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#### MATERNAL INFLUENCES ON THE CALF RUMEN MICROBIOME AND SUBSEQUENT IMPACTS ON PERFORMANCE AND EFFICIENCY

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# **IMPLICATIONS**

Cow-calf producers have done an exceptional job at managing and maintaining cows to wean efficient and profitable calves. Nutrition, health, and genetics have been at the center of cow management practices as it is well known the impact that these facets have on the reproductive efficiency of cattle and the impact these factors can have on the calf itself. However, one area that is more recently being considered as an important aspect of the cowcalf interface is the rumen microbiome. This includes the impact of the cow on not only the early microbiome in the calf (i.e. the influence of licking, suckling, consumption of colostrum, etc.) but also what aspects of gestation may influence the calf rumen microbiome. This area is fairly new to livestock research but has been highly publicized in human medicine.

To continue to improve efficiency of cow-calf operations, every avenue that affects the management of the cow and the subsequent impact on the calf should be considered. Thus, it becomes imperative to understand what maternal factors influence the rumen microbiome at all stages of development from birth through maturity and the potential impacts on performance of those calves.

## **INTRODUCTION**

Genetic material of the microbes housed in the rumen compose the "rumen microbiome". It is critical to evaluate the microbiome rather than independent microbes due to the interactions that these various microbes have with each other, the feed consumed, and the host tissue (rumen wall) itself. The ability of ruminant livestock to convert low quality feedstuff into high quality end-products is largely due to the role that the microbes housed in the rumen have in digesting the feed. Nearly 70% of the ruminant animal's energy is produced via microbial fermentation (Flint and Bayer, 2008), or the breakdown of feedstuffs into small components and converting those into volatile fatty acids (VFA) that can then be used by the host. While the rumen is not functional until nearly 4-6 weeks of age (Church, 1988), and the rumen microbiome shifts rapidly during this initial period, the early microbiome is responsible for production of VFA that impact rumen development (Flatt, 1958; Suárez et al., 2006) and ensures proper absorptive capacity for the mature ruminant. Thus, the evaluation of these early microbiomes is necessary to understand.

We are discovering that the microbes housed in the rumen have impacts on the host far beyond digestion and generation of energy for the host. There are connections between these microbes and many aspects of performance and productivity of the host itself. It is also important to understand the potential "programming" effect of the microbiome. Or rather, the potential for gestational/maternal factors to impact the microbiome in the calves and have lasting impacts on their health and performance.

#### **DEVELOPMENTAL FACTORS**

It is well known that the rumen is very underdeveloped at birth. The abomasum, or the true stomach, comprises the majority of the ruminant complexes at birth. The rumen develops and grows rapidly in early life, which is supported and regulated by the microbes that are also developing in the rumen during this time. As calves begin to consume feed other than milk (nibbling on hay, getting creep feed, etc.) their microbiomes also begin to increase in number and productivity dramatically. The actual process of rumination is believed to begin around 4-6 weeks of age but depends on the amount of feed those calves are consuming to stimulate this development. Even though rumination is not occurring immediately, the rumen microbes found in the pre-ruminant is critical to gut development and facilitates the establishment of the immune system. Very few VFA are produced at this stage suggesting the host is not relying on ruminal fermentation for energy, yet these limited VFA play a critical role in the development of the rumen itself.

The effect of stage of development on the rumen microbiome has been characterized in many different species. In calves the day 1 and day 3 microbiomes have a decreased species richness compared to that at day 28. This suggests more microbes are present at day 28 than at day 1 or 3. This is not surprising as there are only a few microbial species present during the pre-ruminant phase but as the calves begin to transition into a functional ruminant, a more complex microbiome is required in order to facilitate digestion of more complex feedstuff. Not only does the number of different species identified (i.e. richness) differ but the compositions also differ quite dramatically. This can be seen not only by the day 28 microbiome but the day 1 and day 3 microbiomes also differ quite dramatically (Figure 1). As the calf matures species diversity and composition continue to change. When comparing samples from day 28 calves to the rumen profile at weaning, species richness is greater at day 28 than in the rumen at weaning. This may be due to the stabilization of the rumen microbiome at weaning which is reflective of calves having a more consistent balance of milk, hay, creep feed, etc. The day 28 calves may just be starting to ruminate and are consuming feed other than milk which requires a variety of microbial species to colonize the rumen to allow proper digestion. However, a stabilization of these species is evident at weaning. The composition of these microbiomes are also quite different (Figure 2) with a more balanced and less variable microbiome present at weaning than that at day 28. These data are not new to the field of rumen microbiology, but they do highlight the importance of stage of development and the rumen microbiome. Once the rumen stabilizes making prolonged changes in the rumen microbiome become increasingly difficult. Thus, targeting the early microbiome may be the most opportune time to make changes in the microbiome of the rumen with the increased potential in those changes persisting into adulthood.

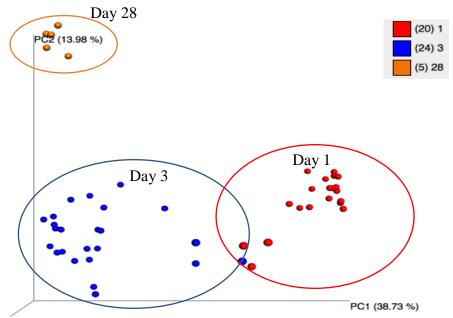


Figure 1. PCoA Plot of beta-diversity in the early rumen of calves of day 1 (red), day 3 (blue), and day 28 (orange) of age. The separation of these three sets indicates differences in the rumen microbial populations at each stage of development. The more spread out these points are within a day, indicate more variation in the microbiomes at that day. The day 28 points appear to cluster more tightly suggesting a less variable microbiome compared to the day 1 or day 3.

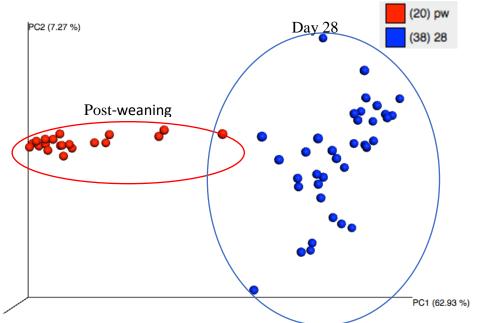


Figure 2. PCoA Plot of beta-diversity in the maturing rumen of calves of day 28 (blue) and post-weaning (red). Spatial separation of these two groups indicate differences in the composition of their rumen microbiomes. Additionally, the tighter clustering of the post-weaning points indicates less variation in the microbiome compared to day 28.

# MATERNAL FACTORS

The area of fetal programming stems from the "Barker Hypothesis" which indicates that when the fetus experiences changes in nutrition and other factors during critical periods of development, there can be long term impacts on the outcome of that offspring (Caton and Hess, 2010). Past research has shown the impact that over- and under-nutrition can have lasting impacts on calf birth weight, growth, physiology, and carcass characteristics (Meyer et al., 2010; Radunz et al., 2012; Meyer and Caton, 2016). However, the microbiome is not commonly considered in the "programming" research, perhaps because technology to adequately study these microbial communities have only become readily available and affordable somewhat recently.

In humans, many maternal factors have been determined as critical in the development of the gut microbiome. These factors include maternal nutrition during gestation, use of antibiotics or pre-/pro-biotics, delivery method (vaginal vs. caesarean), and others. After delivery, aspects of the early rearing has impacts on the development of the gut microbiome as well, including breast-fed vs. formula fed. In livestock, the influence of maternal factors has not been as thoroughly investigated to date. Somewhat recently, our labs have worked to explore the impact that maternal gestational nutrition, delivery type, maternal genotype, and rearing environment have on the development of the calf rumen microbiome and the long-term impacts on feed efficiency (Austin et al., 2018; Cunningham et al., 2018b; Cunningham et al., 2018a).

## **GESTATIONAL NUTRITION**

Beef cows in the Western United States often experience periods of nutrient restriction during gestation due to the overlap of gestation with harsh weather conditions and limited forage availability and quantity. Although many producers are feeding hay during these periods and sometimes offering supplementation, the increase in nutritional requirements due to the demand of the growing fetus make it difficult to meet these requirements during the entirety of gestation. The economics of providing hay and supplementation is also a limiting factor when striving to provide adequate nutrition.

Gestational nutrition can affect fetal organogenesis and specifically the development of the gastrointestinal tract (GIT; Duarte et al., 2013). Thus, these effects on the GIT physiology could also lead to alterations in the gut microbiome. When cows were nutrient restricted during late gestation effects in the week-old calf rumen microbiome were detected. The restricted cows were only mildly restricted (less than half a BCS less than the control group) yet these differences were present. The alpha-diversity metrics which indicate the "richness" of the microbiomes did not differ between calves born to nutrient restricted cows or those born to cows fed to meet requirements. However, the beta-diversity metrics did differ, which describe the compositional differences between the microbiomes of the calves born to nutrient restricted dams versus the controls.

# MATERNAL BREED INFLUENCE

There is a vast amount of data evaluating the role of the host (the cow or calf) genome in the rumen microbiome. Breeding programs and genetic selection have been employed to maximize productivity of the offspring by evaluating the genetic makeup of the dam and sire. There are several reports of breed influences on the rumen microbiome as well. This concept strengthens the idea of a host genetic/rumen microbiome connection.

When comparing the microbiome of calves born to Angus or Charolais cows various impacts on the calf rumen microbiome were discovered. In the early rumen microbiome (day 1, 3, and 28) the richness, or variety of microbes found in the rumen, did not differ between the two breeds. However, the composition of the microbiomes in their entirety were different between the Charolais and Angus calves within the first 4 weeks of life. These compositional differences suggest that some microbes were present in different amounts in one breed versus another. Differences in richness become apparent by weaning when the Charolais calves had more variety in their rumen microbiomes compared to Angus calves. These data suggest that maternal breed (and host genotype) can be a factor in the rumen microbial profiles.

## **MODE OF DELIVERY**

Although caesarean sections are not practiced often, or desired for that matter, in cow-calf operations, understanding the influence that delivery method has on the developing rumen microbiome is important to consider. This data can lend support or information that might help us better understand how much the birthing process influences what microbes are present in the early calf rumen. In humans, there has been extensive work on this area as caesarean deliveries are more common in humans than in livestock. The data from humans indicates a dramatic difference in the gut microbiome of infants born via caesarean versus those born vaginally and hypothesize that those differences (decrease richness) may be responsible for some long term metabolic/immune related complications as those infants mature.

Caesarean born calves often have decreased species richness, which is not surprising as many of the microbes we expect to find in the early rumen come from the birth canal. This decrease in richness however, does not persist as the animal matures and those calves born via caesarean are able to "recover" in terms of species richness. However, the implications that that early change may have on health, performance, and efficiency are still unclear. Compositionally, the microbiomes differ between calves born via caesarean or vaginally, but this difference does appear to subside as the animal matures, at least for a period of time.

## **REARING METHOD**

The ideal situation in a cow-calf operation is for a vaginal birth and the cow raises that calf until weaning. When calves are reared artificially on milk replacer there are several factors that differ between that rearing environment and what environment they would have been exposed to reared on their dam. The initial difference is in the differences of the milk that the calves receive. Many compositional differences exist between milk replacer and milk produced from the cow, but the udder also contributes to the "effect". The calves are exposed to different microbial sources via the milk itself and the delivery method (bottle vs. teat). There is also a social factor to consider when evaluating rearing methods. Calves are often seen mimicking their dam at the feed line and learning behaviors of consuming hay, even before their rumens are functioning in a way to properly digest this hay. When calves are separated from their dam and raised in a pen in a barn they may have companions or perhaps not. Regardless, this difference in social experiences could alter their eating behaviors which would subsequently impact the development of the rumen and the microbiome within.

In a study aimed at understanding what impact rearing might have on the rumen microbiome, calves were separated from their respective dam the day after calving and reared on milk-replacer. These bottle reared calves did not differ in the first 28 days in terms of the richness of their microbiomes but the composition of those microbiomes did differ compared to calves reared on their dam. The rumen microbiome was perhaps less impacted early on by rearing method and this may be because all calves received colostrum from their dam as they were not removed until 24 hours after calving.

#### **FEED EFFICIENCY**

Variation in the rumen microbiome has been associated with divergence in feed efficiency in livestock (Hernandez-Sanabria et al., 2010; Ellison et al., 2017; Guan et al., 2017). There are several avenues that could explain this variation in the microbiome with feed efficiency. There are known differences in feed efficiency based on the type of diet (i.e. concentrate vs. forage based), which could be partially driven by the microbial shifts that enable cattle to consume these different types of diets. The rumen microbes are also responsible for the production of methane and the amount of methane produced is associated with efficiency. The energetic cost of producing methane makes it an inefficient process, thus these microbial species that produce methane could be directly related with efficiency.

In the studies mentioned previously regarding maternal breed, delivery method, and rearing type, all of these factors were also evaluated in a post-weaning feed efficiency test. Performance of these calves differed by breed with Charolais being more efficient in terms of residual feed intake compared to Angus calves. The microbiome of these two breeds also differed more dramatically post-feed test than in the samples collected earlier (pre-weaning). While at this stage of development species richness did not differ between any of the factors (breed, delivery, or rearing) the composition of these microbes were strikingly different between breeds. The composition of the bottle reared, caesarean section, and those calves born vaginally and reared on their dam all had unique microbiomes at this stage, indicating long-term impacts of these effects on the rumen microbiome. When calves were ranked by residual feed intake into a high efficiency (low residual feed intake) and low efficiency (high residual feed intake) the microbial composition between these two groups differed

dramatically. This supports the evidence that the microbiome is related to feed efficiency, which allows the potential for manipulations of the rumen microbiome to influence feed efficiency.

# <u>GESTATIONAL MATERNAL FACTORS AND THE RUMEN MICROBIOME. IS</u> <u>THERE REASON TO INVESTIGATE THIS?</u>

The rumen has long been considered sterile at the time of calving, with rapid colonization occurring after this point. However, in humans and other non-ruminant species, there has been a plethora of data to suggest that perhaps the gut isn't as sterile as we had once believed. This has not been definitely proven in non-ruminants or ruminants, yet is a growing area of interest. Certain areas of the gestating female harbor a unique microbiome including the vagina, placenta, and amniotic fluid (Aagaard et al., 2014). These microbiomes may be influencing the development of the gut microbiome, even during gestation. The infant meconium (first stool) is interestingly similar to that of the placental microbiome, suggesting a potential influence on that early microbiome via the placental microbiome. These data have not been confirmed in cattle, yet these investigations are currently underway. The contribution of the placenta's function to the developing calf is not a new concept, however the attempts to describe a unique microbiome and its overlap with the calf meconium is.

If these microbiomes due indeed exist in cattle and there appears to be overlap with the developing rumen microbiome, the question still remains as to why producers should care about this. The most intriguing aspect of this concept is that there is the potential to "program" the developing rumen microbiome and set that calf up for improved health and performance in its lifetime via alterations during the development of the rumen microbiome. There is a long way to go before we can confirm this potential, yet producers have effectively managed the nutrition of gestating cows to give the calf the best start in early life to hopefully influence its lifetime productivity, why not consider the microbiome as another avenue to "kick start" the calf's production potential?

## **CONCLUSIONS**

The tools required to adequately study and evaluate the rumen microbiome are expensive and mostly limited to a laboratory setting. However, advances in this technology are making the reality of a "chute-side" testing method more believable in the coming years. But, until the time where we can effectively evaluate the microbiome in an affordable and logistically feasible manner, we can continue to understand what aspects of management can impact the rumen microbiome. We can strive to more clearly understand the impact that the cow has on the calf rumen microbiome and develop strategies to optimize this interaction to set that calf up for a more productive life. Every avenue of improving efficiency is being considered at this time when demands are high yet resources to produce are limited. The rumen microbiome may be yet another aspect of the cow's physiology to consider when selecting for and managing for improved efficiency.

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