THE IMPACTS OF DIET AND PREDICTED FEED EFFICIENCY ON PERFORMANCE, ULTRASOUND CARCASS CHARACTERISTICS, AND THE GASTROINTESTINAL MICROBIOME IN GROWING ANGUS HEIFERS

by

KRISTEN LEE PISANI

(Under the Direction of Todd R. Callaway and Timothy D. Pringle)

ABSTRACT

The objective of this research was to determine differences in the gastrointestinal microbiota of Angus heifers that were divergently selected for feed efficiency and how changes in diet impacted these microbes and host animal feed efficiency. The first experiment evaluated animal performance and ultrasound carcass characteristics of heifers during a high-grain diet and a high-forage diet, to determine if heifer feed efficiency was dependent on diet or genetic selection and how their interaction affected ultrasound carcass characteristics. The second experiment evaluated the rumen and fecal microbiome in terms of heifer feed efficiency on grain versus hay to determine how the microbiota changed with different diets within efficiency groups.

INDEX WORDS: Microbiome, Angus Heifers, Residual Feed Intake, Feed Efficiency

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BSA, The University of Georgia, 2019

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2021

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May 2021

DEDICATION

I'd like to dedicate this thesis to my nephews, Greyson and Connor, and my niece, Payton. I want this to show you all that leaving home is okay and doing something different than we're used to is okay. You don't have to go to school, but if you choose to, make it be in something you love; there is so much more to being successful than just money. Don't get stuck in the "Long Island Bubble"… unless you want to!

I'd also like to dedicate this to my Grandma McDonald, my entire life she encouraged me to go against the grain and do what I felt was right. I know if she were here today, she would be extremely proud that I took that advice and ran with it. I am so grateful for the support she provided me, so I want to dedicate this to her because she is such a big part of where I am today (writing a very large document).

ACKNOWLEDGEMENTS

There are many people who I would like to acknowledge at this time. First and foremost, my parents. Without your love and emotional (as well as financial) support, I truly could not have made it through so many years of schooling. Mostly, I want to thank you for trusting me when I made the decision to pursue a new field.

My boyfriend Grant and my dog Leroy. The two of you are what got me through many nights of writing, research planning, schoolwork, and even more frustration. Graduate school was a challenge but the two of you made it fun, and I'm so grateful I have been able to share this time with you (Grant).

There are many people within the University of Georgia who I would also like to acknowledge:

Dr. Pringle and Dr. Callaway, thank you for taking a chance on me and giving me the opportunity to study what I love (and being nice when I come up with crazy ideas). Thank you both for your wisdom, encouragement, and support. But most importantly, Dr. Pringle thank you for showing me animal science. After the first week of your "Introduction to Animal Science" class, I changed my major. I truly would not be where I am today (sitting in my cubicle at the department) if it were not for your passion, wisdom, and kindness.

To Kaleb Marchant, thank you for encouraging me to go to graduate school, guiding me, and advising me during the process of finding an assistantship. Most importantly, thank you for

teaching me how to work cows like a boss. If it weren't for you I wouldn't know how to drive a tractor, how to castrate bull calves, or how to manage cattle – all of the things I love.

To the farm crews of the Northeast Georgia Research Center in Calhoun, and the Eatonton Research Farm, I do not think I could thank you both enough. Without your commitment to research at UGA, my project and many others, would not be possible. You put in the hard work and made my job easy, thank you!

To Anna Scott, Taylor Krause, Madison Walker, and Jon McDonald thank you for your dedication to my project and for showing up when I needed so much help (and for being frustrated with me). Also to Darren Seidel, thank you for a lot of time on the phone with me coaching me through lab analysis, rumen collection, pump repair, statistics, and job advice – but more than anything, you gave me my first taste of research and a passion to come up with new ideas.

To Christina Welch and Jeferson Lourenco, my non-committee, committee members. Thank you so much for your support throughout the entire process of this thesis. From research planning, paper editing, statistical analysis, and everything in between. This thesis is just as much yours as it is mine, I am so grateful for the help of all of you during this time

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Feed costs represent a significant fraction of the total cost of beef production (Archer et al., 1999), because of this there was a push to develop cattle that required less feed to produce more end product, this was accomplished by selecting for feed efficiency. Measuring feed efficiency has been difficult due to traditional production practices (Archer et al., 1997); until the recent development of feed intake systems (Wells, 2015). The introduction of these feed intake systems increased data available on feed efficiency and led to the development of the expected progeny difference (EPD) for residual average daily gain (RADG) and dry matter intake (DMI) (Northcutt, 2010). Some intake systems, like GrowSafe^{TM}, allow feedlot operators and producers to observe other measures of feed efficiency, like residual feed intake (RFI).

Selecting cattle for favorable RFI became a tool for individual beef producers to increase cattle efficiency because of its independence of body weight and weight gain (Koch et al., 1963). Residual feed intake is calculated as the difference between actual and expected feed intake by cattle based on size and growth rate (Herd & Bishop, 2000). Within a herd, cattle that consume less feed than predicted for their average daily gain (**ADG**) have negative-RFI (**n-RFI**) and are considered more efficient. Cattle that consume more feed than predicted for their ADG haven positive-RFI (**p-RFI**) and are considered less efficient (Herd et al., 2003; McCann et al., 2014). Knowing the impacts of growth and intake on feed efficiency has led producers utilize residual RADG and DMI as selection criteria when breeding beef cattle.

Expected progeny differences (**EPD**) provide estimates of the genetic value of cattle as parents and EPDs for feed efficiency are calculated by geneticists using animals fed high-grain rations (Greiner, 2009). Expected progeny differences for body size and feed intake provide benefits to feedlot operators and packers; however, in cow-calf operations where cows and heifers spend a majority of their lives on pasture, literature fails to demonstrate whether selecting for grain-driven EPDs will positively impact the feed efficiency of cattle on pasture.

Changes in diet alter the microbiome within the gastrointestinal tract (**GIT**), which is fundamental in digestion (Callaway et al., 2010; Noel et al., 2019). The rumen contains an entire ecosystem of organisms, including: bacteria, archaea, fungi, and protozoa that ferment and decompose host animal feedstuffs (Zhou et al., 2018). Multiple studies indicated differences in rumen microbiota may be a driving force in host animal RFI (Carberry et al., 2012; Cantalapiedra-Hijar et al., 2018; Li et al., 2019; Welch et al., 2021). Additionally, hindgut microbial populations have been linked to RFI although its specific role in efficiency is unknown (Welch et al., 2020).

The microbial population has also been associated to differing performance in carcass traits (Krause et al., 2020); however, carcass traits for breeding herds cannot be estimated on the rail. Utilizing ultrasound equipment can allow for estimation of carcass characteristics in live animals. The data collected for backfat thickness (**BF**), area of the longissimus muscle (**REA**), and intramuscular fat (**IMF**) are possibly correlated with RFI (Williams, 2002; Lancaster et al., 2009). Previous studies revealed conflicting results of correlation between RFI and carcass characteristics (Nkrumah et al, 2004; Lancaster et al., 2009; Hafla et al., 2013). These differences are believed to be due to differing energy concentrations in diets (Mao et al., 2013). In order to better understand the role that gastrointestinal microbiota have in host animal feed efficiency and

carcass characteristics, and how diet further impacts these microbes and efficiency, we performed a series of experiments investigating these relationships.

The objective of the current research was to determine differences in the gastrointestinal microbiota of Angus heifers that were selected for divergent feed efficiency and how changes in diet impacted these microbes and host animal feed efficiency. The first experiment evaluated animal performance and ultrasound carcass characteristics of heifers during a high-grain diet and a high-forage diet, to determine if heifer feed efficiency was dependent on diet or genetic selection and how ultrasound carcass characteristics were affeted. The second experiment evaluated the rumen and fecal microbiome in terms of heifer feed efficiency on grain versus hay to determine how the microbiota changed with different diets within efficiency group. We hypothesized that there would be observable differences in the microbiota and carcass characteristics of growing heifers selected for divergent feed efficiency regardless of diet.

CHAPTER 2

LITERATURE REVIEW

Feed costs represent a significant fraction of the total cost of beef production (Archer et al., 1999), because of this there has been a push to develop cattle that require less feed while maintaining high levels of end product, by selecting for feed efficiency. Measuring feed efficiency has been difficult due to traditional production practices; until the recent development of feedlot feed intake systems. The introduction of these feed intake systems increased data available on feed efficiency and led to the development of the expected progeny difference (EPD) for residual average daily gain (RADG). While the development of an EPD that incorporates calf weaning weight, postweaning gain, and predicted dry matter intake (Northcutt, 2010) is a simple way for cattle producers to select for a herd that is more feed efficient, these EPD are measured on animals fed a feedlot ration (high grain). During different stages of production, cattle are fed different diets; a high grain ration only represents one sector of cattle production.

In the breeding herd, cows spend a majority of their productive lives on pasture, like cow-calf operations in Georgia, and these grain-driven feed efficiency EPDs may not be as impactful. Changes in diet impact the microbial population that is present within the gastrointestinal tract of cattle. These microbes are responsible for the initial fermentation and decomposition of cattle feedstuffs, and provide energy for their host animal to use. Meaning that when the microbial population is altered, so is the energy that is available to the animal. For this reason, it is plausible that animals selected for efficiency on grain may not be equally efficient on

forage. This review of the literature aims to find probable connections between feed efficiency and microbial populations present within beef cattle.

Measures of feed efficiency

Feed efficiency is a complex biological trait which combines feed intake (the input) with production (the output) (Archer et al., 1999). While the basis of feed efficiency is constant (intake compared with output), there are multiple approaches to determining feed efficiency. The two most utilized measures of feed efficiency are feed conversion ratio (FCR) and residual feed intake (RFI). Both FCR and RFI require that individual feed intake be measured for each animal. In cattle production, where most animals are fed in groups, determining individual intake may not be possible (Archer et al., 1997).

Feed Conversion Ratio

Average daily gain (ADG) and daily dry matter intake (DMI) are typically used to measure feed efficiency traits that are ratio-based, most often as feed conversion ratio (which can be expressed as the amount of feed required for a unit of gain (F:G) or the amount of gain per unit of feed (G:F) (Carstens & Tedeschi, 2006). While FCR is moderately heritable (0.29) ,because of its potentially negative effects it may not be the most effective measurement of efficiency on a genetic basis (Arthur et al., 2001). Koots et al. (1994) found that animals with a high genetic potential for growth rate, are assumed to have lower FCR (improved efficiency), and an increased potential for mature size. So while selecting for low FCR values, producers are indirectly selecting for larger animals, with larger maintenance requirements (Nkrumah et al. 2004). This prompted Archer et al. (1999) to point out that although FCR may be a relevant measure of efficiency in the production of growing animals, if feed requirements in the breeding

herd increases then the gains of the market progeny will be offset by the added maintenance costs of the breeding herd, and there will be little to no progress in system efficiency.

Residual Feed Intake

Defining feed efficiency involves using an animal's weight and feed intake and coupling that with a linear regression equation. Expected feed intake is determined as a factor of weight; the expected feed intake is then compared to the animal's actual intake to determine residual feed intake (Kennedy et al., 1993; Koots et al., 1994). For an animal to be classified as efficient based on RFI, it must consume substantially less feed than less efficient cattle without noticeable changes in growth rates or body weights (Bingham et. al, 2009).

RFI is moderately heritable (0.26 – 0.43) (Herd et al., 2003; Crews, 2005) meaning within herd RFI can be used as a selection tool for producers. However, unlike FCR, RFI has no correlation associated with growth performance or cow size (Archer et al., 1999; Crews, 2005) . In a study by Herd et. al (2003), a single generation was divergently selected by postweaning RFI between 8 to 12 months of age. The results of the study demonstrated favorable correlated changes in average daily feed intake (9.2 ± 0.2 vs. 9.8 ± 0.2 kg/d), RFI (-0.20 ± 0.11 vs. 0.17 ± 0.10 kg/d), and Feed:Gain ratio (7.0 ± 2.0 vs. 7.6 ± 0.2 kg/kg) in Angus feedlot steers. Thus selecting for animals with low postweaning RFI values could potentially lead to a decrease in feed intake and improve the feed efficiency of growing animals.

Another way in which RFI diverges from FCR is in repeatability. Archer et al. (2002) found that when offering a roughage based diet to heifers and later to the same animals as mature cows there were strong genetic correlations for RFI (r = 0.98). Similarly, Kelly et. al (2010) found that the magnitude of association between the growing and finishing phases in heifers was much greater for RFI than gain to feed. While RFI has been found to be consistent across an

animals productive lifespan, there is animal-to-animal variation that is not yet fully understood (Cantalapiedra-Hijar et al., 2018). An investigation into the biological and physical determinants of between-animal variation of feed efficiency in growing beef cattle, is necessary to appropriately select for feed efficiency in beef cattle herds.

Variation in RFI

Feeding Behavior

It has been well documented that there is a relationship between feeding behavior and efficiency, in terms of both G:F and RFI, indicating that feeding behavior can account for some of the variation between high efficiency, and low efficiency animals (Bingham et al., 2009; Kelly et al., 2010; Cantalapiedra-Hijar et al., 2018). Feeding behavior can be measured using different types of equipment and some of the differences in behavior could potentially associated with the equipment types used as well as breed type, sex or diet (Bingham et al., 2009). Regardless of these differences, key feeding behavior metrics include meal frequency and duration of feeding (Cantalpiedra-Hijar et al., 2018).

One hundred fifteen Brangus heifers were assigned to pens with Calan-gate feeders and their feeding behavior was recorded on camera (Bingham et al, 2009). Frequency of feeding was measured as a head-down feeding event, and duration was calculated as the sum of the duration of the individual head-down feeding events; head-down eating rate was also calculated as the average daily DMI divided by head-down feeding duration. High-RFI (low efficiency) heifers consumed 1.92 kg/d (22.5%) more a day, on average, than low-RFI heifers. Previous studies confirm the findings of this study that RFI is positively correlated with daily DMI, but is independent of growth and body size (Carstens et al., 2006; Kelly et al., 2010). Bingham et al. (2009) found that heifers with high-RFI had head-down durations that were significantly (P

<0.001) shorter than low-RFI heifers, indicating that the most-efficient animals spent more time feeding per day, than less efficient animals.

Hafla et al. (2013) examined the feeding behavior of 24 Bonsmara heifers that were divergent in RFI (n= 12 high, n= 12 low) and bred. The selected heifers were bred and the pregnant females were fed a chopped hay diet where their individual intake and feeding behavior was measured using GrowSafe bunks for 77 days. Forage use, body composition, physical activity, and heart rate were compared to RFI. To measure feeding behavior, bunk visit frequency and duration was recorded and then clustered into meal events. Meal criterion, which is the longest non-feeding interval that is still part of a meal, was determined for each animal and fitted to a model to compute individual animal meal data, consisting of frequency, duration and meal size (Hafla et al., 2013). These findings confirmed the conclusions of Bingham et al. (2009), that residual feed intake was strongly correlated with DMI (P<0.01; 0.62, Hafla et al., 2009). However, Hafla et al. (2013) found that the frequency of bunk visit events was not affected by RFI, although pregnant females classified as low RFI heifers spent 26% less time at the bunk compared to females with high RFI as heifers. Additionally, pregnant females in the low RFI group consumed smaller meals (P=0.04) and tended to have slower meal eating rates (P=0.06) compared to those classified as high RFI. Collectively, these results indicate that heifers maintain RFI classification throughout their lifespan and individual feeding behavior can help to explain the decreased DMI in more efficient females.

Body Composition

Lean tissue requires less energy per unit of gain than fat which is why body composition may also contribute to variation in RFI (Carstens & Kerley, 2009). Barsarab et al. (2003) studied the effects of divergent selection for RFI on body composition, through ultra-sounding as well as

carcass evaluation on 176 crossbred steers over two years. A multiple linear regression analysis of metabolic mid-point weight, average daily gain, and gain in empty body fat, and water revealed variation of actual feed intake by 67.9, 8.6, 3.9, and 1.1% respectively. Further, a simple correlation analysis across years showed a positive relationship between RFI and gain in empty body fat, while gain in protein was not significantly correlated to RFI. This suggests that steers with high RFI gain empty body fat at a higher rate than low RFI steers.

Richardson et al. (2001) used yearling Angus steers from a preliminary RFI breeding study and divided them into low RFI and high RFI groups which were fed in automated self-feeders, and the impact of RFI on metabolism and body composition were observed. Changes in body composition showed low RFI steers gained more protein than high RFI steers, and significantly less total carcass fat than high RFI steers (Richardson et al., 2001). A correlation exists between sire EBV (expected breeding value) for RFI and traits for dissected subcutaneous, intramuscular fat, and non-carcass fat, indicating that selection for increased RFI had slightly increased the amount of fat in these depots (Richardson et al., 2001). These correlations showed that less than 5% of the variation in parental RFI was explained by variations in fat and lean measures of body composition on progeny. Additional body composition changes were seen in external organs and bones. Low RFI steers had more external organs and bones as a percentage of their final liveweight ($P<0.1$, $P<0.01$), there was also a trend for low RFI steers to have longer intestines ($P<0.10$) than high RFI steers. Changes in measures of protein and fat, external organs, and bones suggest that changes in body composition occur when selecting for RFI.

Rumen microbiota

In the rumen – the first chamber of the cattle's four chambered stomach—exists an entire ecosystem of diverse microscopic organisms, including: bacteria, archaea, fungi and protozoa.

This ecosystem is responsible for the fermentation and initial decomposition of feedstuffs that cattle consume. The by-products of this fermentation and decomposition act as an energy source for the host animal to absorb and use. This is why recent studies suggest that differences in rumen microbiota are associated with feed efficiency (Carberry et al., 2012; Cantalapiedra-Hijar et al., 2018; Li et al., 2019).

Carberry et al. (2012), divergently selected 28 growing beef heifers for RFI (14 high-RFI, 14 low-RFI). These selections were made with data based off a low forage (LF) diet. Heifers were allocated to a high forage (HF) diet for 44 days, and were then turned out onto pasture for 56 days before resuming the same low forage diet for 35 days. During these feeding periods, individual animal intake was recorded to create RFI rankings. Throughout the course of the study, the heifers maintained their initial ranking for RFI, and a distinct interaction between the rumen microbiota was found with RFI. A DDGE profile analysis was performed using the Dice similarity coefficient ($D_{sc)}$; this analysis calculates the similarity between profiles. Rumen fluid from HF heifers revealed two distinct clusters between L-RFI and H-RFI, suggesting the existence of differences in bacterial population of H-RFI and L-RFI animals. These clusters were not present when LF rumen fluid was analyzed. Additionally, host feed efficiency was found to have an effect on specific ruminant microbe, *Prevotella*. H-RFI animals were observed to have greater relative abundance of *Prevotella* compared to L-RFI animals ($P<0.05$). Carberry et al. (2012) also reported an association between feed efficiency phenotype and rumen microbial diversity.

McGovern et al. (2020) analyzed rumen fluid from animals divergently selected for RFI to identify microbial and rumen fermentation markers – volatile fatty acids-- that may be associated with RFI status. After feeding contrasting diets (high-concentrate, grass silage, zero-

grazed grass, and high-concentrate again) rumen fluid was collected on the ten highest (HRFI) and ten lowest (LRFI) ranking animals within breed during each dietary phase; after running a 16S rRNA amplicon sequence and metabolic profile the results were inconsistent than those found in previous studies. McGovern et al. (2020) reported no differences across diets in microbial diversity or rumen fermentation profile between HRFI and LRFI animals. Guan et al. (2008) had previously reported that differences in rumen microorganisms were found to likely be associated with RFI in cattle, which was confirmed by Welch et al. (2020), in which it was noted that differences microbial diversity (richness) were observed between low and high RFI groupings. It is possible the variation in these results is due to differences in the stage of the animal's life when sampling was conducted, as these some of these studies observed cattle in a growing phase, while others observed a finishing phase.

Because of the small body of literature on this subject and the variation between present studies, further research is needed to more clearly discern the relationship between the rumen microbiota and its possible impact on phenotypic RFI in beef cattle.

Hindgut microbiota

It is well documented that the microbiome of the rumen is distinct from the microbiome of the hindgut portion of the gastrointestinal tract (GIT) (Guan, 2008; Welch et al., 2020). While there is active research investigating the impacts of feed efficiency on the rumen microbiota, little is known about the impact of the hindgut microbiota on the host animal's RFI ranking (Myer et al., 2015). Recent studies have shown that certain bacterial and archaeal families in the hindgut of cattle may be a driving force in the feed efficiency of the host animal (Welch et al., 2020), meaning the hindgut may have the potential to be manipulated to impact host feed efficiency or measured to predict it.

Welch et al. (2020) collected samples from the rumen, cecum, and feces of Angus steers from a commercial feedlot at slaughter. This study sought to observe the relationship between bacterial populations from these segments of the ruminant gastrointestinal tract (GIT) and the host animal RFI. Following a DNA extraction and gene sequencing, six bacterial families were significantly correlated ($P< 0.05$) with RFI in the cecum and feces. Four of these bacterial families (Ruminococcaceae, Mogibacteriaceae, Christensenellacae, and BS11) were negatively correlated with RFI in both the cecum and feces; while Succinivibrionaceae in the cecum and Bifidobacteriaceae in the feces were positively correlated with RFI. Collectively, these results demonstrated that more efficient steers contained bacterial populations that produced more end products which could be more rapidly absorbed by the host animal.

Lopes et al. (2019) conducted a similar study using 27 Nelore steers whose rumen, cecal, and fecal contents were collected at slaughter and extracted for DNA sequencing. Following analysis, microbial communities were compared against rankings for RFI. Results showed that bacterial and fungal alpha diversity (Chao1 richness, Simpson's diversity and Shannon's diversity) and beta diversity in fecal samples did not significantly vary between RFI groups ($P>0.05$ and $P>0.0001$, respectively). However, more detailed analysis of operational taxonomic units (OTU) revealed bacterial and fungal OTU's that were unique to each efficiency group. The results of this study draw connections between bacterial and fungal populations within the fecal microbiome that are unique to Nelore steers classified as efficient and inefficient at slaughter.

McGovern et al. (2020) questioned that the association between microbial communities and RFI classification would only be significant at certain stages in a beef animal's productive life, and therefore would vary with changes in diet, environment, and age. Welch et al. (2020) explored this in a study that observed the fecal microbiome of Angus steers divergently selected

for feed efficiency (through RFI) and marbling ability from weaning until slaughter. An analysis of DNA sequencing revealed that *Ruminococcaceae, Rikenellaceae,* and *Christensenellaceae* had abundances that were numerically greater in feces of efficient steers throughout their entire productive life. Conversely, inefficient steers had greater abundances of *Bifidobacteriaceae* and *Lactobacillaceae*, indicating a potential negative impact on feed efficiency. Additionally, the study revealed that microbial diversity in the hindgut was strongly correlated with feedlot RFI how so?, and was highest in the most efficient steers.

Relationship between RFI and ultrasound carcass traits

It has been well-documented that selecting for RFI has an impact on gain and body size in cattle. However, several studies have also demonstrated that RFI impacts the rate of fat deposition (Lancaster et al., 2009). Fat deposition rates can impact the time at which an animal is ready for slaughter, the quality of the carcass, and breeding value in cows and heifers (Williams, 2002). Utilizing ultrasound equipment can allow for estimation of carcass characteristics in live animals. Ultraound data collected on backfat thickness, area of the longissimus muscle, and intramuscular fat have been correlated with RFI (Williams, 2002; Lancaster et al., 2009).

Lancaster et al. (2009) sought to estimate phenotypic and genetic correlations with RFI and ultrasound measurements of 12^{th}-13^{th} rib fat thickness (BF), longissimus muscle area (LMA), and percent of intramuscular fat (IM) in Brangus heifers. After intake data was measured for 70 days and ultrasound measurements at the start and end of trials, the results revealed that while there were only weak correlations between the ultrasound carcass data and RFI, BF and LMA did play a significant role in dry matter intake (DMI) variation across heifers. Dry matter intake is the most appropriate method to compute RFI on individual animals. More efficient cattle may be slightly leaner as measured by BF, but there is little or no relationship between RFI

and IM (Lancaster et al., 2009). These results are somewhat different than those reported by Nkrumah et al. (2004) who reported no significant correlations between RFI and LMA or IM; however significant positive correlations ($P<0.05$) were observed between RFI and average ultrasound BF. Hafla et al. (2013) also found a tendency for gain in BF depth to be greater in heifers classified as less efficient, however the study also revealed a significantly ($P<0.01$) greater LMA in high RFI heifers compared with low RFI heifers.

The differences observed are believed to be due to differences in energy concentration in diets. Mao et al. (2013) performed a study using a diet more reflective of feedlot ration in Angus and Charolais steers. In Angus steers on a high grain ration, it was observed that RFI was correlated with ultrasound LMA, and BF (0.04 ± 0.05 to 0.07 ± 0.05, respectively). In Charolais steers RFI was also weakly correlated with BF and LMA (0.19 ± 0.06 , 0.03 ± 0.07, respectively).

Although there is variability in the current literature, it is clear that phenotypic RFI is not independent from ultrasound measurement of BF or IMF. Further research is needed to define whether RFI has a significant impact on LMA. More research must also be performed to decipher what role diet has on the correlation between RFI and ultrasound carcass composition variability.

Diet impact on microbial populations

Rumen microbiome

The rumen microbiome is a well-studied microbial ecosystem that plays a key role in nutrient processing for its host animal (Paz et al., 2018). The diverse pool of microbes consists of bacteria, as well as protozoa, archaea, and fungi (Wirth et al., 2018). These microbes can be assigned to different functional groups according to their main energy source—starch degraders,

fiber degraders, etc. (Henderson et al., 2015). Recent studies have displayed the presence of a dominant (core) ruminal microbiome in a large selection of ruminants (Jami & Mizrahi, 2012; Henderson et al., 2015; Wirth et al., 2018). The impact of diet on the rumen core and non-core microbiome has been explored and linked to host animal health, productivity, and environmental footprint (Zebeli & Metzler-Zebeli., 2012; Yáñez- Ruiz et al., 2015; Gruninger et al., 2018).

Acidosis is a condition of the rumen most commonly from a rapid increase in short chain volatile acids and subsequently a decline in pH, as a result of the fermentation of starch (Nagaraja & Lechtenberg., 2007). Ruminal acidosis is known to have a negative impact on animal productivity and in some cases can be life threatening to the host animal. Among the first to evaluate this metabolic disorder was Hungate et al. (1952), who established that the feeding of a grain based diet decreased the presence of cellulolytic bacteria and protozoa and gave rise to other organisms like *Streptococcus bovis* and lactic acid bacteria.

A study by Petri et al. (2013), examined the effects of an acidotic environment on the core rumen microbiome as well as during a transition from a forage-based diet to a concentrate rich diet. The results showed that for each dietary treatment group, the "core taxa" was different and was diet dependent. For example, forage-fed animals had a distinctive core microbiome from that of the grain-fed animals. Additionally, it was noted that animals fed a forage or forage-mixed diet had greater diversity in OTU's than those fed a grain-based diet. Overall, it was found that the core microbiome is stable across a range of diets and even a severely acidotic event, and those bacteria that emerge or undergo significant changes in population can serve as indicators of metabolic disorders such as acidosis.

Henderson et al. (2015) also found the presence of a stable core microbiome in ruminant animals. While the study observed 32 different species and sub-species of foregut fermenters,

there were significant findings in the Bovidae family. Like Petri et al. (2013), the results showed that bacterial communities are distinctive to forage-fed and grain-fed bovines. Specifically, it was noted that *Clostridiales* and *Fibrobacter* were most abundant in bovines fed forage, and interestingly *Butyrivibrio* was most abundant in samples from bovines fed mixes of forages and concentrates. Much like Hungate et al. (1952), as the concentrate content of the diet was increased, the abundance of *Fibrobacter* – a cellulolytic bacteria—decreased. While the core microbiome may have some importance, it appears diet is the major determinant of bacteria community structure, rather than host.

Hindgut microbiome

The microbiome of the ruminant hindgut has been studied for its profound impact on animal physiology, productivity, food safety, and environmental impact (Durso et al., 2010; Shanks et al., 2011). Like the rumen, there is also a "core microbiome" for the entire bovine GIT that is independent of diet (Durso et al., 2010). For example Durso et al. (2010) examined DNA from fecal samples collected in feedlot beef heifers, it was found these beef cattle shared many taxa with the bovine community described for dairy cattle, although their abundance differed.

The microorganisms outside of that "core microbiome" are subject to change based on dietary changes. Shanks et al. (2011) explored the impact of diet on fecal bacteria in beef cattle from four different geographic locations and three management groups (forage fed, processed-grain fed, and unprocessed-grain fed). The study concluded that management practices were more influential than geographic location on fecal microbial ecology. For example, the data showed that the fecal microbiomes of animals within the same management practice groups were more closely associated with one another than with animals from other management groups. Additionally, animals within the same geographic region subjected to different management

practices were not similar in their fecal microbiomes. Alteration of the fecal (and presumably hindgut) microbiome as a product of diet, more so than geographic location, indicates that diet can potentially be used to manipulate the microbial population of the hindgut.

Kim et al. (2014) further investigated the role of diet on the fecal microbiome by collecting samples from animals fed 3 different diets – Steers fed finishing diet; steers fed late growing diet; heifers fed early growing diet. Analysis showed that the bacterial communities in feces were greatly affected by dietary differences. 176,692 OTUs were identified, and only 2,359 were shared across the three diet groups. Specifically there were distinct differences between animals fed a high grain and a high forage diet, once again suggesting the role that a forage versus grain fed diet can have on the fecal microbial community.

The microbiome of fecal matter is seen as especially important because of its potential environmental impacts (i.e. waste runoff, methane emissions) (Durso et al., 2010) and food safety concerns like fecal shedding of foodborne pathogens such as *E.coli* O157:H7 or pathogenic strains of *Salmonella* (Durso et al., 2010; Shanks et al., 2011; Kim et al., 2014). With changes in diet heavily altering the microbial population present in the hindgut, there is a potential to formulate diets with the intent of manipulating fecal microbial populations to decrease the potential hazardous effects outlined above.

Vaginal microbiome

Reproductive success is essential for the survival of all species; however, for cattle producers reproductive success is essential for the survival of their business. It is estimated that the early cost of female infertility, abortions/stillbirths, dystocia, retained placentas, and metritis/pyometra is $441 to $502 million for beef producers, and $473 to $484 million for dairy producers (Bellows et al., 2002). These losses are influenced by many factors including disease

states, sex gametes, and genetics related to male and female fertility. However, these factors cannot account for all reproductive related losses (Clemmons et al., 2017). While the microbiome of the gut, oral cavity, and skin has been well studied, the microbiome of the reproductive tract has little information available. Recent studies have found that the vaginal microbiome could be used as a biomarker in bovine reproduction, and thus could provide an unexplored aid in reproductive losses for the cattle industry (Deng et al., 2019; Appiah et al., 2020).

It has been well documented across species that the vaginal microbiome is influenced by a number of factors including levels of sex hormones, breed or race, age, sexual behaviors and health status (Huang et al., 2014; Appiah et al., 2020). The human vaginal microbiome has a well-documented "core" that recent studies have reported fluctuations outside of these aforementioned factors. Diet is one factor that is gaining increased attention in human vaginal microbiome research.

Women have a unique vaginal microbiome that is unlike any other mammal, dominated by bacteria from the genus Lactobacillus; in the human vagina the relative abundance of lactobacilli is typically >70% whereas in other mammals it rarely comprises more than 1% vaginal microbiota (Miller et al., 2016). Miller et al. (2016) tested the hypothesis that humans have a larger abundance of lactobacilli as a result of humans high level of starch consumption, compared to other mammals. The study found that the vaginal microbiomes of women have exceptionally high levels of glycogen, whose breakdown products are a main energy source for lactobacilli; in addition to glycogen, female vaginal tracts also contain α-amylase which is responsible for breaking glycogen down to a form useable by lactobacilli; it is well known that

carbohydrate ingestion increases glycogen in the liver and skeletal muscle, so it is reasonable to believe glycogen could increase in the vagina by a similar mechanism.

A study by Song et al. (2020) investigated the effects of multiple influences on the vaginal microbiota, most notably diet. The study compared the vaginal microbiome of women who had a vegetarian diet against those who were nonvegetarians; the results showed that vegetarians had a vaginal microbiome that was more diverse than those of nonvegetarians. This reveals that long-term diet and energy metabolism does influence the vaginal microbiome. This is supported by multiple studies that reveal obesity and diets high in fat, and low in vitamins A, C, E, and D are associated with an increased risk of bacterial vaginosis (Brookheart et al., 2019; Neggers et al., 2007; Thoma et al., 2011).

Multiple studies have found an association between diet and the vaginal microbiome of women. Beef cattle research suggests that the vagina plays a possible role in populating the rumen microbiome (Deng et al., 2019); however, little research has been conducted on the impact of dietary changes on the vaginal microbiome in cattle. More research needs to be done to investigate the potential impact diet may have on the vaginal microbiome in cattle, and how this can impact reproductive losses within the beef industry.

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CHAPTER 3

IMPACTS OF DIET ON CHANGES IN RESIDUAL FEED INTAKE AND ULTRASOUND CARCASS CHARACTERISTICS OF ANGUS HEIFERS SELECTED FOR DIVERGENT FEED EFFICIENCY[1].

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To be submitted to *The Journal of Animal Science*

ABSTRACT: Twenty-four Angus heifers were selected for divergence in feed efficiency based on their sire's residual average daily gain EPDs (*n* = 12/efficiency group(**EG**) , and were individually fed to measure residual feed intake (**RFI**). The objective of this study was to determine if feed efficiency ranking in growing heifers on a feedlot ration (ME = 1.33/Mcal/lb) is the same when the same heifers are fed a forage ration (ME = 0.91/Mcal/lb). Heifers were fed each diet for 28 d in 2 consecutive periods, using Calan-gate feeders. On d 0 and 28 of each feeding period ultrasound carcass composition traits were measured to observe compositional changes that occurred as a result of diet or efficiency. Residual feed intake was computed as the differences between actual and expected feed intake based on the residuals from the linear regression of dry matter intake (DMI) and mid-test metabolic weight (body weight$^{0.75}$) on actual feed intake. Residual feed intake was significantly impacted by EG (P = 0.05). The mean RFI for high – and low- efficiency heifers were -0.186 and 0.186 ± 0.6577, respectively. However, EG did not significantly impact other animal performance data or ultrasound carcass characteristics. Actual RFI did not change based on diet (P = 0.53). Thirty-three percent of heifers had a difference of more than 1 SD in RFI as diets changed. Diet did significantly impact ultrasound ribeye area (**UREA**), ratio of muscle to fat (**RATIO**), intramuscular fat (**UIMF**), and fat thickness (**UFT**) in the first period, and UIMF and UFT in the second period (P < 0.05). Selecting heifers for improved feed efficiency based on their sire's feed efficiency EPD (on a high-concentrate diet), resulted in lower RFIs regardless of whether their diet was primarily concentrate or forage.

KEY WORDS: beef cattle, diet, feed efficiency, forage, residual feed intake, ultrasound

INTRODUCTION

Selecting cattle for favorable residual feed intake (**RFI**) is a tool for individual beef producers to increase cattle feed efficiency because of its independence of body weight and weight gain (Koch et al., 1963). Residual feed intake is calculated as the difference between actual and expected feed intake by cattle based on size and growth rate (Herd & Bishop, 2000); within a herd, some cattle will have more favorable RFI values than others. Cattle that consume less feed than predicted for their average daily gain (**ADG**) and metabolic weight have a negative-RFI (**n-RFI**) and are considered more efficient. Cattle that consume more feed than predicted for their ADG and metabolic weight have a positive-RFI (**p-RFI**) and are considered less efficient (Herd et al., 2003; McCann et al., 2014). An alternative feed efficiency measure that is currently available in the form of a breeding value is residual average daily gain (**RADG**) and dry matter intake (**DMI**) to predict gain and identifies animals that more on the same amount of feed resources.

Feed efficiency expected progeny differences (**EPD**), like RADG EPD, provide estimates of the genetic value of cattle as parents and are calculated by geneticists using animals fed high-grain rations (Greiner, 2009). Expected progeny differences for body size and feed intake provide benefits to feedlot operators and packers; however, in cow-calf operations where cows and heifers spend the majority of their lives on pasture, literature fails to demonstrate whether selecting for grain-driven feed efficiency will impact the feed efficiency of cattle on pasture. While the impact of diet on efficiency is unknown, the impact of diet on carcass characteristics in beef cattle is well-studied (Roberts et al., 2007; Fitzsimmons et al., 2014; Del Bianco Benedeti et al., 2016). Utilizing ultrasound technology can allow for estimation of carcass characteristics in live animals. The data collected on ultrasound measures of backfat thickness (**UBF**), area of

the longissimus muscle **(UREA)**, and intramuscular fat (**UIMF**) are possibly correlated with RFI (Williams, 2002; Lancaster et al., 2009). Previous studies revealed conflicting results of the relationship between RFI and carcass characteristics (Nkrumah et al, 2004; Lancaster et al., 2009; Hafla et al., 2013); however, these different outcomes appear related to differing energy concentrations in diets (Mao et al., 2013). Therefore, the objective of this study was to determine if the RFI ranking of growing heifers, genetically selected to differ in feed efficiency, changed with dietary changes, and to compare ultrasound body composition characteristics of those heifers.

MATERIALS AND METHODS

Animals, Facilities, and Diet

The heifers used in the present study are from the eighth generation of a genetic selection program involving Angus cattle being selected for RADG and intramuscular fat (Detweiler et al., 2019). All heifers ($N = 24$) were born and raised at the Northwest Georgia Research and Education Center (Calhoun, GA) where they were reared in a pastured-based system until approximately 9 months of age, when they were transported to the Eatonton Beef Research Unit (Eatonton, GA). Using data from the selection program, heifers were selected based on their sire's feed efficiency measures (RADG, DMI, and RFI EPDs) . Heifers sired by bulls with high feed efficiency were classified as high efficiency (**HI**) and heifers sired by bulls with low feed efficiency were classified as low efficiency (**LO**). Heifers were separated into sire feed efficiency groups (**EG**, $n = 12$ heifers/group), stratified by body weight (BW 275.5 kg $\pm$ 48.3) to two dietary treatments (grain to hay or hay to grain). Within a dietary treatment, heifers were assigned to 1 of 5 pens (4 heifers/pen) or, due to facility constraints, 1 of 4 individual pens (1 heifer/pen), which will be referred to as pen 6 for the remainder of the study.

Heifers had free access to water and were individually fed 1 of 2 treatment diets using Calan-gate Feeders (American Calan Inc., Northwood, NH). Diet 1 was a high concentrate diet (**Grain**; ME= 1.33/Mcal/lb; Table1**)**. Diet 2 was a hay diet (**Hay;** ME= 0.91/Mcal/lb; Table 2.**)**. During a 21-d period, heifers were trained to eat from the Calan-gate feeders and were adapted to their respective diets before the beginning of the study.

The study was divided into two consecutive 28-d feeding periods (the first feeding period is referred to as **P1**, and the second feeding period as **P2**). For P1 pens 1, 2, and 3 were offered *ad libitum* access to diet 1, while pens 4, 5, and 6 were offered *ad libitum* access to diet 2. After P1, both groups were transitioned to the alternative diet for the remainder of the study. Both groups were fed to allow 10% refusal of feed. Feed refusal and body weight were measured weekly to calculate daily DMI and ADG. Residual feed intake was calculated as the difference between actual DMI and the DMI predicted from multiple linear regression of DMI on mid-test metabolic weight ($BW^{0.75}$) and ADG using the following model:

$$DMI = \beta0 + \beta1\text{mid-test } BW0.75 + \beta2ADG + €,$$

where β0 is the y-intercept, β1 is the partial regression coefficient of mid-test $BW^{0.75}$, β2 is the partial regression coefficient of ADG, and € is the error term.

On d 0 and 28 of both periods, real-time carcass ultrasound data were collected. Ribeye area, 12th-rib backfat thickness, and intramuscular fat percentage images were collected using an Aloka 500-V ultrasound unit with a 17.2 cm, 3.5 MHz linear probe (Corometrics Medical Systems, Wallingford, CT) on the right side of the animal between the 12^{th} and 13^{th} ribs. Images were then interpreted using Beef Information Analysis Pro Plus software (Designer Genes USA, Harrison, AR).

Statistical Analysis

All analyses were performed in Minitab (v 19.1; MiniTab LLC.) using animal as the experimental unit. A factorial design was used to analyze the effect of diet and EG in a replicated 2 × 2 factorial arrangement. Resulting in four treatment combinations: HI, grain; HI, hay; LO, grain; and LO, hay. The main effects of EG, diet and their interaction were measured using the GLM procedure within ANOVA. The proportion of heifers within EG that changed their RFI ranking from grain to hay by 1 SD, 0.5 SD, and <0.5 SD were calculated. Results were considered significant at $P \leq 0.05$.

RESULTS

Intake, Performance, and Feed Efficiency

There were no EG× Diet interactions on heifer performance ($P > 0.53$; Table 3). There were no EG main effects ($P > 0.18$) on performance data, except LO heifers had a greater ($P = 0.05$) RFI than HI. There were no diet main effects on performance data ($P > 0.52$), except average DMI and ADG were greater on grain than hay ($P < 0.01$).

Figure 1 displays individual heifer RFI on grain and hay. The solid black line represents RFI on grain and bar represents RFI on hay. Green color indicates a decrease in RFI (more efficient) from grain to hay, red indicates an increase in RFI (less efficient). The shade of the color indicates the degree of change. The darkest shade indicates a change of 2 SD or greater, the second darkest indicates a change of 1 SD or greater, the medium shade indicates a change of less than 1 SD, and the lightest shade indicates a change of 0.5 SD or less. Residual feed intake from grain to hay varied with a mean RFI of 0.0949 ± 0.6577. From grain to hay, 8.0% of heifers increased 2 SD or greater, the remaining changed in either direction. 25% changed by 1 SD or greater, 29% changed by less than 1 SD, and 38% changed by 0.5 SD or less.

There was no change in rank (Fig. 2) for either efficiency group, with a mean of -0.186 ± 0.631. In the HI group, 25% of heifers RFI changed by more than 1 SD [95% CI (0.05, 0.57)] and 75% changed by less than 0.5 SD [95% CI (0.43, 0.95)]. In the LO group, 42% changed by less than 1 SD [95% CI (0.15, 0.72)] and 58% changed by less than 0.5 SD [95% CI (0.28, 0.85)].

Ultrasound Carcass Characteristics

There were no two or three-way interactions on all ultrasound body composition ($P > 0.65$), except a Diet × Day in REA and FT in both periods ($P < 0.01$; Table 4). Ribeye area, FT, and RATIO were all greater on d 28 when heifers were fed grain. There were no EG main effects for all ultrasound carcass characteristics during both periods ($P > 0.15$). There were no Day effects ($P > 0.13$) on ultrasound carcass characteristics except, in both periods FT was greater on d 28 than d 0, and REA was greater on d 28 than 0 in P2 ($P < 0.04$). Heifers fed grain had greater mean values of all characteristics in P1 ($P < 0.03$), and IMF and FT in P2 than hay-fed heifers ($P < 0.03$).

DISCUSSION

The range in initial body weight in the present experiment is similar to other studies examining RFI in growing heifers, previous studies have recorded average initial body weights of 265.4 kg ± 10.93 (Bingham et al., 2009; Damiran et al., 2018), indicating the heifers used in the present study were of average body weight for a growing heifer study. As expected, ADG was influenced by diet; however, on average, heifers on the hay diet gained no weight (mean = -0.48 kg/d). This is potentially due to the extreme nutrient requirements of growing heifers to gain weight; although the hay was average quality, it still may not have had enough energy or protein for adequate growth.

Average daily DMI was not influenced by EG, these findings are unexpected due to previous studies. Typically, low-RFI heifers have lower DMI than high-RFI heifers and strong correlations are present between RFI-status and DMI (Carstens et al., 2006; Bingham et al., 2009; Fitzsimmons et al., 2013; Hafla et al., 2013). In the present study, the lack of correlation between EG and DMI could be due to differences in energy concentrations. Lawrence et al. (2012), found a positive correlation between RFI and DMI during an 84-d RFI measuring period on grass silage and concentrate; however, when heifers were transitioned to a pasture diet, DMI and RFI were not correlated. McDonnell et al. (2016) observed RFI across 3 different diets (grass silage, pasture, and TMR) and found RFI and DMI were only positively correlated when animals were fed TMR. Potentially, this could mean that DMI is correlated with RFI-status only when the animal is fed a diet containing concentrates. This corroborates our results which indicated ADMI is impacted by diet, but not RFI status.

Due to the nature of the data, statistical analysis on RFI within efficiency groups was unable to be performed; however, there were numerical changes in rank in RFI between EG. There is no apparent pattern in these changes, although there were two heifers that displayed differences which may warrant further investigation. In the HI group, the heifer which had the smallest RFI on grain, had the greatest RFI on hay. In the LO group, the heifer with the second highest RFI on hay, had the lowest RFI on grain. These findings may imply that heifers with extreme performance on grain, may have the opposite extreme on forage, and vice versa. Similar changes in RFI have been attributed to changes in temperature, Duranna et al. (2012) observed efficiency of heifers classified as high, medium, and low RFI over two periods of contrasting temperatures (i.e. extreme cold and extreme heat). Between these two periods, 41% of heifers had changed their RFI ranking, 28% of these exhibited extreme RFI changes (high to low, or low

to high). Collectively, these findings may imply heifers with extreme RFI may be more sensitive to dietary or environmental changes.

Additionally, all observed changes and differences may be attributed to the microbial populations within the gastrointestinal tract, recent studies suggest that differences in the gastrointestinal microbial population are associated with feed efficiency (Cantalapiedra-Hijar et al., 2018; Li et al., 2019, Welch et al., 2020). The microorganisms present throughout the entire gastrointestinal tract belong to different functional groups according to their main energy source—starch degraders, fiber degraders etc. (Henderson et al., 2015); and thus, dietary changes should result in alteration of the bacterial communities. While diet was not impactful on RFI in our study, it is likely diet was impactful on the microbial communities within the rumen and hindgut which may have altered RFI.

Contrary to previous studies, there were no observed differences in ultrasound carcass data due to efficiency. Studies by Nkrumah et al. (2004), Santan et al. (2012) and Mao et al. (2013) all found correlations between feed efficiency and FT, and some tendencies for REA and IMF. Lancaster et al. (2009) performed a 70-d feeding trial where ultrasound carcass data was collected in conjunction with feed intake and found only weak positive correlations between ultrasound carcass data and RFI. In the present study, the lack of interaction could potentially be due to differences in energy concentration in the diets or potentially the length of the feeding periods. The present study had a timeline similar to that presented by Lancaster et al. (2009), meaning 72-d may not be a long enough period of time to see large changes that could be correlated back to feed efficiency.

Efficiency groupings based off of sire feed efficiency EPDs (RADG, DMI, and RFI) was accurate in predicting actual RFI of the heifers and could be a good tool for selection if

producers have access to the facilities required for RFI measurement. Since actual RFI was not impacted by changes in diet, it would appear that producers will not negatively impact their breeding herd by selecting sires based on feed efficiency EPDs measured on a feedlot diet. Due the implications, further research needs to be performed to address the changes in RFI during diet change observed for the most extreme animals. Additionally, the microbial population of the gastrointestinal tract needs to be explored during dietary changes while feed efficiency is being measured to further explore connections between changes in RFI and diet.

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Table 3.1. Composition of grain ration (diet 1)

Nutrient	%
Dry Matter	83.9
% DM	
Crude Protein	17.5
Acid Detergent Fiber	15.3
Neutral Detergent Fiber	28.7
Total Digestible Nutrients	76.2
Ash	5.29
Calcium	0.15
Phosphorus	0.47
Metabolizable Energy, Mcal/lb	1.33

Table 3.2. Composition of bermudagrass hay (diet 2)

Nutrient	%
Dry Matter	79.6
% DM	
Crude Protein	13.5
Acid Detergent Fiber	38.7
Neutral Detergent Fiber	70.2
Total Digestible Nutrients	55.3
Ash	7.22
Calcium	0.46
Phosphorus	2.16
Metabolizable Energy, Mcal/lb	0.91

Table 3.3. Animal performance in response to diet by efficiency group (EG)

	HI[1]		LO[1]					P-value		
	Hay	Grain	Hay	Grain	Minimum	Maximum	SEM	Diet	EG[7]	EG*Diet
Initial BW[2], kg	275.69		275.69		234.96	315.70	3.13	1.00	1.00	1.00
Final BW[2], kg	351.90		354.71		300.28	412.77	3.97	1.00	0.73	1.00
MMW[3], kg	62.39	61.60	62.19	63.13	51.52	72.03	0.67	0.96	0.63	0.53
ADMI[4], kg DM/d	5.46	8.82	5.76	9.47	4.22	11.99	0.31	<0.01	0.18	0.97
ADG[5], kg	-0.09	2.01	-0.05	2.05	-0.68	3.31	2.24	<0.01	0.79	0.97
RFI[6] kg DM/d	-0.21	-0.17	0.09	0.29	-1.21	1.35	0.09	0.53	0.05	0.68

Mean animal performance data by efficiency group and diet treatment.

[7]Efficiency groups: [1]HI = predicted high efficiency heifers; [1]LO = predicted low efficiency heifers.

[2]BW = body weight.

[3]MMW= midpoint $BW^{0.75}$ (metabolic weight).

[4]ADMI = average dry matter intake, daily.

[5]ADG = average daily gain.

[6]RFI = residual feed intake.

Table 3.4. Mean values of ultrasound carcass characteristics from d-0 and d-28 of P1 and P2 in response to diet by efficiency group

Period 1

	LO[1]				HI[1]								
Ultrasound	Hay		Grain		Hay		Grain			P-Value			
Data	D-0	D- 28	D-0	D-28	D-0	D-28	D-0	D-28	SEM	Diet	Day	EG[6]	Diet*Day
REA[2], in^2	7.50	7.10	8.18	9.54	7.37	6.39	8.18	9.89	0.20	< 0.01	0.17	0.68	< 0.01
IMF[4], %	4.16	4.16	4.66	4.66	4.18	4.18	5.17	5.17	0.17	0.03	1.00	0.44	1.00
FT[5], in	0.14	0.15	0.19	0.26	0.13	0.13	0.18	0.26	0.01	< 0.01	0.01	0.45	< 0.01

Period 2

	LO				HI								
Ultrasound	Hay		Grain		Hay		Grain			P-Value			
Data, cm^{-1}	D-0	D- 28	D-0	D-28	D-0	D-28	D-0	D-28	SEM	Diet	Day	EG	Diet*Day
REA, in^2	9.15	9.42	8.55	10.56	9.39	9.17	7.92	9.63	0.15	0.67	< 0.01	0.15	< 0.01
IMF, %	5.93	5.83	4.46	5.12	6.01	6.41	4.67	5.85	0.24	0.03	0.24	0.38	0.42
FT, in	0.25	0.24	0.15	0.25	0.25	0.23	0.15	0.22	0.01	< 0.01	0.04	0.41	< 0.01

[2,3,4,5] The mean values of ultrasound ribeye area, ratio of fat to muscle, intramuscular fat, and backfat thickness between the 12th and 13th ribs were calculated for the LO and HI efficiency groups, in both periods.

[6]Efficiency groups: [1]LO= predicted low efficiency heifers; [1]HI= predicted high efficiency heifers.

[2]Diet × EG ($P > 0.10$); Day × EG ($P > 0.39$); Diet × Day × EG ($P > 0.37$)

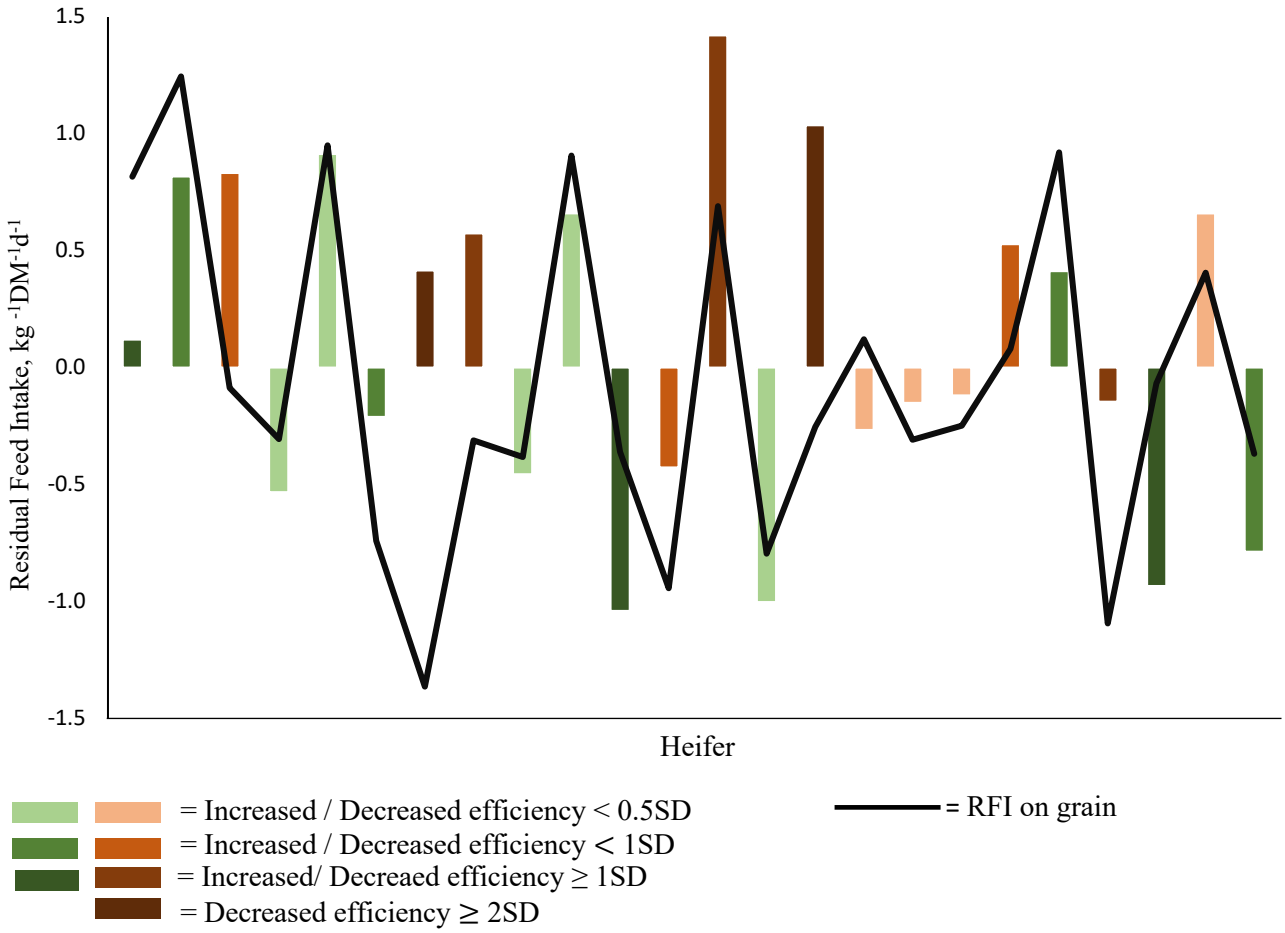

Figure 3.1 Individual heifer residual feed intake (**RFI**) on grain and hay.

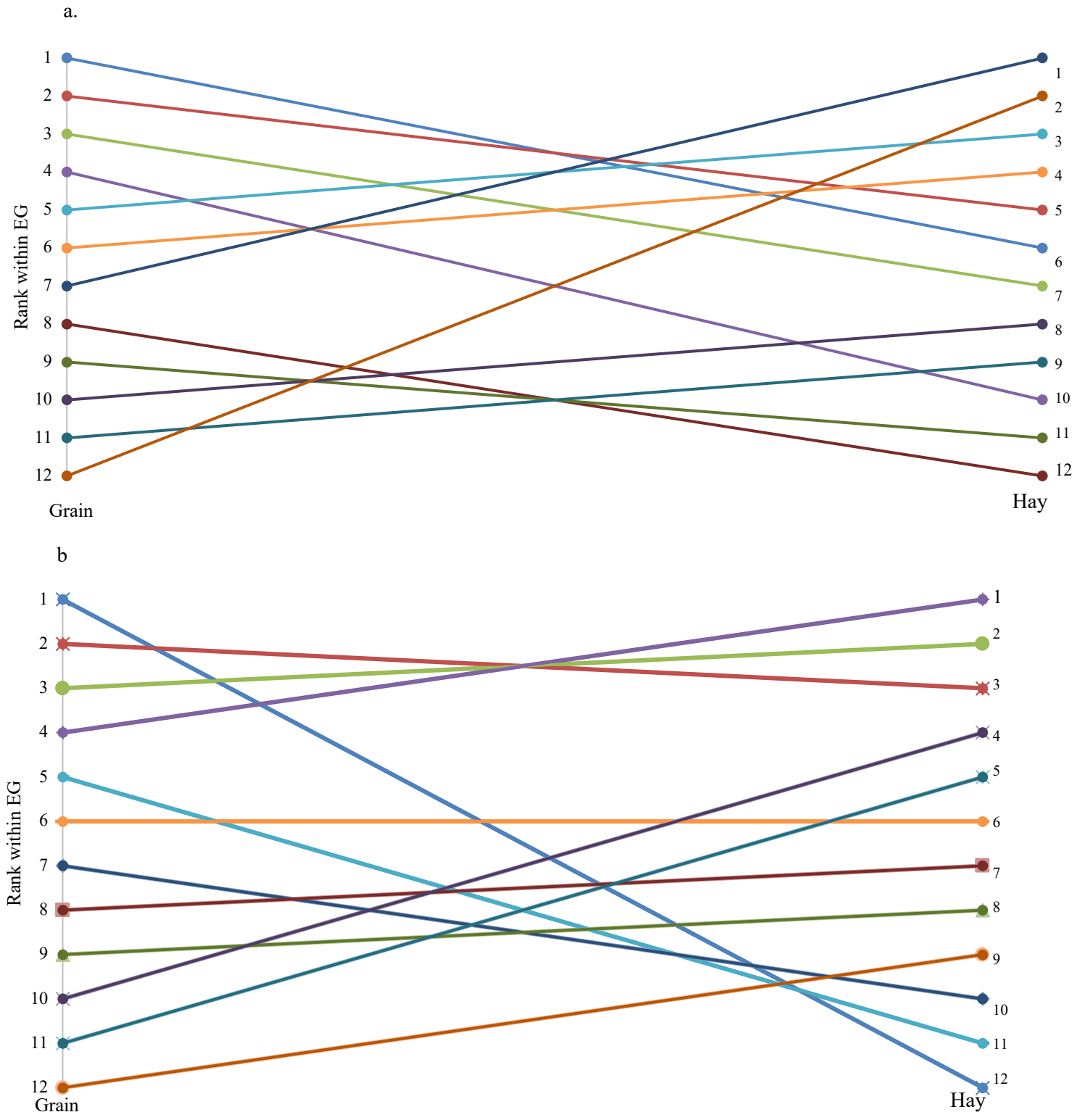

Figure 3.2. Change in rank from 1 (most efficient) to 12 (least efficient) for heifers in the HI efficiency group (a) and LO efficiency group (b)

CHAPTER 4

EVALUATION OF THE RUMEN AND FECAL MICROBIOME ON FEED EFFICIENCY IN ANGUS HEIFERS FED TWO CONTRASTING DIETS[1].

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To be submitted to the Journal of Animal Science

ABSTRACT: Twenty Angus heifers were selected from a larger study that measured residual feed intake (**RFI**) in heifers selected for expected feed efficiency based on their sire's residual average daily gain (**RADG**) and dry matter intake (**DMI**). Over two, 28 d feeding periods, heifers were individually fed a hay diet (ME = 0.91 Mcal/lb), or a grain diet (ME= 1.33 Mcal/lb; *n* = 5/ efficiency group/dietary treatment). On d 0 and 28 of both feeding periods, rumen and fecal samples were collected and DNA was extracted and sequenced by 16S rRNA gene analysis. The objective of this study was to quantify changes in the microbial population within the rumen and feces,with changes in diet and feed efficiency. A general linear analysis was used that included alpha diversities and bacterial abundances using efficiency line and diet as main factors. Overall, diet was the most impactful ($P < 0.039$) on changes in alpha-diversity indices, with all diversity measures being greater in heifers fed hay, compared to those fed grain. The most prevalent phyla in the rumen were *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Verrucomicrobiota* and *Spirochaetota*. All phyla populations in the rumen were influenced by diet in both periods, except for *Firmicutes* and *Actinobacteria* in period 2. In period 1, *Spirochaetota* were more abundant in high efficiency heifers classified as low-RFI. Further analysis of data is required to draw stronger conclusions about the microbial population of the rumen and hindgut on beef cattle feed efficiency; however, diet was more impactful on alpha-diversity and bacterial abundance at the phylum level, than was RFI classification.

KEY WORDS: beef cattle, diet, rumen, microbiome, residual feed intake

INTRODUCTION

Selecting cattle for favorable residual feed intake (**RFI**) became a tool for individual beef producers to increase cattle feed efficiency because of its independence from body weight and weight gain (Koch et al., 1963). Residual feed intake is calculated as the difference between actual and expected feed intake of cattle based on size and growth rate (Herd and Bishop, 2000); within a herd, some cattle will have more favorable RFI than others. Cattle that consume less feed than predicted for their average daily gain (**ADG**) are classified as negative-RFI (**n-RFI**) and are considered more efficient. Cattle that consume more feed than predicted for their ADG are classified as positive-RFI (**p-RFI**) and are considered less efficient (Herd et al., 2003; McCann et al., 2014). Seeing impacts of growth and intake on efficiency, producers utilize residual average daily gain (**RADG**) and dry matter intake (**DMI**) as selection criteria when breeding beef cattle.

Expected progeny differences (**EPD**) provide estimates of the genetic value of cattle as parents and RFI EPD are calculated by geneticists using animals fed high-grain rations (Greiner, 2009). Expected progeny differences for body size and feed intake provide benefits to feedlot operators and packers; however, in cow-calf operations where cows and heifers spend a majority of their lives on pasture there is little evidence about the impact of selection using grain-driven feed efficiency on the efficiency of cattle on pasture. Dietary changes alter the microbiome throughout the gastrointestinal tract (**GIT**), which is impacts the amount of energy and protein that can be harvested from the ruminant diet (Callaway et al., 2010; Noel et al., 2019).

The rumen contains a complex microbial ecosystem including: bacteria, archaea, fungi, and protozoa that ferment feedstuffs (Zhou et al., 2018). Multiple studies indicated differences in rumen microbiota may be a driving force in host animal RFI (Carberry et al., 2012;

Cantalapiedra-Hijar et al., 2018; Li et al., 2019; Welch et al., 2021). Additionally, hindgut microbial population was recently linked to RFI status, although its impact on animal production efficiency remains unknown (Welch et al., 2020). Previous studies have not explored how efficiency changed in the cow herd with diet and how the microbial population of the GIT in ruminant animals affected these shifts in efficiency. Therefore, the objective of this study was to determine whether ruminal and fecal microbial populations based upon different diets impacted heifer efficiency.

MATERIALS AND METHODS

The procedures outlined in this study were approved by the University of Georgia's Animal Care and Use Committee (AUP #: A2020 07-008-Y1-A0).

Animals, Facilities, and Diet

The heifers used in the present study were selected from a feed intake study measuring the effects of diet on expected RFI and ultrasound carcass characteristics (Pisani et al., 2021). Heifers were separated by predicted feed efficiency (n = 12 heifers/ RFI class), stratified by body weight (**BW** 275.5 kg $\pm$ 48.3), and assigned to 1 of 5 pens (4 heifers/pen) or due to facility constraints, 1 of 4 individual pens (1 heifer/pen), which will be referred to as pen 6 hereafter. Heifers sired by bulls with high feed efficiency were classified as high efficiency (**HI**). Heifers sired by bulls with low feed efficiency were classified as low efficiency (**LO**).The heifers had constant access to water and were individually fed 1 of 2 diets using Calan-gate Feeders (American Calan Inc., Northwood, NH). Diet 1 was a feedlot-like ration, high in concentrates (**Grain**; ME= 1.33 Mcal/lb; Pisani et al., 2020**)**. Diet 2 was a forage-based ration consisting of only bermudagrass hay (**Hay;** ME= 0.91 Mcal/lb; Pisani et al., 2020**)**. For 21 d the heifers were

trained to eat from the Calan-gate feeders and were adapted to their respective diets before the beginning of the study.

The study was divided into two consecutive feeding periods of 28 d each with a 21 d transition time between feeding periods. In this paper, the first feeding period is referred to as P1, and the second feeding period is referred to as **P2**. For P1 pens 1, 2, and 3 (**group 1**) were offered *ad libitum* access to diet 1, while pens 4,5, and 6 (**group 2**) were offered *ad libitum* access to diet 2. Following P1 both groups were transitioned for 21 d over to the alternate diet for P2. Both groups were fed to allow 10% refusal of feed. Feed refusal and body weight were measured weekly to calculate daily DMI and ADG. After P1, RFI was calculated and 20 heifers were selected (n = 10 heifers/ diet) by most extreme RFI status (n = 10 n-RFI, n = 10 p-RFI).

Rumen Fluid, Fecal, and Blood Collection

On d 0 and 28 of both periods blood, rumen, and fecal samples were collected for all 20 heifers. Fecal samples collected using the hand-grab method were placed in 50-mL sterile conical tubes and stored on ice before being stored at -20°C until further analysis. Rumen fluid collection was performed by esophageal tubing using a weighed metal perforated probe and an electric vacuum pump. About 200mL of fluid was collected, placed in 50-mL sterile conical tubes, and placed on ice before being stored at -20°C until further analysis. Blood samples were obtained from the coccygeal (tail) vein and placed in a cooler until processing later that day. Blood samples were centrifuged at 2000 × g at 4°C for 10 minutes. Using a plastic pipette, the serum layer was removed from the resulting sample and placed in a 1.5 µL microcentrifuge container and stored at -75°C.

Analysis of Volatile Fatty Acids

Rumen fluid samples were thawed and vortexed for 30 sec to produce homogenized samples which were added (1.5 mL) into a centrifuge tube. Feces (1g) were diluted with 3 mL of distilled water, and placed into 15-mL conical tubes. Tubes were vortexed for 30 sec to produce a homogeneous sample and 1.5 mL of the mixture was transferred to microcentrifuge tubes. Tubes were centrifuged at 10,000 × *g* for 10 minutes. Supernatant (1 mL) was transferred to a new centrifuge tube and combined with 200 µL of a metaphosphoric acid solution (25% v/v). Each sample was vortexed for 30 sec to ensure proper mixture and stored at -20℃ overnight. The following morning, samples were thawed and centrifuged at 10,000 × *g* for 10 min**.** Supernatant was placed into polypropylene tubes with ethyl acetate in a 2:1 ratio of ethyl acetate to supernatant. Samples were vortexed for 10 sec and allowed to settle for 5 min to optimize separation. 600 µL of the top layer was transferred into screw-thread vials for analysis of the volatile fatty acid (VFA) concentrations. A Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector and a capillary column (Zebron ZB-FFAP; 30 m x 0.32 mm x 0.25 µm; Phenomenex Inx., Torrance, CA, USA) was used for VFA analysis. This equipment utilized helium as the carrier gas. Sample injection volume was 10 µL. The column temperature started at 110℃ and gradually increased to 200℃. The injector and detector temperatures were set to 250℃ and 350℃, respectively.

1.2 DNA Extraction and Sequencing

Deoxyribonucleic acid (**DNA**) was extracted from the samples following the procedures described by Welch et al. (2020) with slight modifications. Briefly, this procedure uses 250 µL of sample placed in 2-mL Lysing Matrix E tubes (MP Biomedicals LLC, Irvine, CA, USA),

which are homogenized using a QIAGEN vortex adapter (QIAGEN, Venlo, the Netherlands) to disrupt the cells. Enzymatic inhibition was achieved by using InhibitEX Buffer (QIAGEN, Venlo, the Netherlands), and DNA elution and purification were carried out using a spin column and a series of specialized buffers according to manufacturer's specifications (QIAamp Fast DNA Stool Mini Kit; QIAGEN, Venlo, the Netherlands). Determination of DNA concentration and purity in the resulting eluate was performed spectrophotometrically using the Synergy LX Multi-Mode Microplate Reader in conjunction with the Take3 Micro-Volume Plate (BioTek Instruments Inc; Winooski, VT, USA). Samples with a minimum volume of 100 µL and 10 ng/µL of DNA were stored at 4 °C until the following day. Samples that failed to meet these requirements were rejected and subjected to a new DNA extraction cycle.

Following DNA extraction, samples were taken to LC Sciences Biotech (Houston, TX) for library preparation and 16S ribosomal ribonucleic acid (**rRNA**) gene sequencing. The library preparation step included polymerase chain reaction (**PCR**) replications using the forward: S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and reverse: S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) primer pairs (Klindworth et al., 2013), followed by a PCR clean-up using AMPure XP beads (Beckman Coulter Life Sciences, Indianapolis, IN, USA). A second PCR step was then carried out to attach Illumina's indices and sequencing adapters (Nextera XT Index Kit; Illumina Inc., San Diego, CA, USA), followed by another PCR clean-up step using AMPure XP beads. Following this final library clean up, the library was quantified using qPCR, and the nucleotides were sequenced using an Illumina MiSeq instrument and a MiSeq v3 reagent kit (Illumina Inc., San Diego, CA, USA). A well-characterized bacteriophage PhiX genome (PhiX Control v3 Library; Illumina Inc., San Diego, CA, USA) was used as a control for the sequencing runs.

The sequencing data was demultiplexed and converted to FASTQ files. Pair-end reads were set and merged using BBMerge Paired Read Merger v37.64 with default sensitivity and an expected insert size of 500 bp. The files were analyzed using QIIME pipeline v1.9.1 (Caporaso et al., 2010), quality-filtered according to the default values provided in QIIME's script "multiplpe_split_libraries_fastq.py", merged into a single file, and converted into the FASTA format. Sequences were clustered based on operational taxonomic units (**OTU**) at 97% similarity using the Uclust OTU picking method and the Greengenes database (gg_13_8_otus). Samples that did not align to PyNAST were excluded from the analysis. Sequencing depth was set at 3,148 sequences per sample.

2.5 Statistical Analysis

All analyses were performed using the rumen fluid and feces collected as the experimental unit. A replicated 2 × 2 factorial arrangement was used to analyze the effect of diet, RFI, and their interaction on the microbial population and VFAs. Residual feed intake was calculated as the difference between actual DMI and the DMI predicted from multiple linear regression of DMI on mid-test metabolic weight ($BW^{0.75}$) and ADG using the following model:

$$DMI = \beta0 + \beta1\text{mid-test } BW0.75 + \beta2ADG + €,$$

where β0 was the y-intercept, β1 was the partial regression coefficient of mid-test $BW^{0.75}$, β2 is the partial regression coefficient of ADG, and € was the error term.

Resulting in four treatment combinations: HI, grain; HI, hay; LO, grain; and LO, hay. Data were analyzed in Minitab v.19.2020. Alpha-diversity and phylum data were collected using the GLM procedure, where diet, efficiency, and their interaction were factors and microbial measures were the response. Pearson correlations were also made between microbial populations and VFA concentrations in ruminal and fecal contents.

Results

Evaluation of ruminal microbial population

There were no Efficiency group (**EG**) × Diet interactions for all alpha-diversity indices, except in P2 there were trends for Operational taxonomic units (**OTUs**) and FaithPD ($P = 0.084$; $P = 0.074$, respectively; Table 1). In P1, OTU and FaithPD tended to be greater in heifers fed hay than grain, but there were no differences between EG. Conversely, in P2 OTU and FaithPD were not different between diets for HI heifers; however, LO heifers fed hay had greater OTUs and FaithPD than when fed grain. There were no EG main effects ($P > 0.311$) on any alpha-diversity measures in P1 or P2. There were no day effects ($P > 0.171$), except in P2 Shannon diversity and evenness ($P < 0.046$) were greater on d28 than 0. All alpha-diversity measures were greater in hay than grain ($P < 0.001$) throughout both periods.

Over 30 phyla were detected within the samples, and the most prevalent were: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. There were no EG × Diet interactions ($P > 0.220$; Table 5) for the four phlya, except a trend in P1 for Firmicutes ($P = 0.086$) to be greater in HI heifers fed grain. LO heifers fed grain had a percentage of Firmicutes that was not different from HI heifers in the same dietary treatment, or from heifers in either EG fed hay. There were no efficiency main effects ($P > 0.171$) on any of the four phyla in either period. On d 28 of both periods Firmicutes was greater ($P < 0.002$) similarly, in P2 Actinobacteria was greater ($P = 0.024$) on d 28 than 0. Conversely, Proteobacteria was greater ($P = 0.002$) on d 0 than 28 in P2 and tended ($P = 0.098$) to do the same in P1. There were diet main effects ($P < 0.009$) for all phlya in P1, and for Bacteroidetes and Proteobacteria ($P = 0.006$, $P = 0.002$, respectively) in P2. In P1 Proteobacteria, Firmicutes, and Actinobacteria were greater ($P < 0.001$) in heifers fed grain than hay, while Bacteroidetes was increased ($P < 0.001$) in heifers hay rather than grain. Two other

phyla of some significance were Verrucomicrobiota, and Spirochaetota,. The abundance of Verrucomicrobiota was greater ($P < 0.001$; Fig. 1) in heifers hay versus grain, in both periods. Spirochaetota, in P1was greater ($P < 0.024$; Fig. 2) in HI than LO heifers; in both periods heifers fed hay had greater ($P < 0.019$) percentage of Spirochaetota than those fed grain.

Discussion

Dietary changes in cattle can cause dramatic shifts in rumen pH and consequently the microbial population that resides within it (Hungate, 1966).By shifting diets, energy available to the host animal in the form of dietary substrate (e.g. CHO) is altered, but so is energy available to the host derived by the microbial fermentation in the rumen and hindgut. Dietary differences that alter the microbial ecosystem endproducts can alter the host animal's feed efficiency .

Most differences in alpha diversity were a result of different diets, except for richness and Faith's Phylogenetic Diversity which differed because of the interaction of diet and efficiency group. Regardless of the order in which they were fed (period), hay diets resulted in increased diversity, evenness, and richness. These present results differ from those of Kocherginskaya et al. (2007), who found in an in-vitro study of ruminal bacterial populations of steers fed corn or hay diets that Shannon and Simpson diversities were greatest in rumen fluid from steers fed the corn diet. Petri et al. (2012) also saw an increase in diversity in Angus heifers fed a high concentrate ration without forage, as opposed to a high concentrate ration with forage. Petri et al. (2013) observed the ruminal microbiome of Angus heifers during a transition from forage to concentrate and during an acidotic challenge; the results of DGGE analysis in combination with pyrosequencing, revealed greater diversity of OTU's in forage and mixed forage diets compared to those that were high in grains, which is similar to results in the present study. Kocherginskaya et al. (2007) and Petri et al. (2012) both utilized 16S PCR-DGGE, this same method was also

applied by Petri et al. (2013); however an additional Roche 454 pyrosequencing (an early Next Generation Sequencing technique) increased sequencing reads and provided a more in depth coverage of the rumen microbiome. Further investigation into the relationship between microbial diversity and diet, using more advanced sequencing technology such as that utilized in the present study, is necessary to further uncover relationships between diet, the rumen microbial population, and feed efficiency.

Connections between alpha-diversity and diet were apparent in the current study; however, there was no observed relationship between alpha-diversity and feed efficiency. Contrary to the current findings, previous literature has found a distinct relationship between the diversity and richness of the ruminal microbiome and feed efficiency (Shabat et al., 2016). A previous study by Shabat et al. (2016) examined the ruminal microbiome of 78 Holstein Friesian cows which represented the most efficient and lest efficient of their herd. The biodiversity results indicated that lower richness and diversity was linked to increased feed efficiency. Similarly, Welch et al. (2020) examined the ruminal, fecal, cecal microbiomes of Angus steers selected for divergent feed efficiency, following 16s rRNA sequencing a decrease in evenness was associated with an increase in cattle feed efficiency. Shabat et al. (2016) reported that the ruminal microbiomes of more efficiency dairy cows had lower alpha diversity but produced more host-relevant metabolites. The differences between the current study and Shabat's results may be due to the feed efficiency evaluation period. Heifer growth, feed intake, and feed efficiency in beef cattle using the GrowSafe system was performed and it was determined that to accurately measure ADG, DMI, feed conversion ratio, and RFI were 63 35, 42, and 63d, respectively (Wang et al., 2006). Meaning the 28-d period in the present study may not have been long

enough to accurately determine RFI and observe differences in the ruminal microbiome as it applies to feed efficiency.

Similar to other studies, the main phylum observed were Firmicutes, Bacteriodetes, Proteobacteria, and Actinobacteria (Myer et al., 2015: Myer et al., 2017;Bi et al., 2018; Li et al., 2019). Two additional phyla, Verrucomicrobiota and Spirochaetota, were also somewhat abundant. Verrucomicriota was greater in rumen fluid from heifers fed hay, bacteria from this phyla have been recognized as aerobic methantrophs (Dedysh & Dunfield, 2011) meaning phyla mostly only grow on methane and in some instances on methanol, formate, formaldehyde, and methylamines . In the present study, Spirochaetota were associated with efficiency and diet; typically bacteria from this phylum increased with increasing levels of dried distillers grain, a high concentrate (Callaway et al., 2010).Genus and species within these phyla have been associated weight gain, methane recycling, and feed efficiency (Dedysh & Dunfield, 2011; Li et al., 2019; Welch et al., 2020); however, analysis of these roles are not complete.

In the present study, there was no distinction between the profiles of HI and LO heifers; this could potentially be due to differences in diet. Carberry et al. (2012) observed rumen fluid from heifers fed a high forage diet (HF) and low forage diet (LF), that were representative of high RFI (H-RFI) and low RFI (L-RFI). When comparing bacterial profiles from HF heifers, there were distinct clusters, suggesting differences between bacterial population in rumen fluid of H-RFI and L-RFI animals existed. In contrast, when bacterial profiles from LF heifers were analyzed there was no consistent segregation of the two RFI phenotypes. Although there were no differences attributed to efficiency, many differences in the ruminal microflora can be attributed to diet. The observed differences in phyla due to changes in diet were expected based on past literature. Actinobacteria, Firmicutes, in period 1, was greater in heifers fed grain; Petri et al.

(2013) observed the rumen microbial environment of 8 heifers challenged with acidosis, the results revealed that during acidosis genus' from phyla Actinobacteria and Firmicutes were prevalent; this indicates that Actinobacteria and Firmicutes increased with the inclusion of a high grain ration. However, the role of Firmicutes remains largely unknown, with the Firmicutes:Bacteroidetes ratio has been linked to obesity in mice (Murphy et al. 2010). More information about these phyla and their role in dietary changes can be unveiled once further analysis has been performed to reveal which genera are also present.

Evidence from the current study shows that diet has more direct impact on the ruminal microbiome than did RFI classification. However, further analysis must be performed on the VFA concentrations as well as the remaining microbial population data in order to make connections between these dietary shifts and host animal feed efficiency.

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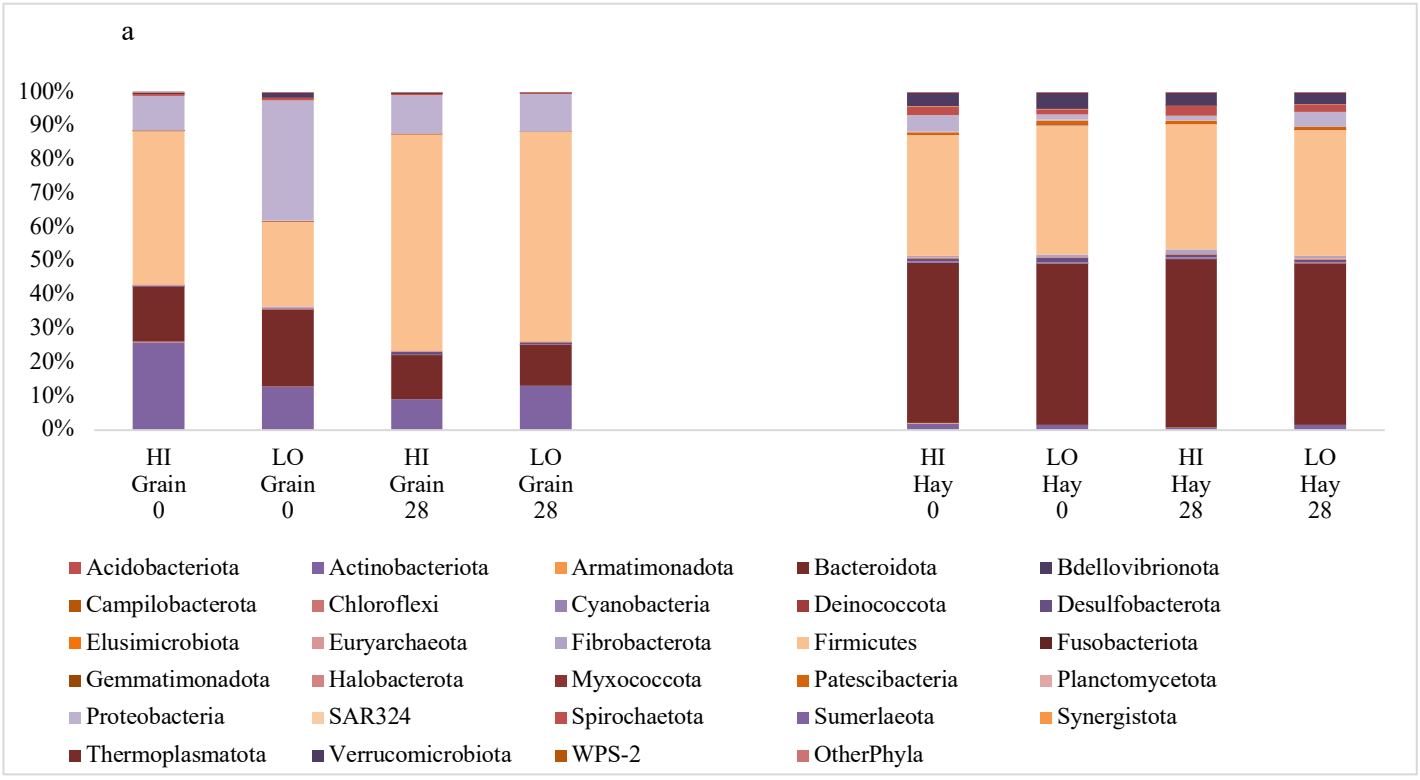

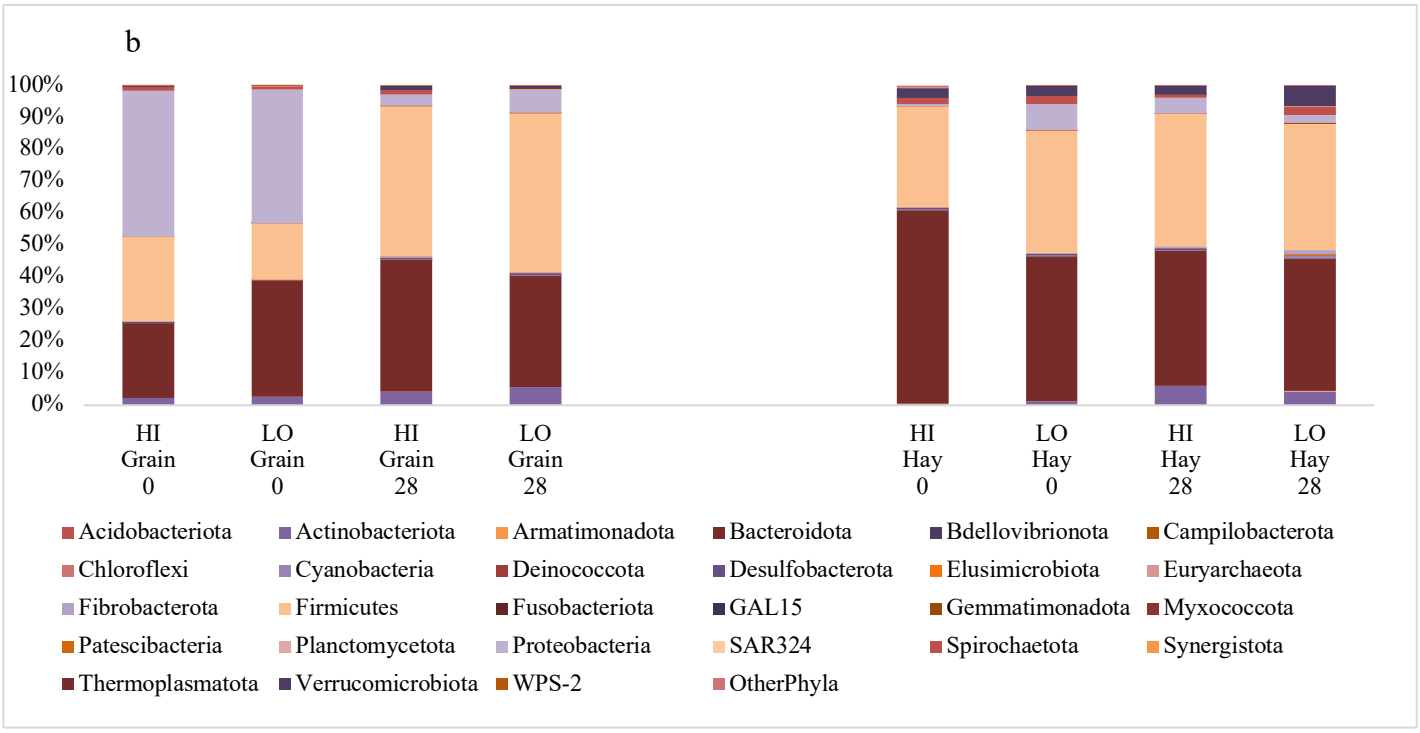

Figure 4.1. Abudances of phyla in the rumen

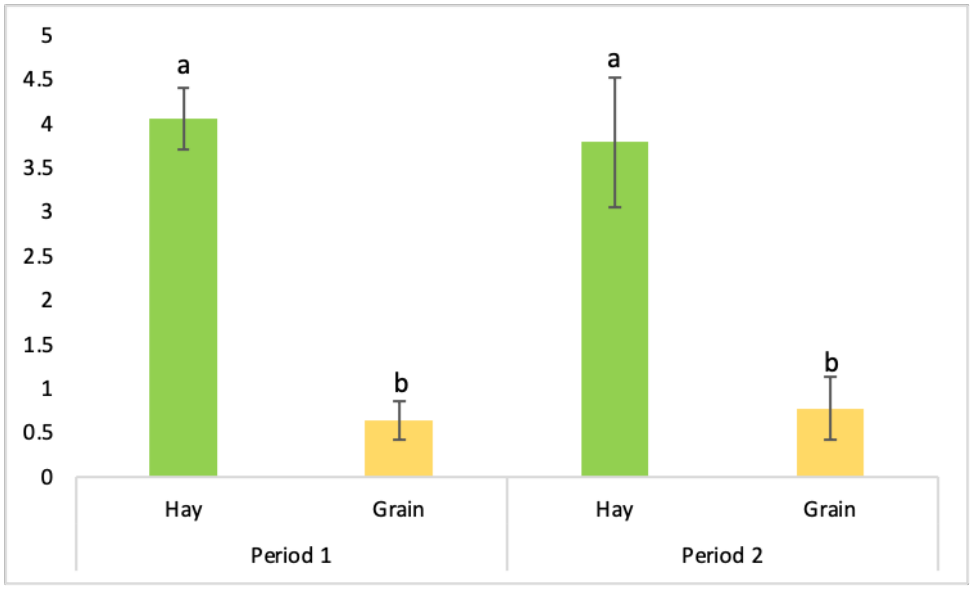

Figure 4.2. Abundance of phylum Verrucomicrobiota in heifers fed grain vs. hay

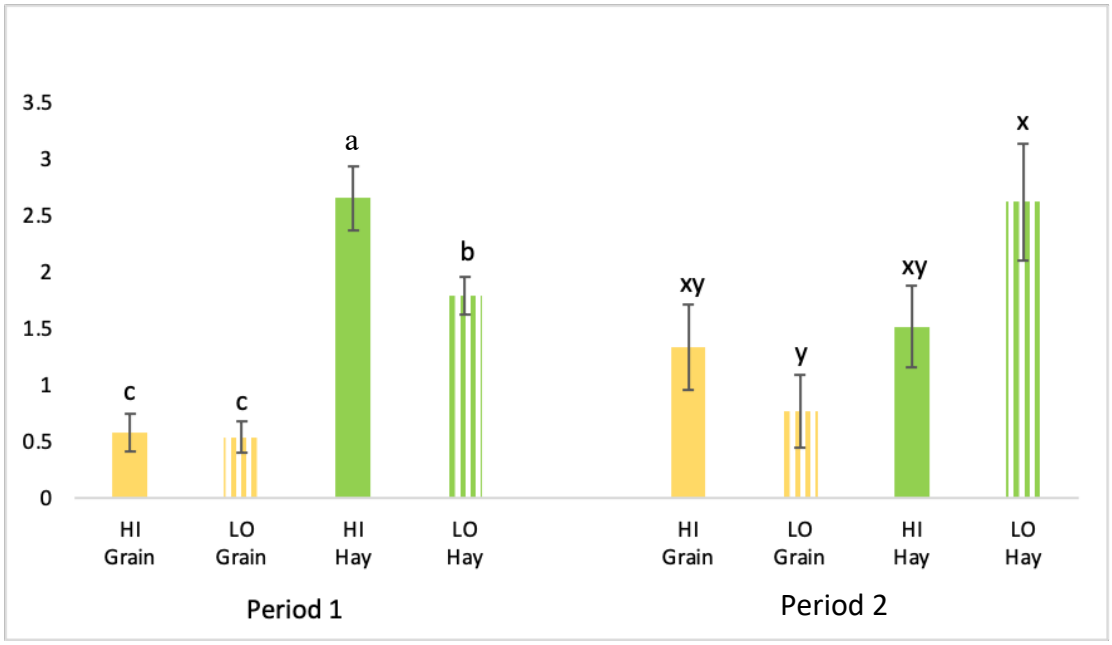

Figure 4.3. Abundance of phylum Spirochaetota in high efficiency and low efficiency heifers fed grain vs. hay.

Table 4.1. Abundances of four main phyla observed in the rumen in between diets and efficiency groups

Period 1

	LO				HI					*P*-value			
	Hay		Grain		Hay		Grain						
Phylum, %	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	SEM	Diet	Day	Efficiency	Efficiency*Diet
Firmicutes	38.230	37.760	25.050	62.130	35.750	37.210	45.500	64.020	2.560	<0.001	0.002	0.171	0.086
Bacteriodetes	47.620	47.710	22.590	12.130	47.350	49.770	16.370	13.050	2.840	<0.001	0.256	0.721	0.473
Actinobacteriota	1.577	4.260	13.020	13.100	2.040	1.413	26.110	11.360	11.210	0.009	0.239	0.234	0.220
Proteobacteria	1.627	1.603	35.600	11.060	4.840	0.836	10.330	9.190	17.650	<0.001	0.098	0.409	0.378

Period 2

	LO				HI					*P*-value			
	Hay		Grain		Hay		Grain						
Phylum, %	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	SEM	Diet	Day	Efficiency	Efficiency*Diet
Firmicutes	38.200	39.570	17.450	49.650	31.170	41.280	26.430	46.750	2.340	0.520	<0.001	0.960	0.460
Bacteriodetes	45.200	41.440	36.400	34.870	60.600	42.260	23.300	41.290	2.550	0.006	0.760	0.606	0.223
Actinobacteria	1.357	4.380	2.768	5.640	0.361	6.040	2.768	4.230	4.525	0.620	0.024	0.840	0.663
Proteobacteria	8.020	2.165	41.800	6.860	0.621	4.880	45.500	3.342	14.140	0.002	0.002	0.852	0.837

CHAPTER 5

CONCLUSION

After evaluating Angus heifers selected for high and low feed efficiency over a grain diet and hay diet, results indicate that diet may be more impactful on cattle performance, rumen microbial population, and ultrasound carcass characteristics, than feed efficiency status. Although, predicted feed efficiency was a good indicator of heifer RFI. The heifers fed hay had increased microbial diversity, evenness, and richness, and had more prevalence of the phyla *Bacteroidetes* and *Verrucomicrobiota*. Further analysis must be performed on the VFA concentrations as well as the remaining microbial population data in order to make connections between these dietary shifts and host animal feed efficiency.

In the first study, our results indicated that efficiency group based off of sire RADG, DMI, and RFI was accurate in predicting actual RFI and could be a good selection tool for producers interested in improving their herds RFI. The lack of interaction between efficiency group and dietary treatment indicated that by selecting for grain driven EPDs for efficiency, producers may not be negatively impacting their breeding herd on pasture. But due to the potential negative implications, further research needs to be performed to address the changes in RFI during diet change observed for the most extreme animals.

Preliminary results from the second study demonstrated differences in microbial population influenced by diet. Heifers fed hay had increased diversity, evenness, and richness which in past literature has been associated with a decrease in efficiency. Comparison against additional animal performance data, as well as additional microbiome data, must be performed in

order to make that implication. Phyla such as *Actinobacteria*, *Firmicutes*, and *Proteobacteria* were more abundant in the rumen of heifers fed grain in the first period; while *Bacteroidetes* and *Verrucomicrobiota* were more abundant in the rumen of heifers fed hay. Additonally, one phyla, *Spirochaetota*, was found in greater abundance in high efficiency (HI) than low efficiency (LO) heifers in P1, and in both periods was found in greater abundance in the rumen of heifers fed hay. As stated, further analysis and comparisons between microbiome data, VFA, and heifer performance are required to make more decisive conclusions on the role of the microbial population of the rumen in host animal feed efficiency.

Collectively, our results indicate that diet may be a greater, direct influence on host animal feed efficiency. Further analysis of the data is required to determine impact of the microbial population at the genus level, as well as in the hindgut. Determining the role of the bacterial population on host's feed efficiency could allow for the manipulation of these microbial populations to improve the feed efficiency of beef cattle.