

Can Understanding the Gastrointestinal Microbiome REALLY Change the Way We Raise Dairy Cows?

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ABSTRACT

Over the past ten years there has been an increasing amount of information derived from Next Generation Sequencing, to the point that talk of the “microbiome” is now everywhere. We have unearthed vast amounts of information about the composition of the gut microbial population in cattle, and new methods have shed new light on how individual microbes impact cow health and performance. The rumen of cattle is incredibly complex and full of interactions within the microbial ecosystem and between the microbes and their host. These webs of interactions are difficult to quantify, so they do limit the effectiveness of microbiome analyses to a degree. The lack of quantitative relationship measuring of these relationships is similar to attempting to use an elementary school class photograph to predict outcomes for the individual members of a class and their impact on society as a whole. We must develop our understanding of the relationships between microbes and the host more deeply to understand the ecological niche.

INTRODUCTION

Dairy cattle live in a symbiotic relationship with a complex microbial population in their rumen and lower gastrointestinal tract that allows cattle thrive on cellulose containing diets that monogastric animals cannot utilize (Hungate, 1966, Russell, 2002). The microbial population is a great resource of enzyme activity that can degrade fiber, starch and sugars, and is comprised of bacteria, fungi, and protozoa (Russell, 2002). Typically bacteria that are found at a population greater than 10^{9-10} CFU/mL, are thought of as the most important microorganisms in the highly dense microbial community of the rumen, however the degradative activity of the individual microorganisms plays an often overlooked and undervalued role in ruminant nutrition. Although the rumen microbial ecosystem has been well studied generally, there is a large gap in our understanding of how the microbes interact with substrates, fermentation pathways utilized, rates of substrate fermentation, as

well as host factors. Thus, it is apparent that our knowledge and intellectual models of how the rumen microbial population functions is a vast oversimplification, and theoretical concepts may be based on the actions of a single organism. But this clearly ignores the subtle interplay of the complex biological and biochemical actions and interactions within the gastrointestinal tract. In this discussion we will describe the important link between the gut microbiome and host and summarize the need for a complex “unified field theory model” that can be used to understand how to better feed our cattle through better manipulating the ruminal microbial ecosystem.

MICROBIAL INEFFICIENCIES OF ENERGY AND PROTEIN CAPTURE

The environment of the rumen and gastrointestinal tract is anaerobic (lacking oxygen), which means that the resident gastrointestinal microbial population must generate energy (ATP) using fermentation (Russell and Hespell, 1981, Russell, 2002, 2007). Volatile fatty acids (e.g., acetate, propionate, and butyrate) are short-chain fatty acids (2 to 6 C in length) and are the most important end product of ruminal fermentation, which are absorbed from the rumen into the blood stream (Hungate, 1966). In addition to VFA, H_2 , CO_2 , and CH_4 are all produced by the ruminal population, however these endproducts are not utilized by the host (van Houtert, 1993). Methane is released from the animal through eructation and can represent a loss of 5 to 7% of the digestible feed energy from dairy cows (Johnson and Johnson, 1995, Arndt et al., 2015). Greenhouse gases such as methane are a significant environmental threat that negatively impact the productivity of the host, and ruminal methane production is largely dependent on feed intake and dietary composition. If we can find a way to reduce this dietary energy loss then we can increase the animals’ energetic efficiency, which will ultimately increase food availability, while simultaneously lowering the environmental footprint of cattle production (Bradford, 1999, Thornton, 2010). However, to do this, we must understand the interactions of the microbial ecosystem that lead to methanogenesis.



Ultimately, the protein and amino acids required by cattle are provided by microbial cells (also known as microbial crude protein [MCP]), but this important process is inefficient due to the low energy (ATP) return associated with the fermentation process. Synthesis of MCP by ruminal microorganisms is further reduced by the need of the microbes to direct some of the limiting ATP toward maintenance functions, as well as production of reserve carbohydrate synthesis as well as energy spilling (wasteful) reactions that occur when nutrients are present at sub-optimal ratios (Russell, 2007, Hackmann and Firkins, 2015). Rumen protozoa also impact the efficiency of ruminal MCP synthesis because protozoa act as predators that feed upon bacteria, ingesting MCP and recycling it as their own microbial protein (Hino and Russell, 1987, Broudiscou et al., 1997). As much as 50% of dietary protein is degraded to non-protein nitrogen in the rumen, and is subsequently recycled as urea (Hino and Russell, 1987, Wells and Russell, 1996, Reynolds and Kristensen, 2008). The constant recycling of MCP is a further inefficiency characteristic of microbial growth in the rumen (Reynolds and Kristensen, 2008).

It has become increasingly clear that the microbial population of the gastrointestinal tract impact host physiology and body composition, most clearly impacting host obesity (Turnbaugh et al., 2006, Ley et al., 2008, Shabat et al., 2016, Costea et al., 2018). Impacts of the gastrointestinal microbiome demonstrated in monogastrics also include effects on mental health, hormone levels, as well as immune status (Savelkoul and Tijhaar, 2007, Lyte, 2013, Ha et al., 2014, Penha Filho et al., 2015, Logan et al., 2016).

Interactions between the host and the resident gastrointestinal microbiome are bidirectional and demonstrate the powerful impact of environmental and social stressors (Callaway et al., 2006, Dowd et al., 2007, Bailey et al., 2010, Freestone and Lyte, 2010). As methodologies improve, it is apparent that more organisms will be detected, and their activities and ecological niches will be assigned (Liang et al., 2015, Neves et al., 2017, Henderson et al., 2019).

SO WE KNOW MORE ABOUT THE MICROBIOME... WHAT DOES THAT MEAN FOR FEEDING MY COWS?

Examinations of the microbiome of the gastrointestinal tract have primarily been similar to a “class photo” of the microbial population at any given time and dietary conditions (Dowd et al., 2008, Callaway et al., 2009, Williams et al., 2010, Schären et al., 2018, Seshadri et al.,

2018, Lourenco et al., 2019, Wallace et al., 2019). We can think of the biological activity of the microbes as the “behavior” of the members of the “photo” taken by Next Generation Sequencing, thus microbial activity (e.g., fermentation endproducts, fermentation pathways, feeds degraded, and rates of fermentation) is critical to our understanding of what to expect from the microbiome. The “behavior,” “career choice,” and “work ethic” of these grammar school classmates can be translated loosely to the microbial/biochemical world in the form of specific activity of substrate utilization (e.g., starch utilization, protein deamination) and/or endproduct formation. It is impossible to predict lifetime impacts on society from faces in a class photo, it is equally improbable to be able to draw equivalent predictions about microbial populations based solely upon their presence/population numbers in the gastrointestinal tract. While currently the only conclusion that can be drawn from a class photo about any individual is “they were there,” microbiome analyses have a similar lack of correlative/predictive ability when applied to the complex populations of the gastrointestinal tract. Caution must be applied to interpreting microbiome data at the present time, but we are noting trends and correlations coupled with microbial activity and endproduct formation (Shabat et al., 2016, Sasson et al., 2017, Lyons et al., 2018, Muñoz-Vargas et al., 2018, Stewart et al., 2019, Wang et al., 2019).

Changes in the diet, microbial population, and host physiology all alter ruminal function and microbiome composition, and these effectors have been evaluated in a compartmentalized fashion, rather than as part of an intrinsically linked, multi-layer ecosystem. In order to fulfill the promise of Next Generation Sequencing, we must increase our holistic understanding of the biochemical functions of the ruminal microbes as well as the populations of ruminal microbes. The specific metabolic and degradative activities of the ruminal microbes for dietary substrates under a variety of conditions (e.g., starch degradation in the presence of a forage-based ration) is needed. While the biochemical pathways and metabolic activities are documented for many organisms, we lack knowledge about how each pathway directly, and indirectly affects the endproducts of the fermentation and how this affects host animal energetic status and physiology.

Before we can truly harness the potential of the gastrointestinal microbiome, we must develop a unified microbiome field theory for feeding cows that is based upon correlations between diet, the microbiome com-



position, host genetics, environmental conditions, the host immune system, and animal production needs and demands (e.g., high production levels). Inclusion of all these factors will elucidate our understanding of the bidirectional interactions between the microbiome, fermentation endproducts (e.g., production and utilization of VFA, ammonia, amino acids, and other substrates), host animal physiology, production parameters, immune status, animal health, and food safety.

Fermentation clearly has a direct impact on the animal's physiological status, including fetal growth, lactation, and milk composition (Jami et al., 2014, Li et al., 2017, Weimer et al., 2017). First lactation Holstein cows at the same stage of lactation contained three dominant ruminal bacterial phyla: *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Jami et al., 2014). Although 151 genera were detected, only the 42 most abundant genera were examined, a decision that is rational but also highlights the concept that population numbers do not tell the entire story of impact of a microbial population. The degradative specific activity (enzyme activity/min/mg of bacterial protein) of bacteria species differs in substrate utilized, rate, extent, and pathway utilized. The ruminal environmental niche of protein degradation best illustrates this. Ruminal protein degradation was thought to be primarily performed by *Bacteroides rumenicola* (now *Prevotella rumenicola*, *bryantii*, and *albensis*), however the specific activity of *B. rumenicola* was lower than that of whole ruminal fluid (Russell, 1983, Russell et al., 1983, 1991, Yang and Russell, 1992, 1993). This disconnect demonstrated that there were other unknown protein degrading bacteria in the rumen, and researchers found bacteria that obligately fermented amino acids (did not ferment sugars) at a very rapid rate (>10 times the rate of degradation of other isolated ruminal bacteria) (Russell et al., 1983, Russell and Strobel, 1988, Chen and Russell, 1989). These obligate amino acid fermenting bacteria (e.g., *Clostridium aminophilum*, *C. sticklandii*, and *Peptostreptococcus anaerobius*) were present at 10^6 cells/mL in the ruminal population, yet were responsible for more than 25% of the ruminal protein degradation.

Nutrient crossfeeding is one of the most important interactions that occurs at all levels of networking in the rumen (e.g., removal of endproduct inhibition, B vitamin production), and because some of these nutrients are needed at very low concentrations the abundance of bacteria will not necessarily be representative of their importance to a healthy rumen ecosystem (Schultz and Breznak, 1979, Bradford et al., 2016). Therefore,

suggesting that the most numerous species are more important to the host physiology than other members of the ruminal bacterial population, is akin to using that 3rd grade class photo to draw conclusions about the career arcs of members of that snapshot.

The composition of the microbial ecosystem in the gut of dairy cows changes throughout the gestation and lactation periods, but the changes in populations and microbial activities remains unclear (Pitta et al., 2016, Lyons et al., 2018, Muñoz-Vargas et al., 2018). Next Generation Sequencing has demonstrated that the composition of the rumen microbiome changed by age, as well as lactation stage (Henderson et al., 2015, Pitta et al., 2016). Results have demonstrated that as dairy cows are changed from a non-lactation diet to a lactation diet, their microbial composition changed, likely in response to the dietary change as well as the demands of early lactation (Pitta et al., 2016). When cows were changed to a dry cow diet, *Proteobacteria* populations increased, while *Firmicutes* decreased. Furthermore, as cows aged, the ratio of *Bacteroidetes:Firmicutes* and *Bacteroidetes:Proteobacteria* decreased, which was negatively correlated to milk fat yield (Pitta et al., 2016). Previously, significant shifts in the microbial populations of different aged Holstein cows were observed during the transition period (Indugu et al., 2017). Further changes in the microbial population of lactating cows can be affected by feeding direct fed microbial yeast products (AlZahal et al., 2017).

Calves are born essentially as monogastric animals and maternal contact (including microbes of the skin and udder) quickly colonizes the calf with maternal microbes; milk spilled from the esophageal groove (as well as any hay or grain consumed) provides a substrate in the rumen for bacterial fermentation (Malmuthuge et al., 2015, Poutrel and Rainard, 2018). As the young ruminant increasingly consumes solid feed and forages, the ruminal microbial population ferments more substrates to produce VFA, which results in an increase in the growth and development of the rumen tissue. In calves up to 42-d-old, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were identified as the most abundant phyla, but between day 14 and 42, there was an increase in *Bacteroidetes* and a corresponding decrease in *Firmicutes*, and *Proteobacteria* (Li et al., 2011). This study only compared the 45 most abundant genera due to an assumption that the most abundant genera represented the “core microbiome” of the pre-ruminant calf (Li et al., 2011). The ruminal microbiome matures as the calf ages, and microbial diversity increases al-

lowing the microbiome to utilize an widening spectrum of substrates (Malmuthuge et al., 2015). Further studies have also brought to light the use of waste milk as a potential source of calf ruminal microbes (including pathogens and antimicrobial residues), which can have a significant impact on animal health and food safety (Edrington et al., 2012, Edrington et al., 2018). Interestingly, recent research has demonstrated that the calf may not be born as “sterile” as was previously thought, particularly in regard to transmission of *Salmonella* to calves, with vertical transmission from dams being reported (Hanson et al., 2016).

Throughout the life cycle of dairy cows, a variety of stresses are encountered. Stress has been shown to have a significant impact on the microbial population of the gut of food animals (Carroll and Forsberg, 2007, Dowd et al., 2007, Bailey et al., 2011, Verbrugghe et al., 2012), and the immune system has in turn been shown to impact the microbial population of the gut (Rostagno, 2009, Bailey et al., 2011). Dairy cattle are susceptible to increased incidence and severity of both metabolic and infectious diseases during the periparturient period (Sordillo, 2016). The incidence and severity of both metabolic and infectious diseases during the periparturient period are also linked to the efficiency of the immune system. It was suggested that correlating nutrition and immune functions could optimize immunity during times of increased pathogen susceptibility such as stresses and periods immediately after calving (Sordillo, 2016). Disruptions in the resident ruminal microbial community structure (a dysbiosis) can create an opening for enteric pathogen colonization (Shreiner et al., 2015, Muñoz-Vargas et al., 2018), such as *Salmonella* which can be a threat to both human and animal health. While it appears that cattle at freshening are more likely to shed *Salmonella* (Fossler et al., 2005), other researchers found that *Salmonella* prevalence was highest one week after calving compared to three weeks after calving (Muñoz-Vargas, Opiyo et al. 2018). Due to the variation in pathogen shedding across farms, unfortunately this study was not able to correlate the onset of shedding with changes in the diversity of the fecal microbiome.

Glucogenic precursors, such as propionate, have been frequently used in the treatment and prevention of excessive lipid mobilization associated with ketosis (Piantoni and Allen, 2015, Piantoni et al., 2015). Administration of glucogenic precursors limits adipose mobilization (lipolysis) enough to support the cow to adjust to the dramatically increased energy demand during early lactation (Piantoni and Allen, 2015). A

diet high in starch results in an increase of propionate produced by ruminal microbes, however, it also results in an enhanced inflammatory response within the host (Plaizier et al., 2012). This enhanced response can be due to the development of clinical ruminal acidosis, or a disruption of the epithelial barrier in the gut, which is termed “Leaky Gut Syndrome” (Murugesan et al., 2014, Castro et al., 2016). The question remains, how can we manipulate the microbiome to produce adequate levels of propionate to limit excessive lipid mobilization, while producing acid levels low enough to prevent clinical acidosis and Leaky Gut Syndrome?

Foodborne pathogens are commonly found in the gastrointestinal tract of cattle, and cow fecal material can pose a risk to public and environmental health as it can carry many human and animal health pathogens. Cows serve as a reservoir for the foodborne pathogenic bacteria *Salmonella*, *Listeria*, and Shigatoxigenic *E. coli* (the best known member is *E. coli* O157:H7). These microorganisms are transmitted to humans primarily via a fecal oral route by consuming contaminated food products, or directly coming into contact with contaminated animals, feces, or surfaces (Döpfer et al., 2012, Berry et al., 2017). The incidence and shedding of these microorganisms are influenced by factors such as diet, water source, age, physiological status, geographical regions, season, stress, disruptions in intestinal microbiome, and management practices (Berry and Wells, 2012, Berry et al., 2017, Muñoz-Vargas et al., 2018). Further transmission of foodborne pathogens can happen from migratory and native birds, flies, and water troughs (Smith et al., 2008, Carlson et al., 2011, Schuster et al., 2013, Wasala et al., 2013, Callaway et al., 2014). Therefore, all changes in microbiome composition must take care to not introduce a dysbiotic situation that foodborne pathogens can exploit to colonize the gut.

CONCLUSIONS

Next Generation Sequencing has provided a much deeper and broader understanding of the microbial ecology of the rumen and intestinal tract of cattle. However this great new horizon of information has not proven to be a panacea to improving cattle nutrition. It is clear that the gut microbial population impacts food safety, animal health, production parameters, metabolomics, and host immunity, but it is equally clear that these impacts are more interconnected between host and the microbial consortium than we previously appreciated. It has become increasingly apparent that there are more variables and unintended consequences that can impact animal performance. It is time that we in



ruminant nutrition must move beyond the use of a “class photograph” approach to describe the rumen microbial population, but instead we must understand the biological functions of the gastrointestinal microorganisms. Without understanding what is going on, we continue to attempt to predict the impacts of diet or environmental changes on life outcomes using a high school yearbook. It is time for us to develop a comprehensive “unified field theory model” that can explain the behavior the rumen microbial ecosystem, its endproducts, and impacts on the host dairy cow to be able to maximize our production efficiency and profitability for producers.

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