development of strategies to manipulate the microbial community structure should include a range of microbes considered to be beneficial in mature dairy cattle, especially when this approach is used to promote GIT development, an issue of importance for calves. Species specificity may be a critical factor in the selection of microbes to promote or support GIT function. One study conducted using sterile mice evaluated the effect of inoculating the mice with microbes from mice, microbes from humans, or microbes from rats (Chung et al., 2012). They found that the effect of donor source (mice vs. human vs. rat) had a major effect on development of the gastrointestinal tract in terms of immune system development and establishment of the microflora. Only mice inoculated with microbes from mice had an immune system that was considered to develop normally, while those inoculated with microbes from human or rat microflora had immature GIT development. While species dependency has not been confirmed in cattle, these data may suggest that strategies to promote GIT development by improving the microbial community structure should consider a diverse microbial profile and incorporate microbial species that are components of the core microbiome. Unfortunately, this area has received very little research attention to date.

Finally, there is a consensus that diversity of the microbial community structure is an important feature of a healthy microbial community (Heiman and Greenway, 2016). Antimicrobial treatment, to treat infection, has been shown to modify the microbial community structure, providing a situation post-treatment which may further challenge the host (Oh et al., 2016). This may be a particular challenge with in-feed antimicrobial use but still may be relevant with injectable administration routes (Zhang et al., 2013). While providing in-feed antimicrobials is not an approved practice for lactating dairy cattle, there are times when calves could consume antibiotics if they are consuming waste milk. Antibiotic feeding reduces the microbial diversity (Oh et al., 2016) in the GIT and may result in the establishment of an undesirable microbial community. Under such a scenario, it may be beneficial to develop strategies to facilitate establishment of a stable microbial community structure.

Conclusions

The microbial community helps to support feed digestion and provides essential nutrients for dairy cattle. The role of the microbial community structure needs to be expanded to consider its role to support GIT development and immune system tolerance and development. It is clear that individual cows have a distinct microbial community and that similarities in the microbial community can be detected within cows across diets and among cows. These key groups are considered to be the core microbiome, and there appears to be an association between the abundance of key genera and important production outcomes, such as the yields of milk and fat. While it may be beneficial to manipulate the microbial community composition, communication between the microbes and host may provide a resistance to such manipulation. However,

the post-natal period for calves or following antimicrobial therapy may be two key time points for when the composition of the microflora could be manipulated. Manipulations should include a broad spectrum of microbial genera specific to the production setting. Future research is needed to confirm whether strategies to manipulate the microbial community structure result in positive benefits to the health, development, and productivity of dairy cattle.

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