AN EXPLORATION OF BIOLOGICAL MECHANISMS THAT IMPACT INTAKE AND FEED EFFICIENCY IN THE GRAZING ANIMAL

A Thesis

by

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ABSTRACT

Biological mechanisms that potentially contribute to residual feed intake (RFI) have not been fully understood in the grazing animal. The objective of this study was to determine the differences of RFI measured in confinement (RFIc) or grazing (RFIg) on animal performance. Animals were previously classified in confinement as high RFI (HRFIc), or low RFI (LRFIc) and subsequently under grazing as high (HRFIg) or low (LRFIg). Effects of forage quantity on dry matter intake (DMI), and biological mechanisms that contribute to variations in RFI were investigated using ultrasound, carcass traits and bacterial populations.

Bulls were allotted to replicate bermudagrass pastures at low (LSTK) or high (HSTK) stocking intensities and heifers grazed one Ryegrass pasture. Ruminal microbial content was collected and profiled using bacterial tag-encoded FLX amplicon pyrosequencing technique. In 2009, bulls were harvested directly off the pasture and ultrasound and carcass measurements were determined. Data were analyzed using PROC GLIMMIX of SAS. Linear regressions were obtained using PROC REG to estimate RFI.

In 2009, there was a difference for LRFIc bulls in F:G (P=0.032), and HRFIg bulls on LSTK had an interaction for ADG (P=0.043). HRFIg bulls had greater intakes regardless of STK (P=0.003). In 2010, HRFIc bulls remained heavier throughout with the greatest DMI (P=0.0095). There were no differences for any traits for 2010 RFIg bulls. At a LSTK, HRFIg bulls tended (P>0.05) to have a lighter gastrointestinal tract

(GIT) weight (P=0.093) while liver weight (P=0.072) tended to be heavier for all bulls. The small intestine was heavier for LRFIg bulls (P=0.09) on a HSTK. There was an interaction for microbial bacteria identified in the rumen in 2009 on hemicellulolytic (P=0.048), starch (P=0.025), and pectinolytic (P=0.057) degrading bacteria. HRFIg bulls at a LSTK had a greater percentage for amylolytic and pectinolytic degrading bacteria (P=0.008 and P=0.051, respectively) in the large intestine. There were no interactions for any substrates in 2010.

DMI (P<0.0001) was greater each year for HRFIc heifers and was greater (P=0.0168, P=<0.0001, P=<0.0001) each year respectively for LRFIg heifers. No differences were found for initial BW, final BW, MetBW, and ADG in the RFIc or RFIg classes.

HRFIg bulls with the greatest forage availability consumed more and had heavier GIT, but it is still unclear how the microbial fauna affected the efficiency among RFI phenotyped bulls

DEDICATION

This thesis is dedicated to my immediate and extended family and my friends and co-workers. My personal support system is the greatest of anyone I know. Without all of you here to provide supportive advice, unconditional love, even sometimes a much-needed distraction or to just listen to me, this would have been the most challenging 3 years of my life. You have all been my own little army to help me accomplish anything I set out after. Of course, the two people that have provided the most emotionally, spiritually, and financially without questioning "*What in the world?!"*, are Mom and Daddy. I love you guys, I hope to never disappoint you. You have instilled a mind set to never give up on anything I want and to never give up on something I start.

"Whether you think you can, or think you can't, you're probably right." Henry Ford

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CHAPTER I

INTRODUCTION

The cattle industry strives to achieve the greatest profit with the least amount of input. Particularly, the beef cattle industry has always been confronted with the challenge of providing at a profitable margin adequate feedstuffs to meet animal requirements as suggested by the National Resource Council (NRC). The United States Department of Agriculture's (USDA) 2007 Agricultural Census reported that the United States has more than 760,000 beef cattle ranches with more than 32 million head of cattle (USDA, 2007). For these ranches, the most quantifiable inputs for each operation are the feed costs. Although information is available about new and more cost effective feedstuffs, researchers, and producers have to continuously work together to identify and develop the most sustainable methodologies and techniques to help the animal industry.

The USDA Agricultural Census (2007) reported that from 2002-2007, the greatest increase in cost for producers raising beef cattle in the United States was feed, with a 45% rise over those five years. In response to the rise in production costs, producers have started to maintain animals under grazing conditions for a longer period of time. Maturing beef cattle animals spend more than 70% of their lifetime on forages to mature and gain weight, whereas cows and bulls remain on pasture their entire lifetime (Rouquette et al., 2009). As the numbers of animals kept under grazing increases, the producer has developed a secondary problem. The total acres of farmland

per total US land area (acres) has decreased 1.4% (USDA Agricultural Census), meaning that land area for keeping cattle is decreasing.

We have to find more efficient ways to continue producing meat animals. For example, animal scientists have made great strides with ionophores. Originally used to control internal parasites in the poultry industry. They are now utilized to improve feed efficiency in cattle by reducing the dry matter intake (DMI) with little effect on average daily gain (ADG) of cattle in feedlots or increase ADG for cattle on pasture by adjusting ruminal microbial populations. Monensin is an example of an ionophore that is marketed as a methane inhibitor, but is also known to reduce dietary protein deamination and therefore likely reduces N excretion when appropriate amounts of feeds are fed to animals (Russell and Strobel, 1989; Tedeschi et al., 2011). Similar responses were found with condensed tannins. Originally, condensed tannins were considered anti-nutritional because of their ability to bind to proteins, metal ions, and polysaccharides and reduce DMI. However, potential benefits are improved ADG, reduced gastro-intestinal parasitism and methane emissions (Waghorn, 2008; Krueger et al., 2010; Tedeschi et al., 2011). Agricultural scientists and professionals in the industry continuously work to identify newer and stronger methodologies for animal efficiency and implementing them with the goal to continue to provide food and fiber for the world's growing population.

Conventionally, producers are looking at maximizing their outputs such as live weight, carcass weight, or meat quality while having the same end goal in mind: to be profitable. Profitability is a function of both outputs and inputs and any amount of reduction in feed input can improve feed efficiency and increase profit. Selecting

animals with lower energy requirements and superior feed utilization would potentially reduce production cost. It has been suggested that intake is the most distinct variable in determining an animal's performance. The rate of intake can be regulated by digestibility of the forage, which is based on a plant's rigid composition of fibrous mechanisms (Illius, 1998; Romney and Gill, 2000). The composition of the forage consumed by the cattle can alter how effectively an animal will be able to retrieve the nutrients needed from the plant. Thus, factors such as quality and quantity of the forage along with animal behavior and physiological status can affect their DMI. Mayes and Lamb (1984) were the first to investigate the possibility of using n-alkanes (plant waxes) as a method to predict the DMI. Their research followed Body and Grace (1981), who studied the methodology of using long chain fatty acids as an indigestible fecal marker. Mayes and Lamb (1984) determined that n-alkanes are more inert than long chain fatty acids and are simpler to analyze. Dove et al. (1988) suggested adding an alkane and heptane solution to powdered cellulose in gelatin capsules. Although equally accurate as previous techniques, it is perhaps more easily and rapidly prepared. Many researchers have successfully estimated intake using the n-alkane technique in sheep (Mayes et al., 1986; Dove et al., 2000) and dairy cattle (Dillion and Stakelum, 1989; Unal and Garnsworthy, 1999). Olivan et al. (2007) confirmed the reliability of the n-alkane technique including the C_{32}:C_{33} technique to be used in predicting the DMI of grazing cattle.

Another trait that is important for beef cattle production is feed efficiency. Feed efficiency is the conversion of feed into animal products (e.g., meat, milk) varying

between animals. Feed efficiency is not a directly measurable trait (Koch et al., 1963) Feed to gain ratio (F:G) has been used as an indicator trait for efficiency and is computed from the DMI and ADG. The F:G ratio has been used in selection programs, but Koots et al. (1994) reported a negative correlation between ADG, body weight (BW) and F:G, indicating increases in growth rate and mature animal size (Herd and Bishop, 2000). Originally proposed by Koch et al. (1963), residual feed intake (RFI), as expected, is phenotypically independent of the constituent traits BW and ADG, unlike F:G. RFI has been used more frequently for measuring feed efficiency on an individual animal basis and can be used as an alternative form of measuring feed efficiency instead of F:G.

Ruminant animals have a symbiotic relationship with anaerobic microorganisms (bacteria, protozoa, and fungi) in the rumen. These microorganisms are capable of digesting complex plant polysaccharides, such as cellulose and starch, into volatile fatty acids that can be used by the ruminant animal as an energy source. Only 1% of gastrointestinal bacteria collected have been identified utilizing a variety of DNA

techniques (Nocker et al., 2007b). The bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) technique (Dowd et al., 2008a) is a modern technology that can help to clarify the diversity of rumen microbes. A better understanding of the inhabitants of the ruminant gastrointestinal tract will open new opportunities for explaining the influence they have on animal health and production.

The rumen has an advantageous characteristic that other animals lack. Inside the rumen, ingested feed can be hydrolyzed and the microbial enzymes from the rumen breakdown complex dietary polysaccharides to usable end-products (e.g. sugars and VFA) (Beever and Mould, 2000). Due to this distinctive trait, and that grasses and other forages provide nutrients at a low cost, it is no surprise they comprise the diet for most ruminant animal systems (Wilkins, 2000). The pyrosequencing technique can be utilized to identify which specific bacteria are in the rumen and how they influence animal performance.

Objectives

The first objective was to estimate the intake of grazing animals using the n-alkane technique during the optimal fecal collection period to estimate DMI and to further determine residual feed intake of grazing animals (RFIg) on Coastal bermudagrass or Ryegrass.

The second objective was to investigate the relationship between residual feed intake (RFI) previously determined in confinement conditions (RFIc) and RFIg.

The third objective was to investigate the effects of forages of varying qualities and quantities on the DMI and digestibility of forage in RFI-phenotype-indexed cattle while grazing. Forage DMI was determined for grazing cattle on Coastal bermudagrass or Ryegrass using the alkane technique as detailed in the first objective.

The forth objective was to identify factors that contribute to the variation in RFI rank of grazing cattle by examining ultrasound and carcass traits and using the pyrosequencing technique to identify bacterial populations for individual animals.

CHAPTER II

LITERATURE REVIEW

Feedstuff prices can fluctuate drastically depending on the time of year, the weather, and national and international markets. Weather is a major factor and although it can be forecasted, it is difficult to prepare for season long droughts or consistent days of rain. The feedstuffs purchased by a producer may not even come from the area or the region in which the cattle are being raised. Therefore, the national commodities market influences purchase price for the small farmer in the southern or western United States. As the United States has seen over the past few years, each year has provided much variance on the price of livestock feed. Some major factors include competition for feedstuffs from other industries such as the competition for corn as a feed stock for the ethanol industry, or the use of cottonseed hulls by the oil and gas industry.

Forage-based beef systems have been facing greater challenges in recent years. These systems are dealing with difficult economic conditions as a result of increased costs in feedstuffs for making feedlot rations, fertilizer for pastures in production of hay or for grazing purposes, and increased fuel prices for transportation and harvest. Reports from the USDA-ERS in 2009 discussed the challenge faced by feedlots from the increase in corn prices. Cattle were rejected at the feedlot level and opted to keep animals on pasture for a longer period or to even finish them on forage (Quanbek and Johnson, 2009). Potential meat animals, prior to feedlot entry, can be kept longer on pasture alongside the animals that are on pasture 100% of their lifetime, cows without

calves, and adolescent cattle and bulls. However, a secondary drawback is that the amount of national agriculture land area decreased 21% from between the 1997-2002 to 2002-2007 reporting periods. Pasturelands are continuously converted to developed lands, rangeland, CRP land, cropland, forestland or water areas. From 1982-2007, 40% of the national pastureland was converted to these other land uses. Although some of these lands are converted to pastureland, there was still about a 9% loss of the total acreage of pasture from 1982-2007 (USDA-NRI, 2007).

The productivity level of the land has become more important as animal productivity per area has become a concern in order to maintain the food supply. It has always been a challenge to sustain pastures with good quality and quantity of forage year round. Rouquette et al. (2009) reported that, of the land-grant universities surveyed, 70% of their research dealt with forage physiology and management. He also reported that, of universities surveyed, 60-70% of research efforts were toward emphasis on grazing and animal performance, forage utilization systems, and forage cultivar evaluations. This research is important to producers in order to make better decisions and remain aware of new technologies available to increase production levels.

Residual Feed Intake and the Grazing Animal

Measures of Feed Efficiency

Since more than two-thirds of beef animals remain on pasture and require additional feed inputs, it would be optimal if production operations could identify animals that have lower maintenance energy requirements. Maintenance efficiency is recognized as the feed energy requirement for zero BW change (Ferrell and Jenkins,

1985). For a typical beef herd, the feed energy for maintenance represents 60-75% of the total energy requirement of an individual breeding cow (Klosterm, 1972; Ferrell and Jenkins, 1985). Although this is important, there are difficulties associated with this measurement. Typically, a producer's goal is to increase weight gain on their cattle as fast as possible. According to Taylor et al. (1981), it can take up to 2 years to obtain an authentic measure of maintenance. While it can be performed in a shorter period, there is a greater chance for error. This is impractical for a significantly sized operation due to the large requirement of resources.

For decades now, researchers have developed and improved upon various measures of feed efficiency. Normally feed intake is correlated with output traits, therefore making it difficult to isolate each. The inputs and outputs are different depending on the age, breed, sex, etc., therefore making it difficult to measure production system efficiency on individual animals. There are a variety of indices that exist for describing livestock feed efficiency. However, to make comparisons between individual or groups of animals, the input and production outputs for a specified cycle of production are used (Archer et al., 1999). Since beef cattle herd producers typically have two goals, restocking the breeding herd and/or producing animals for slaughter; adjustments have to be made for the selected index.

Feed efficiency is defined as the ratio of gain in BW, or gross efficiency, to feed input; or as the inverse, feed to gain ratio (F:G) (Koch et al., 1963). The F:G is a gross measurement of feed intake to weight gain. As mentioned, F:G is a commonly used measurement for feed efficiency for the industry and scientists (Nkrumah et al., 2004).

It has been well documented that F:G is associated with both phenotypic and genetic traits such as ADG and BW (Koots et al., 1994; Herd and Bishop, 2000). Brelin and Brannang (1982) summarized 4 studies of beef cattle and all demonstrated strong genetic correlations (-0.1 to -0.95) between growth rate and F:G in beef cattle. Although selection for improved F:G may improve efficiency for the growth and finishing phases of beef production, it may not directly influence the overall profitability of the entire production system. Koots et al. (1994), reported that F:G has a negative correlation with ADG and BW. Herd and Bishop (2000) reported that selecting for reduced F:G and improved feed efficiency is associated with increased BW and contributes to increased maintenance energy requirements and higher maintenance costs on cow herds.

Residual Feed Intake

Residual feed intake (RFI) is a current measurement used to calculate feed efficiency based on animal intake. The RFI is the difference between an individual animal's expected consumption and the amount consumed, based on average growth rate and average metabolic BW, is referred to as RFI. Koch et al. (1963) suggested RFI as an alternative to F:G as an attempt to solve the problems associated with F:G such as the negative correlation with ADG and BW. Residual feed intake is a measure of feed efficiency and it is computed as the difference of the actual feed intake and the feed intake predicted using the mean BW and ADG. Koch et al. (1963) suggested that feed intake could be adjusted for BW and ADG by separating feed intake into 2 components: 1) the feed intake expected for the specified level of production and maintenance, and 2) a residual portion. This residual portion can help identify which animals deviate from

their expected level of feed intake. Animals that consume less feed than expected, but also have the same or better performance than their counterparts are considered efficient (low, negative RFI), whereas their contemporaries consume more feed than expected and are considered inefficient (high, positive RFI). Efficient animals yield numerous benefits, including decreased dry matter intake (DMI) without affecting daily gain, a decrease in feed input and a decrease in cost. The RFI is calculated by a linear regression of DMI on ADG and metabolic BW ($MBW^{0.75}$):

$$y = \beta0 + \beta1(ADG) + \beta2(MBW) + RFI$$

where y is DMI, β0 is the regression intercept, β1 is the partial regression of daily intake on ADG, and β2 is the partial regression of daily intake on BW expressed as metabolic BW (MBW).

The RFI is a measurement that is used to calculate the expected feed intake and by definition is phenotypically independent of production traits such as ADG and BW. RFI is more desirable than F:G because the values can also be used to compare animals at different physiological states and different segments of the industry (Crews, 2005). Although this method is a more capable fit to identify efficient animals that are an appropriate size, it is costly and time consuming. For those reasons, commercial beef cattle operations are slow to adopt RFI as a selection tool.

Individual animals are able to be compared using RFI because of its independence of the production traits ADG and BW, unlike the other previous types of measurements (Herd and Arthur, 2009). The RFI may be correlated to overall

production system feed efficiency. Since the period of measuring for production has been adjusted, it is more likely to remove some effects of measurements, which complicate interpretation of gross efficiency (Archer et al., 1999). Although RFI is independent of ADG and BW, there is still variation in the RFI of animals. Previous researchers have reported that RFI is moderately heritable (Nkrumah et al., 2007; Crowley et al., 2010).

Genotypic residual feed intake is genetically independent of production, allowing it to more likely reflect genetic differences between feed intake and production. However, to obtain the genetic relationship between feed intake and production, it is required to calculate genotypic RFI, or to predict correlated responses in feed intake and production for selection based on phenotypic RFI. Information about genetic relationships between feed intake and production is limited for most animal production systems (Archer et al., 1999).

Since RFI is only moderately heritable (Herd et al., 2003), there are other factors that are associated with variations in RFI. Herd et al. (2004) and Richardson and Herd (2004) summarized that there are 5 major processes that influence variation; 33% is composed of heat increment of feeding (9%), digestion (14%), body composition (5%) and physical activity (5%), while the remaining 67% is from other processes (protein turnover, ion pumping, proton leakage). More recently, Herd and Arthur (2009) have redefined some of these mechanisms adding feeding patterns (2%) and redistributing other originally defined processes (Figure 2.1).

Determining individual animal uniqueness requires multiple measurements including those depicted in Figure 2.1. However, due to the difficulty of assessing DMI on grazing animals, few experiments have been performed in regards to these biological mechanisms in grazing animals. Based on previous experiments the use of n-alkanes has proven to be a reliable technique for reporting a constant prediction of DMI among grazing animals (Aguiar et al., 2012).

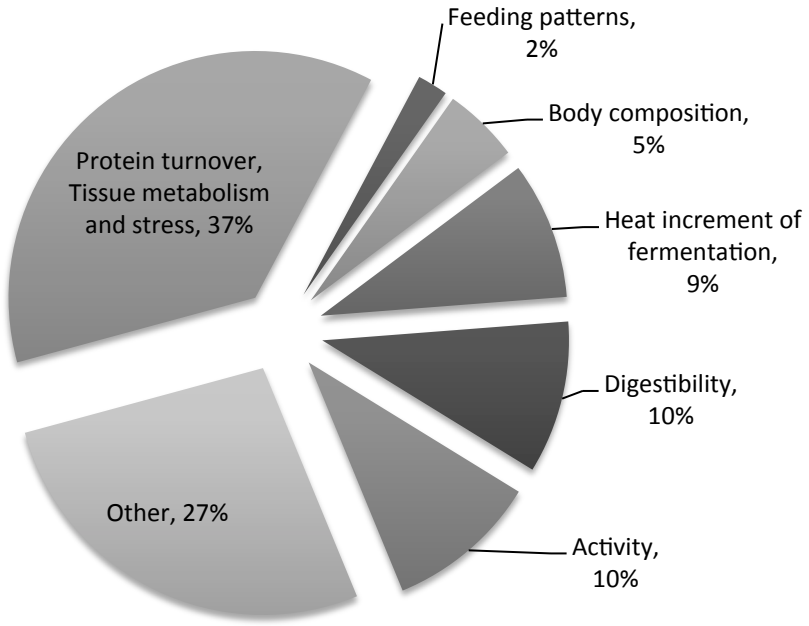

Figure 2.1. Biological mechanisms that contribute to variations in residual feed intake (Herd and Arthur, 2009).

Residual Feed Intake in the Confinement or Grazing Animal

Most researchers have used RFI as a measure of feed efficiency on confined animals. It is more difficult to measure intake in grazing systems because it is necessary

to predict feed consumed. There have been numerous experiments performed using confined feeding technologies. Little is known of the differences in grazing forage intake (Herd et al., 2003).

In a study by Herd et al. (1998), 41 lactating cows had been previously tested as young heifers on a pelleted ration and classified as low postweaning RFI or high RFI. The objective of their study was to determine if animals previously tested and ranked for postweaning RFI would be similarly efficient as grazing lactating cows on pasture. The low RFI cows did not consume any more feed than the high RFI cows, but they were 7% heavier, had similar subcutaneous fat stores, and reared calves of similar BW (Herd et al., 1998).

In another grazing study by Meyer et al. (2008), RFI had been determined for 42 Hereford heifers that were fed unprocessed alfalfa-grass mixed hay in a GrowSafe 4000E feed intake system. Two grazing experiments followed, using the same animals but as cows. In the first experiment, mid- to late-gestating cows grazed non-endophyte infected tall fescue paddocks. The average DM offered was less for low RFI cows. The BW and body condition scores (BCS) of low and high RFI groups did not differ over the period of the study, but low RFI cows had a 21% lower DMI. For experiment 2, low and high RFI cows and their calves were placed on tall fescue paddocks from late winter to early spring and because of shortage of forage from the winter were also fed pelleted soy hulls daily. However, the forage offered was similar for low and high RFI cow-calf pairs. Again, BW and BCS did not differ during the study between low and high RFI cows, but low RFI cow-calf pairs had numerically lower (about 11%) DMI than high

RFI pairs. Meyer et al. (2008) concluded that there were no intake differences between low and high RFI cows or maybe their methodology and limited number of animals prevented them from detecting differences.

The results of these studies illustrate the reasons that RFI is difficult to measure from a pasture standpoint. Forage in pastures that are intensely grazed begin to lose the quality and quantity of the forage available over a period of time. The base of a plant is higher in lignin and less digestible. In the study from Pieper et al. (1959), the chemical content (e.g. total protein, gross energy, etc.) of the diet consumed decreased as consumption increased. As availability decreases, selectivity of the forage consumed increases. Also as availability decreases, forage height decreases and animals graze lower portions of the plant that typically have poorer nutritional quality.

Determining Forage Consumption

The extrapolation of the forage component quality is only part of understanding the forage constituent of a grazing animal's consumed diet. Quantitatively estimating forage consumption is just as important as knowing the qualitative component. Ruminants on pasture consume the majority of their nutrients through grazing available forages, therefore defining why it is important to further understand animal intake (Lippke, 2002).

The most successful methods used for measuring intake are those that estimate digestibility of a consumed herbage and the fecal output from the same animal. The fecal output is typically a diluted measure from the orally administered marker. However, total fecal collection is laborious and can potentially affect animal behavior

(Dove and Mayes, 1991). With the use of a marker, total collections are not necessary. Estimating total fecal output using markers and measuring digestibility of the diet is the best form (Lippke, 2002). However, Mayes et al. (1986) and Dove and Mayes (2006) concluded that DMI can be estimated without preforming total fecal collections. Increasing the number of fecal samplings will reduce occurrences of measurement error (Malossini et al., 1996).

In order to be an ideal marker the following requirements must be met: a) inert, without toxic effects; b) can not be absorbed or metabolized in the gastro-intestinal tract; c) must not be appreciably bulky; d) must mix and remain distributed in the digesta; e) must not influence gastro-intestinal secretions, digestion, absorption or normal mobility; g) must have chemical properties that allow precise quantitative measurements (Kotb and Luckey, 1972). There are two types of markers: internal and external. Internal markers are indigestible substances that occur naturally in feeds, and external markers are either added to a diet or orally administered to the animal.

The most frequently used marker has been Chromium oxide (Cr_2O_3), an external marker, and is extensively discussed in Dove and Mayes (1991). However, there are errors associated with this technique from the application of a single value for digestibility of the forage, due to the recovery of the marker, and the density of the powder tends to travel at a rate independent of either the particulate or liquid phases (MacRae, 1974; Dove and Mayes, 1996; Malossini et al., 1996).

N-alkanes are long chain hydrocarbons and are a constituent of the cuticular plant surface wax (Van Soest, 1982; Dove and Mayes, 1991). The use of n-alkanes as an

indigestible marker to predict DMI was initially proposed by Mayes and Lamb (1984). It has been proven to be an extremely useful technique for measuring DMI and has been supported by numerous researchers (Mayes et al., 1986; Lippke, 2002; Ferreira et al., 2007; Olivan et al., 2007). They also suggest that n-alkanes are more chemically inert and are also easier to analyze than long chain fatty acids. Markers have been widely used to predict DMI for grazing cattle, most commonly dosing even chained n-alkanes not present in the forage (external marker) and compared to the odd chain hydrocarbon present in the forage (internal marker). Mayes and Lamb (1984) observed that the most abundant alkanes in herbage are odd chain C_{29}, C_{31} and C_{33}. It is widely recognized that alkanes are not fully recovered, however, C_{32} (an external) and C_{33} (an internal) are almost identical at 89% recovery (Mayes et al., 1986). This set is also beneficial to use because it reaches a steady state in 5 days Molina et al. (2004).There is now considerable research proving that as the length of the alkane carbon chain increases, the fecal recovery increases (Dove and Mayes, 1996; Molina et al., 2004; Olivan et al., 2007). However, C_{32} is a preferred external marker due to low concentration levels in the forage (Dove and Mayes, 2006). Although the recovery of alkanes in feces is not complete, estimations can still be made when estimating pairs of alkanes (one natural and one dosed) (Dove and Mayes, 1991).

Controlled-release devices (CRD) were first used with Cr_2O_3 and found to reduce diurnal variation (Ellis et al., 1981). The device is administered orally and predicted to slowly release said marker within the rumen. CRD can also be used with waxes (alkanes) that have been dissolved in a heptane solution and pressed into a capsule.

Also, when dissolved into heptane solution, alkanes can be used as a pour-on to a feed supplement. Dillion and Stakelum (1989) found that dosing twice-daily reduced the diurnal variation in fecal ratios of natural and dosed alkanes opposed to one daily dose.

There is no practical way to measure DMI directly from grazing animals. It is important to know an animal's intake and to be able to reduce the DMI in order to keep operational costs down. Currently alkanes are the strongest methodology available for predicting DMI, and DMI strongly influences residual feed intake.

The in vitro gas production technique (IVGP) was initially developed by McBee (1953) and later refined by Hungate (1966). This technique yields reliable measurements of rates of fermentation of fiber and can be used to determine the available energy of the tested feeds. This study used the in vitro anaerobic fermentation chamber as described by Tedeschi et al. (2009) and Aguiar (2011) From this method, total digestible nutrients (TDN) and metabolizable energy, along with digestible energy and net energy of maintenance and growth can be determined.

Accounting for Microbial Population Changes in the Rumen

Ruminants are classified as such because of their symbiotic relationship with anaerobic microorganisms (bacteria, protozoa, and fungi). Rumen microorganisms are capable of digesting complex plant polysaccharides such as cellulose (an abundant source of energy) and producing substrates (volatile fatty acids — VFA) that are beneficial to the host animal. In reality, the only obligation of the animal, as a host, is to provide a suitable environment for the microorganisms. Although there is a plethora of microorganisms throughout the gastrointestinal tract of a ruminant, it is mainly the

microbiota in the rumen that hold the true symbiotic relationship with the host (Yokoyama and Johnson, 1988). The composition of a diet and the amount and type of complex polysaccharides of a diet will require different microorganisms to degrade each component. Despite the importance of ruminal microbial populations, few studies have thoroughly examined fluctuations in rumen microflora resulting from changes in diet or differing physiological processes of the animal. The limited research effort has been largely due to the level of difficulty and cost of the analysis. Historically, research on rumen microbes has been performed using techniques that are strictly anaerobic and provide a media that simulates the natural habitat (Krause and Russell, 1996).

A more recent method to examine microbial species diversity is DNA sequencing. Pyrosequencing techniques are used to encode rRNA to identify taxonomic groups of organisms. Pyrosequencing has been used to identify and evaluate microbial diversity in a variety of complex ecosystems (Roesch et al., 2007). The 16S rDNA bacterial tag-encoded FLX-titanium amplicon pyrosequencing (bTEFAP) technique has been used in an assortment of different research fields to characterize microbes (Dowd et al., 2008a; Dowd et al., 2008b; Dowd et al., 2008c; Pitta et al., 2010). Information about microbial diversity in the gastrointestinal tract of the human has increased recently due to the 16S-rDNA-based analysis. However, similar information about livestock microbial diversity is not as abundant (Dowd et al., 2008a). This lack of information is due to costly expenses, excessive amount of time needed, and complexity of methodology for traditional culture based methods. With culture-based methods, only approximately 1% of bacteria in the gut has been cultured (Nocker et al., 2007a). The

use of powerful tools like the bTEFAP technique combined with molecular methods is becoming the principal demand to evaluate animal microbes. However, little research has been done to evaluate rumen microbial diversity for cattle grazing on pasture using this technique.

The bovine rumen is composed of a mixed population of bacteria, fungi, and protozoa. The function of the fungi is to penetrate the plant cell wall and solubilize cellulose; however since they attach to feed particles it is difficult to estimate their biomass. About 8% of the total microbial biomass is fungi, and even this is not highly reliable. Due to the limited knowledge base of their activity, their reported contribution of fiber digestion in the rumen is limited (Russell, 2002). Protozoa contributions are also considered to be minimal in terms of fiber degrading activity and are more apt to degrade sugar or starch. There is a considerably greater amount of knowledge about the role of bacteria in the rumen, specifically the role of degrading plant fiber. Rumen bacteria have an advantage because of their large biomass and greater activity within the rumen (Van Soest, 1982).

Bacteria are unicellular organisms and there is not a clear definition of identification of species. Rumen bacteria have been most widely studied over the past 50 years using anaerobic cultivation techniques. These techniques are accountable for 11% of total ruminal bacterial populations as estimated from ribosomal RNA gene libraries (Edwards et al., 2004). Although there are several bacteria recognized as fibrolytic bacterial species, Varel and Dehority (1989) reported that 81.6% are composed of *Fibrobacter succinogenes (33.0%), Ruminococcus albus (46.0%),* and *Ruminococcus*

flavefaciens (2.6%) and are the species referred to as the representative cellulolytic bacteria of the rumen. Their abundance is appropriate since plant fiber is mostly composed of cellulose and hemicellulose (Van Soest, 1982). In addition to cellulolytic and hemicellulolytic bacteria, the rumen harbors species that fall into a range of categories. Amylolytic species are more predominant when diets are high in starch. Some bacteria that degrade complex carbohydrates also are capable of degrading simple sugars and referred to as sugar-utilizing. Pectinolytic species degrade pectin and some possess an enzyme to cleave the pectin chain. Many ruminal bacteria are proteolytic, but few rely solely on protein as a primary source of energy alone. Rumen bacteria that hydrolyze triglycerides and phospholipids are classified as lipolytic bacteria (Yokoyama and Johnson, 1988). These categories are referred to as guilds and can be grouped into fiber carbohydrates (cellulolytic and hemicellulolytic) and non-fiber carbohydrates (amylolytic, pectinolytic, sugar-utilizing, proteolytic and lipolytic bacteria).

The rumen is a diverse community of anaerobic organisms. The diversity of these communities as discussed by Hungate et al. (1964) are: a) ruminates consume complex roughages, b) 'selection for maximum biochemical work' may allow for specialization, and c) natural selection among bacterium altering the community to a point and creating new opportunities for even more bacteria. Because of these differences, it is of interest and benefit to researchers to determine the nutrient requirements of rumen microbes that best benefit the host.

In a recent study by Pitta et al. (2010), 14 fistulated Angus and Hereford cross steers were fed bermudagrass hay (C4, lower in protein and higher in fiber) then

transitioned to grazing wheat forage (C3, higher protein and large soluble fraction). Using 16S based bTEFAP pyrosequencing technique, changes in ruminal bacteria diversity were observed. The most predominant bacterial genera were *Prevotella* and *Rikenella* for both diets. However, after the transition from hay to wheat forage, *Prevotella* became more dominant and the proportion of *Rikenella* decreased. Pitta et al. (2010) demonstrated clear differences in communities between bermudagrass hay and wheat forage. This indicates that rumen microbial adaptation to dietary changes is not limited to major transitions in diet (e.g. forage to concentrate diet)(Tajima et al., 2000). Bacterial diversity is also confirmed for diets of C3 grass hays and C3 perennial legume hay (Tajima et al., 1999; Brulc et al., 2009).

We know that diet alters the bacterial communities, and it has been proven that bacterial communities from similar host animals fed the same diet differ (Brulc et al., 2009). It is of interest to look at a specific class of animal consuming the same diet as its counterpart and how the bacteria community is altered. Zhou et al. (2009), observed that methanogenic communities were more diverse for feedlot fed inefficient animals, however no differences in total population of methanogens among efficient and inefficient animals were detected. They concluded that the diversity of the population might contribute to differences in animal feed efficiency classes.

It is also advantageous to explore the influence of the rumen microbial functions on its host's physiology, however little is understood about this. Hernandez-Sanabria et al. (2010) analyzed VFA and feed efficiency traits (DMI, ADG, and RFI). They observed a significant difference between isovalerate and RFI supporting their

hypothesis of association of microbial diversity, fermentation profiles, and host feed efficiency. High RFI animals had significantly higher proportions of isovalerate compared to low RFI animals. Butyrate and DMI were also had significantly different suggesting that on a low energy diet the substrates involved in the energetic metabolism may be associated with differences in RFI since DMI is a major factor in deriving RFI. As demonstrated in the previously discussed chart by Richardson and Herd (2004), perhaps microbial function could fill a portion of the unknown section.

These studies have been performed on animals consuming a concentrate diet of some form. Few studies have been performed on animals consuming forage type diets; mostly cattle (Weimer et al., 1999; Brulc et al., 2009) and some sheep (Saro et al., 2012). Fewer studies have been performed demonstrating the effects between rumen microbial function and feed efficiency (i.e. RFI).

CHAPTER III

RELATIONSHIPS BETWEEN ANIMAL PERFORMANCE, CARCASS TRAITS, RUMEN MICROBIAL ECOLOGY, AND N-ALKANE PREDICTED INTAKE IN BRAHMAN BULLS WHEN FED IN CONFINEMENT AND UNDER GRAZING CONDITIONS

Overview

Traditionally, cattle producers focus on output performance traits such as weaning weight and ADG. Advances in determining animal efficiency have increased research databases on individual animal performance and why similar animals perform differently; however, little emphasis has been placed on ruminal microbial population characteristics. The first objective of this study was to estimate the dry matter intake (DMI) of Brahman bulls grazing 'Coastal' bermudagrass [*Cynodon dactylon* (L.) Pers.] using the n-alkane technique, and to use this data to determine residual feed intake (RFI) while grazing. The second objective was to investigate the relationship between RFI previously determined in confinement conditions (RFIc) and under grazing (RFIg). The third objective was to investigate the relationship between bulls with identified RFIg and their ultrasound and carcass traits. The fourth objective was to identify bacteria populations in the gastrointestinal tract, using the pyrosequencing technique, in relation to bulls based on their determined RFIg.

Brahman bulls were previously RFI-phenotyped and classified as high RFI (HRFIc) and referred to as inefficient, or low RFI (LRFIc) and referred to as efficient in

a 70 d confinement feeding study. They were then allotted to replicated pastures of low stocking intensity (LSTK) or high stocking intensity (HSTK) for 60 d. In 2009, stocking intensity (STK) per pasture was increased to increase grazing pressure based on previous, similar experimentation. Forage intake was estimated using the alkane ratio technique. Bulls were slaughtered in 2009 directly off pasture and carcass and ultrasound measurements, and ruminal and large intestinal contents were collected. In 2010, rumen content was collected by rumen intubation. Microbial populations were profiled using the 16 rDNA bacterial tag-encoded FLX amplicon pyrosequencing technique.

In the 2009 confinement study, there was a significant interaction for F:G (P=0.032) where LRFIc bulls consumed more but gained less weight. Bulls under grazing in 2009 had an interaction of RFIgxSTK (P=0.043) for ADG where HRFIg bulls on LSTK gained more. There was no interaction in DMI, but HRFIg bulls had a greater intake regardless of STK (P=0.003). In the 2010 confinement study, HRFIc bulls had the heaviest initial BW (P=0.048), final BW (P=0.084), metabolic BW (P=0.054), and also had the greatest DMI (P=0.0095). There were no differences for any traits for 2010 bulls on pasture. From confinement to pasture, 37.5% of bulls on pasture remained at the same RFI confinement rank in 2009, and 56.25% of bulls on pasture remained the same in 2010.

Internal organs and the gastrointestinal tract (GIT) were individually weighed and reported as a percent of shrunk body weight (SBW). The weight of the GIT (P=0.093) tended (P>0.05) to be lighter for inefficient bulls on a LSTK in 2009. There

was a tendency (P>0.05) for the weight of the liver as a percent SBW (P=0.072) and was heaviest for bulls on a LSTK treatment. The small intestine as a percent of SBW was heavier for efficient bulls (P=0.09) at a HSTK.

The bacterial DNA % identified in the rumen in 2009 reported interactions (RFIgxSTK) from hemicellulolytic (P=0.048), starch (P=0.025), and pectinolytic (P=0.057) degrading bacteria. Inefficient bulls at a LSTK had a greater percentage for both amylolytic and pectinolytic bacteria (P=0.008 and P=0.051, respectively) in the large intestine. There were no interactions in the rumen for any substrates for 2010.

In 2009, inefficient bulls under grazing conditions had a greater DMI at a HSTK, but gained more on LSTK. Inefficient bulls at a HSTK had a heavier GIT and greatest percentage of hemicellulolytic degrading bacteria in the rumen, but had less pectinolytic and amylolytic degrading bacteria. In conclusion, inefficient bulls with the least limited forage availability consumed more and have a heavier GIT, but it is still unclear how the microbial fauna affect the efficiency among RFI phenotyped bulls.

Introduction

The USDA cattle on feed report showed that from 2007 to 2008, lightweight cattle feeder numbers decreased from 21% to 17% of total animals in the feedlot. This was not the trend from 2008 to 2009; however, calf crop numbers were down 5% in 2008. From 2009-2010, lightweight cattle were not in high demand for feedlots. With increased cost of corn, feedlots sought out cattle with higher BW off pasture that will potentially require fewer days on feed. This would require the feedlot to feed less corn and ultimately be more cost-effective. Ruminant grazing system producers face numerous challenges, with great importance in animal gain and animal efficiency. Estimating individual animal dry matter intake (DMI) is a challenge for grazing operations, and DMI is a major component for determining efficiency of gain.

Ruminants on pasture consume the majority of their nutrients through grazing available forages thus emphasizing why it is important to further understand grazing animal intake (Lippke, 2002). The use of alkanes has proved to be an effective methodology available for estimating DMI of grazing animals. The use of n-alkanes as an indigestible marker to predict DMI was initially proposed by Mayes and Lamb (1984). The most commonly dosed alkanes are even-chained and not present in the forage (external marker). The ratio of the dosed alkane and an odd-chain alkane from the diet collected in the feces may be used to calculate both digestibility and fecal output; thus, enabling the estimation of DMI (Mayes et al., 1986). It has been widely accepted that alkanes may not be fully recovered (Dove and Mayes, 1996; Molina et al., 2004; Olivan et al., 2007); however, for C_{32} and C_{33} there is 89% recovery (Mayes et al.,

1986). Many researchers have successfully estimated intake using the n-alkane technique in sheep (Mayes et al., 1986; Dove et al., 2000) and dairy cattle (Dillion and Stakelum, 1989; Unal and Garnsworthy, 1999). Olivan et al. (2007) confirmed the reliability of the n-alkane technique including the C_{32}:C_{33} technique to be used in predicting the DMI of grazing cattle animals.

The determination of residual feed intake (RFI) relies on an accurate measurement of DMI. The concept of calculating RFI was first proposed by Koch et al. (1963) as a measurement for feed efficiency on an individual animal basis. Animals with a negative RFI are classified as efficient, and animals with a positive RFI are classified as inefficient. Efficient animals consume less feed than expected based on body weight and growth, while inefficient animals consume more feed than projected. Residual feed intake is calculated using a linear regression of DMI on average daily gain (ADG) and metabolic body weight (MBW). By calculation, RFI is independent ADG and body weight (BW), but numerous other factors potentially contribute to variations in RFI, including 27% assigned to the category "other" (Figure 2.1) (Herd and Arthur, 2009). Rumen microbial functionality could potentially be placed in this category.

Pyrosequencing is a technique used to identify taxonomic groups of organisms. This study used the 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (**bTEFAP**) technique (Dowd et al., 2008a; Dowd et al., 2008b; Dowd et al., 2008c). This technique has been the most recent for rumen bacteria identification and little is known about livestock microbial diversity. This lack of data is due to the high cost and length of the procedure to generate the data (Dowd et al., 2008a). The bTEFAP technique performs a diversity analysis of microbial populations. Most research has been performed on animals consuming a concentrate diet. Few studies have been performed on animals consuming forage type diets: (Weimer et al., 1999; Brulc et al., 2009) and (Saro et al., 2012). Even fewer studies have been performed examining the relationship between rumen microbial function and feed efficiency. Thus, an objective of this study was to determine feed efficiency traits and investigate the relationship between RFIc and RFIg. Another objective was to investigate the relationship between bulls with identified RFIg and their ultrasound and carcass traits, and the identified bacteria populations in the gastrointestinal tract.

Materials and Methods

The Institutional Animal Care and Use Committee of Texas A&M University approved all procedures, prior to the commencement of the trials. These studies were conducted at the Texas A&M AgriLife Research and Extension Center in Overton, Texas, in the Pineywoods vegetation zone in east Texas (32°16′N 94°59′W, with an average rainfall of 100cm, and mean temperature of 30.15°C) during the summers of 2009 and 2010.

In both, 2009 and 2010, purebred Brahman bulls (n=42 and n=33, respectively) were measured for ADG and DMI in confinement. The bulls were fed a restricted commercially available growing ration (Tables 3.1) in confinement using Calan gates. Bulls were fed at 2.8% BW which approximated 98% of ad libitum intake, and feed offered was adjusted weekly based on BW. The RFI under confinement conditions (RFIc) was calculated and the most efficient, negative RFI (LRFI) n=8 and least inefficient, positive RFI (HRFI) n=8 bulls were selected for experiments on pasture. The sixteen selected bulls were stratified into groups and groups were randomly assigned to each of 2 replications of stocking treatments of high stocking intensity (HSTK) or low stocking intensity (LSTK). Stocking intensity (STK) was quantified and expressed by forage allowance (FA). Forage allowance was calculated by dividing total forage dry matter by the collective total animal BW per unit area of land. A single FA was calculated for each stocking rate per year. This "relationship between animal live weight and forage mass per unit area of the specific unit of land being grazed at any one time" is referred to as grazing pressure (Allen et al., 2011).

Table 3.1. Brahman bull diet in confinement

Dietary composition, %[1], (as-fed basis)	2009	2010
Cottonseed hulls (pelleted)	55.0	45.0
Corn	-	40.0
Alfalfa pellets	12.5	-
Soybean meal	10.5	-
Rice bran	10.0	-
Soybean hulls	7.45	15.0
Premix (protein, mineral, vitamin)	4.55	-
Chemical composition (DM basis)		
TDN, %	62.0	65.0
ME, Mcal/kg	2.24	2.35
DE, Mcal/kg	2.73	2.86
CP, %	13.5	11.0

In 2009 the most efficient (n=8) and inefficient (n=8) bulls were stratified into 4 groups with two HRFI and two LRFI per replicate group. They were randomly allotted into two replicate Coastal bermudagrass [*Cynodon dactylon* (L.) Pers.] pastures each of high stocking intensity or low stocking intensity for 60 d. The bulls (initial BW for LRFIg = 374 ± 22.02 kg; HRFIg = 362 ± 92.5 kg) were weighed bi-weekly for 60 d during which two, 10-d intake measurement trials were conducted using the n-alkane ratio method (Dove and Mayes, 2006; Aguiar et al., 2012). Ultrasound measurements were taken on the final day of the grazing study. Bulls were harvested at 16 to 18 months of age direct off pasture without a feedlot residence. At harvest, internal organs were separated, dissected and weighted. Rumen and intestinal fluid was collected for bacteria population identification. Total internal fat was determined by adding the kidney pelvic heart fat (KPH) and physically separated organ fat weights. After a 48-h chill, complete carcass trait data were collected.

In 2010, the most efficient (n=8) and inefficient (n=8) bulls were separated into 4 groups with two HRFI and two LRFI per group. The experimental design for 2010 was altered to increase stocking pressure and reduce available forage for intake. They were randomly allotted into 2 replicate Coastal bermudagrass treatment pastures each of high stocking intensity or low stocking intensity for 60 d. The treatments were in duplicate. The bulls (initial BW for LRFIg = 379 ± 61.6; kg, HRFIg = 422 ± 18.6 kg) were weighed bi-weekly throughout the 60 d study during which three, 10-d intake measurement periods were conducted using the n-alkane ration method (Dove and Mayes, 2006).

Forage Quality and Mass Collections

Forage samples for alkane concentration determination were taken by hand-plucking plant parts from the grazed horizon over a 7-d period beginning 2 d prior to the start of the 5-d fecal collection period. For forage mass measurements forage was hand harvested to ground level from quadrates (4 quadrats per pasture per week). The average forage height (cm) and mass (DM, kg/ha) for the duration of each period for both years are shown in Table 3.2. One period is one-10d intake estimation period. Bulls received an ad libitum amount of fresh water and a commercial mineral supplement (Table 3.3).

Table 3.2. Forage height, and mass for high and low stocking intensity levels during each period of both years[1]

	2009				2010					
Items	Period 1		Period 2		Period 1		Period 2		Period 3	
No. bulls	8	8	8	8	8	8	8	8	8	8
Stock Intensity	High	Low	High	Low	High	Low	High	Low	High	Low
Forage										
Height, cm	17.5	33.3	13.3	29.4	9.68	10.6	7.46	11.7	6.11	11.3
DM, kg/ha	2790	4900	2020	5350	1050	1360	1010	2140	566	2460

[1]Each period is an individual 10-d intake measurement period

Preparation of Corn Gluten as a Marker

Corn gluten pellets were used as the carrier for C_{32} n-alkane and prepared at the Texas A&M AgriLife Research and Extension Center at Uvalde, TX. For each year, 510 doses were prepared according to Aguiar et al. (2012). Using a 2-mm sieve, corn gluten pellets were sieved to separate fines and whole corn kernels. To prepare an individual dose, cleaned pellets were weighed (400 ± 1 g), transferred to a 760-ml Rubbermaid container, and placed in an oven at 75°C for approximately 2 h. Using a 30-ml Minipet Pipettor (VWR, cat # 54848-204), 10 ml of C_{32}-alkane solution was slowly pipetted over the warm corn gluten pellets. The solution was composed of 10 g of C_{32} (Dotriacontane, Aldrich cat # D22, 310-7) in 500 ml heptane (VWR cat # EM-HX0080-6) and heated on low temperature until a transparent solution was formed without any particulate. To prevent clogging, the pipet was flush with warm heptane after every 34 doses had been prepared to clean the pipet. After adding the solution over the corn gluten, doses remained at room temperature approximately 30 minutes to allow the heptane from the solution to evaporate. Doses were then placed in a 75°C oven for approximately 1 h. The dry corn gluten pellets were then placed in paper bags and labeled for each day of each trial. One sample of each set was taken for future standard analysis.

Forage and Fecal Chemical Analyses

After forage nutritive samples were collected, they were frozen for storage purposes, then placed in an oven and dried at 60°C. Prior to extraction for alkane determination and subsequent gas chromatography, both forage and fecal dried samples were ground using a cyclone mill equipped with a 1 mm screen. A sample of corn

gluten was also dried and prepared as above. The daily forage samples for the week were combined to represent each treatment per period. Forage samples and corn gluten were sent to an independent laboratory (Cumberland Valley Analytical Services Inc., Hagerstown, MD 21742) for chemical analysis for the following analyses shown in Table 3.3. For forages, dry matter (DM) was a two-step process. The first step was a partial DM of a whole unground, sample if less than >85% DM, performed according to Goering and Van Soest (1970). For the second step, the oven temperature was modified to 105°C. The second step determines the laboratory DM for a ground sample and is multiplied by the partial DM to determine a total DM, according to the National Forage Testing Association (2002). Crude protein and non-sequential ADF analyses were performed according to AOAC (2002; method 2001.11 and 973.18; respectively). The NDF was determined according to Van Soest et al. (1991). Lignin analysis was performed according to Goering and Van Soest (1970) using 72% sulfuric acid with modifications. Ash was performed according to AOAC (2002, method 942.05).

The in vitro gas production technique (IVGP) as described by Tedeschi et al. (2009) and Aguiar et al. (2011) is a useful tool that describes the fermentability of ruminant feeds. It yields reliable measurements of rates of fermentation of fiber and can be used to determine the available energy of the tested feeds. From this method, the fractional rates of degradation of the forage samples per treatment were determined for 4 and 6 %/h. The digestible (NDF), total digestible nutrients (TDN), digestible energy (DE), metabolizable energy (ME), along with and net energy of maintenance (NEm) and growth (NEg), were determined.

Table 3.3. Chemical composition of corn gluten, mineral supplement[1] and forage during all trials for 2009 and 2010

	2009 Period[2]				2010 Period						
	1		2		1		2		3		
	Stocking Intensity				Stocking Intensity						
Items	High	Low	High	Low	High	Low	High	Low	High	Low	Corn Gluten
DM, %	93.1	93.35	93.05	93.25	94.95	95	95.55	95.55	93.75	93.8	87.8
CP, % DM	16.45	18.8	12.75	15.5	17	18.55	17.75	20.35	19.3	20.2	19.6
SP, % CP	40.75	34.9	43.2	38.15	32.75	28.4	34.0	31.75	35.65	35.7	54
ADF, % DM	32.6	30.85	34.95	31.75	36.7	33.75	36.3	32.6	33.45	29.65	9.3
NDF, % DM	67.4	66.2	69.55	67.3	71.5	70.2	69.8	69.45	66.6	65.35	33.2
Lignin, % DM	4.05	3.7	5.59	4.13	6.425	4.78	6.205	4.74	5.8	4.23	1.91
Sugars, % DM	7.35	7.1	6.7	7.45	3.65	5.15	3	3.2	5.35	5.25	4.9
Starch, % DM	2.6	1.8	4.7	3.2	1.85	2.1	1.75	1.8	2.25	2.55	24.7
Crude fat, % DM	1.8	2.15	1.6	1.9	2.15	2.25	1.7	1.85	1.65	1.95	4.2
Ash, % DM	7.4	7.35	7.15	6.75	7	7.1	7.45	7.35	7.45	6.65	5.4

[1] Composition of mineral supplement: Calcium: 15 (min) and 18% (max), Phosphorus: 7%, salt (NaCl): 19 (min) and 22.8% (max), Magnesium: 1.5%, Potassium: 0.4%, Manganese: 1000 ppm, Zinc: 3000ppm, Copper: 2500 ppm, Selenium: 26 ppm, Cobalt: 20ppm, Iodine: 70 ppm, Vitamin A: 136,077.7 IU/lb, Vitamin D: 13,6077.7 IU/lb, and Vitamin E: 45.4 IU/kg.

[2] Each period is an individual 10-d fecal collection period

Feeding and Feces Collection Procedures

Within each year, bulls were assigned to pastures that were each equipped with a 6-unit Calan gate feeding system. Bulls were previously RFIc ranked and trained to operate doors of the Calan gates while in confinement. Individual bulls were fed 400 g of corn gluten two times per day (0700 h and 1700 h). After a one-week adaptation phase to corn gluten fed in the Calan gate units, bulls were fed corn gluten (400 g) labeled with 200 mg C_{32} n-alkane solution samples twice daily (0700 h and 1700 h) in order to reduce diurnal variation as suggested by Smit et al. (2005). Fecal samples were collected twice daily (0700 h and 1700 h). It was determined by (Aguiar et al., 2012) that collections could be reduced to two per day due to the low variability of the alkane excreted in the feces and which was also in accordance with Malossini et al. (1996). Fecal samples were collected immediately by hand upon defecation on pasture or through rectal palpation. Fecal samples were placed in a -20°C freezer after collection for 24 h. The frozen samples were then removed and individually broken up to allow for more surface area and placed in a 60°C oven for 72 h. There were 2 fecal collection periods in 2009 and 3 in 2010 as shown in Figure 3.1.

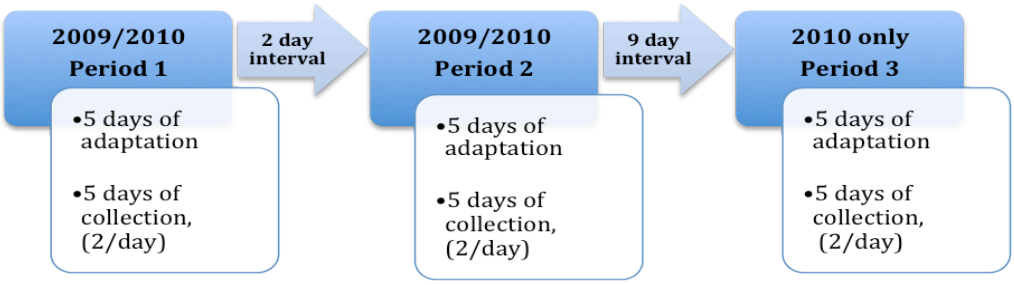

Figure 3.1. Experimental design for each 5-d fecal collection and 10-d intake measurement period

Alkane Analyses

The N-alkane determination in the fecal and forage samples was performed using a gas chromatography system (Agilent 6890N, Santa Clara, CA, USA) with an auto sampler and computer program (Chemstation). A Supelco Special Order SPB-1, fused silica capillary column, 30 x 0.75mm ID x 1.00 μm was used. The settings for the injector were set to add 1.0 μl of sample in a split ratio of 4.3:1 and washed with heptane once pre-injection and twice post-injection. The injector temperature was set at 300°C. Within each analysis, each run included 5 standard samples for calibration. Oven temperature was set at 285°C and held for 12 min, and the detector heater was set at 320°C using a gradient run. Initial temperature was set at 210°C, temperature ramped to 285°C at 20°C/min and held for 5.5 minutes, then amplified to 310°C at 25°C/min and held for 2 min.

Intake Calculations

Intake calculations were based on a 24-h passage rate of forage and n-alkanes dosed with corn gluten (19.6% CP, 33.2% NDF, and 9.3% ADF). According to Dove and Mayes (1996), when calculating intake, the intake of the feed supplement carrying can be disregarded because it is a small proportion of the daily diet. Mayes et al. (1986) suggested that DMI can be estimated for grazing animals by using C_{31} or C_{33} with adjustment for forage C_{32}. For this study, four methods of intake calculations were performed: C_{31} and C_{33} with or without adjustments for C_{32} (C_{31}, C_{33}, C_{31_0}, and C_{33_0}, respectively). The first two calculations accounted for C_{32} in the forage intake equation below:

DM intake = ((Fecal C_{31}/(Fecal C_{32}-Forage C_{32})×Dose value)/Forage C_{31})/1000

The second two calculations assumed that forage C_{32} was insignificant and therefore not accounted for in the forage intake equation below:

DM intake = (((Fecal C_{31} /Fecal C_{32}) × Dose value)/Forage C_{31})/1000

Ultrasound Measurements, Carcass Traits, and

Internal Organ and Gastrointestinal Tract Weights

Ultrasound measurements, carcass traits, gastrointestinal tract (GIT) and internal organ weights were obtained from all 16 bulls in 2009. The real-time ultrasound determination of 12th- to 13th-ribeye area (uREA), intramuscular fat (uIMF), 12th- to 13th-rib backfat thickness (uBF), rump fat, and the kidney pelvic heart depth (uKPH depth) were performed as described by Ribeiro and Tedeschi (2012). Ultrasound images were sent to the National Centralized Ultrasound Processing laboratory (Ames, IA) for analysis.

Directly after the grazing study ended the bulls were shipped to Rosenthal Meat Center at Texas A&M University to be slaughtered. At slaughter, the yield grade and hot carcass weight was recorded per bull. Carcass traits of 12th- to 13th-ribeye area (cREA), 12th- to 13th-rib backfat thickness (cBF), kidney pelvic heart (cKPH), cKPH depth and hump were collected at slaughter. Carcass components were individually weighed and presented as weight per unit of body weight of the bull.

The complete gastrointestinal tract (GIT) and internal organs of the bulls were collected, cooled, and stored in a walk-in refrigerator (2.2°C), and processed in the next

day. The weights of GIT compartments (rumen and reticulum, omasum, abomasum, small intestine, and large intestine) and internal organs (heart, kidneys, and liver) were weighed. Then, physically separable fat was removed from GIT compartments and internal organs, and weighed separately. Each GIT compartment was thoroughly emptied to remove the digesta and weighed. Digesta weight in each compartment was computed by the difference.

Pyrosequencing Analysis

Microbial populations were profiled using the 16 rDNA bacterial tag-encoded FLX amplicon pyrosequencing technique (Dowd et al., 2008a). In 2009, at the end of the grazing study, bulls were harvested at the Rosenthal Meat Center. Two samples (approximately 400 ml) were collected from the rumen and large intestine and frozen for subsequent analysis. In 2010, bulls could not be slaughtered directly after the grazing study. Rumen contents were aspirated with a flexible tube that was inserted orally into the rumen. The contents of the rumen were thoroughly mixed and 2 samples of 400 ml were collected and frozen. As a consequence of the two different methods of collection of rumen contents, the 2010 samples contained a much greater proportion of rumen liquor and considerably less particulate material than the 2009 samples.

For each year, the rumen samples were processed by the Research and Testing Laboratory (Lubbock, TX 79416). Bacterial populations were identified at the genus level and reported as a percentage of the total DNA. Although there were a large number of genera identified, many were found at small percentages. The most predominant bacteria genera (>2%) were used for analyses. Each genus was organized

according to substrate affinities for cellulose, hemicellulose, pectin, starch, protein, and lipids based on Van Soest (1982), Russell (2002), and Dehority (2003). The list of expressive bacteria and substrate affinities are provided in Table 3.4. Because of the organization of these affinities, some genera may appear in more than one guild.

Table 3.4. Expressive bacterial identified at the genus level by 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing and classified according to their affinitive substrate guild

Genus	Substrate guild[1]								
	C	H	St	Pec	Su	Pro	Li	FC	NFC
Anaerovibrio							X		
Bacteroides									X
Blastochloris			X						
Butyrivibrio	X	X		X		X		X	X
Clostridium						X			
Eubacterium			X		X				X
Fibrobacter	X							X	
Lachnospira				X					X
Lactococcus					X				X
Prevotella		X	X	X		X		X	X
Ruminococcus	X	X						X	
Streptococcus			X	X	X				X
Succinivibrio				X					X
Treponema				X	X		X		X

[1]C = Cellulose, H = Hemicellulose, St = Starch, Pec = Pectin, Su = Sugar, Pro = Protein, Li = Lipid, FC = Fiber Carbohydrate, NFC = Non- Fiber Carbohydrate.

Calculation of Residual Feed Intake and Statistical Analyses

Residual Feed Intake

Residual feed intake (RFIc and RFIg) was calculated by a linear regression of DMI on ADG and metabolic BW ($MBW^{0.75}$):

$$y = \beta0 + \beta1(ADG) + \beta2(MBW) + RFI$$

where y is DMI, β0 is the regression intercept, β1 is the partial regression of daily intake on ADG, and β2 is the partial regression of daily intake on BW expressed as metabolic BW (MBW).

Statistical analyses were done with SAS version 9.3 (SAS Institute Inc., Cary, NC). Bulls were sorted by RFIc and classified as low, medium or high RFI based on ± 0.5 SD from mean RFIc within both years. The linear regressions were obtained with PROC REG of SAS. The comparison between previous determinations of efficiency via RFI under confinement (i.e. RFIc) with a subsequent determination of efficiency via RFI under grazing conditions (i.e. RFIg) was performed using PROC REG.

Ultrasound Measurements, Carcass Traits, and Internal Organ and Gastrointestinal Tract Weights

In the 2009 study, a 2-way factorial arrangement of 2 RFI classes (RFI or LRFI) x 2 STK levels (HSTK or LSTK) was used (n=16). The effects of RFIg and STK, and their interaction, on animal performance, ultrasound measurements, carcass traits, rib composition, and internal organ and GIT weights, were analyzed with PROC GLIMMIX using replicate pastures within STK as the random effect. In the 2010 study, the effects of RFIg and STK, and their interaction, on animal performance, were analyzed with PROC GLIMMIX using replicate pastures within STK as the random effect for all traits except F:G.

Pyrosequencing

For the 2009 study, the microbial populations for each GIT compartment (large intestine and rumen) were analyzed separately. For both years, a 2-way factorial arrangement of 2 RFI classes (HRFIg or LRFIg) x 2 STK levels (HSTK or LSTK) was used (n=16). The effects of RFIg and STK, and their interaction on microbial bacteria populations were analyzed per compartment with PROC GLIMMIX. Replicate pastures within STK were the random effect. Individual bull was one experimental unit.

Results and Discussion

Nutritive Analysis of Forage

The digestible NDF for both kp 4 and 6 %/h in both years degraded similarly, however, in 2010 the digestible NDF values were greater suggesting there were greater amounts of cellulose, hemicellulose and lignin in the grazed forage (Table 3.5). The TDN and the remaining dietary energies, DE, ME, NEm, and NEg, were similar across years for both fractional rates for degradation for Coastal bermudagrass. This technique yields reliable measurements of rates of fermentation of fiber and can be used to determine the available energy of the tested feeds. The average values and standard deviation for the two STK treatments for factional rates forage degradation at 4 and 6 %/h time intervals are presented in Table 3.5 for 2009 and 2010.

Comparison of RFIc and RFIg

In the 2009 confinement study, a difference was seen for F:G (P=0.032) where LRFIc bulls had a larger F:G (Table 3.6). There were no differences for initial and final or MBW. The LRFIc bulls had a higher numerical estimated intake, but gained less than HRFIc bulls. There were no differences for ADG or DMI. Other studies have reported that young LRFI animals consumed less feed than their HRFI counterparts (Herd and Bishop, 2000; Arthur et al., 2001a; Arthur et al., 2001b; Nkrumah et al., 2007; Lancaster et al., 2009b; Hafla et al., 2012a).

Table 3.5. Nutritive values[1] of Coastal bermudagrass from low and high stocking intensity levels at multiple time intervals for 2009 and 2010

Year	kp, %/h	STK	Digestible NDF		TDN, (%)		DE, Mcal/kg		ME, Mcal/kg		NEm, Mcal/kg		NEg, Mcal/kg	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2009	4	High	42.8	1.46	63.3	3.17	2.79	0.140	2.29	0.115	1.42	0.104	0.834	0.094
		Low	42.1	4.58	66.1	3.84	2.91	0.169	2.39	0.139	1.51	0.124	0.914	0.111
	6	High	37.7	1.54	58.2	3.29	2.56	0.145	2.10	0.119	1.25	0.111	0.679	0.102
		Low	37.1	4.68	61.0	3.96	2.69	0.174	2.21	0.143	1.34	0.131	0.765	0.119
2010	4	High	48.8	1.34	65.3	2.73	2.88	0.120	2.36	0.099	1.48	0.088	0.894	0.079
		Low	49.9	1.24	68.3	1.96	3.01	0.087	2.47	0.071	1.58	0.062	0.979	0.055
	6	High	43.7	1.32	60.3	3.01	2.66	0.133	2.18	0.109	1.32	0.099	0.743	0.090
		Low	44.5	1.08	63.0	2.20	2.78	0.097	2.28	0.080	1.41	0.072	0.824	0.065

[1] STK=stocking intensity, NDF=neutral detergent fiber, TDN=total digestible nutrients, DE=digestible energy, ME=metabolizable energy, NEm=net energy of maintenance, NEg=net energy of gain

Unlike when bulls were in confinement, a difference was seen in the 2009 grazing study for DMI (P=0.009), and the HRFIg bulls had a greater estimated intake and gained the same as the LRFIg bulls. Herd et al. (1998) reported that efficient and inefficient animals had the same DMI, however, efficient animals were heavier. Meyer et al. (2008) reported no differences for RFI class for BW and only numerical differences for DMI (LRFI cows had 21% lower DMI than HRFI cows) for grazing beef cows. However, the methodology used by Meyer et al. (2008) was relatively imprecise. The bulls had no differences in ADG, and this agreed with a similar study on Brahman bulls grazing bermudagrass by Aguiar (2010). Lawrence et al. (2012) similarly reported no difference for ADG, but also reported no difference for DMI in an n-alkane grazing study.

In 2010, bulls in confinement had different initial BW where LRFIc bulls were lighter than HRFIc (P=0.048). Final BW (P=0.084) and MBW (P=0.054) each had a tendency (P>0.05) for LRFIc bulls to be lighter. The LRFIc bulls also had a significantly lower DMI than HRFIc bulls (P=0.0095) however there was no difference in ADG. There were no differences in 2010 for bulls under grazing conditions in Table 3.6. Comparable to Durunna et al. (2012) there were differences for animals in confinement, but the same traits were not found to be significant for the same animals under grazing conditions. They found that DMI was different for RFI class in confinement, but animals gained the same. During their grazing season animals had the same n-alkane estimated intake and gained the same.

These results disagree with Meyer et al. (2008). They found that RFI phenotyped Hereford heifers fed an ad libitum diet of unprocessed flakes of alfalfa-grass mixed hay using a GrowSafe system, had no significant difference in DMI between RFI groups when animals subsequently grazed tall fescue pasture. Meyer et al. (2008) calculated DMI using a rising plate meter of pre- and post-forage yield to calculate pasture disappearance during the grazing season. It is important to recognize that their method was less accurate, and a possible reason why they did not find differences. Furthermore, Dittmar (2008) reported that Brahman heifers previously ranked as low or high RFI under confinement conditions showed no difference in DMI when they successively grazed irrigated tall fescue (*Lolium arundinacea Schreb*) and annual Ryegrass (*Lolium multiflorum* L.).

Table 3.6. Relationship between RFIc and RFIg for performance traits for both years

	RFIc				RFIg			
Trait[1]	High	Low	SEM	*P*-value	High	Low	SEM	*P*-value
				2009				
No. bulls	8	8			8	8		
Initial BW, kg	285	308	20.7	0.440	362	375	23.7	0.707
Final BW, kg	362	379	25.7	0.643	397	406	21.6	0.776
MBW, kg	75.7	79.8	4.06	0.495	85.8	87.6	3.8	0.739
ADG, kg/d	1.10	1.01	0.08	0.459	0.61	0.55	0.08	0.570
DMI, kg/d	5.44	5.70	0.39	0.643	7.55[a]	5.99[b]	0.36	**0.009**
F:G, kg/kg	4.98[b]	5.67[a]	0.20	**0.032**	13.97	16.18	3.89	0.693
				2010				
No. bulls[2]	8	8			7[2]	6[2]		
Initial BW, kg	339[a]	293[b]	14.9	**0.048**	402	390	23.8	0.702
Final BW, kg	421	376	17.2	**0.084**	434	431	25.6	0.936
MBW, kg	86.0	78.0	2.70	**0.054**	92.2	91.1	4.04	0.832
ADG, kg/d	1.17	1.18	0.11	0.947	0.516	0.661	0.138	0.427
DMI, kg/d	10.4[a]	8.89[b]	0.36	**0.0095**	6.29	5.53	0.344	0.138
F:G, kg/kg	9.13	7.95	0.69	0.246	13.7	11.4	2.77	0.535

[a,b] Within a row, means without a common superscript differ ($P<0.05$).

[1]ADG = Average daily gain for 70d confinement and 60d forage grazing, MBW = mid test metabolic body weight.

[2]Number of bulls for RFIg adjusted for positive ADG.

For both years, the F:G for cattle on pasture was calculated. These results were similar to those found by Lawrence et al. (2012) (12.07 for HRFI and 9.81 for LRFI) for heifers fed a grass silage ad libitum diet. Lawrence et al. (2012) also reported similar ADG (0.60 kg/d for each HRFI and LRFI)and DMI (6.31 kg/d for HRFI and 5.49 kg/d for LRFI heifers) numerical values.

The stocking rate, forage allowance, and ADG for each stocking intensity for both years are in Table 3.7. Unlike Table 3.6, the ADG in Table 3.7 was by STK where as Table 3.6 was across STK and by RFI. Although the stocking rate for the HSTK was greater in 2009, the concentration was greater for high stocking rate in 2010 with a FA = 0.17, and also had the lowest ADG. There were no differences in ADG for RFI phenotyped bulls in 2010 however there was a considerable difference in gain when comparing all 16 bulls at the two STK.

Table 3.7. Forage allowance and stocking rates for each stocking intensity for both years

Year	Stocking Intensity	Stocking Rate[1] animal units/ha	Forage Allowance kg/kg	ADG kg/d
2009	High	21.7	0.38	0.49
2009	Low	4.2	4.25	0.63
2010	High	15.3	0.17	0.14
2010	Low	6.2	1.07	0.82

[1] One bull (animal unit) = 365 kg

Residual Feed Intake Rankings

In 2009, 3 bulls that were ranked as inefficient in confinement remained inefficient on pasture (0 at HSTK and 3 at LSTK), and 3 that were ranked as efficient in confinement remained efficient on pasture (2 at HSTK and 1 at LSTK) (Figure 3.2). Therefore, 37.5% of bulls remained at their same rank for RFI when subsequently tested on pasture. The other 10 bulls switched from HRFIc to LRFIg or from LRFIc to HRFIg (6 at HSTK and 4 at LSTK). In 2010, 4 bulls that were ranked as HRFIc maintained HRFIg (2 at HSTK, and 2 at LSTK), and 5 that were ranked as LRFIc remained LRFIg (3 at HSTK and 2 at LSTK). Therefore, 56.25% of bulls remained at their original RFI rank, which was an 18.75% increase from year 1. The other 7 bulls switched from high RFIc to low RFIg or from low RFIc to high RFIg (3 at HSTK and 4 at LSTK).

In 2009, the majority of bulls did not maintain their previous RFI rank and just over half remained in the same RFI class in 2010. However it is important to consider variability of the original phenotypic RFI (Crews, 2005). This study considered the change from a positive to a negative value, or vise versa; however, there could be potentially insight to differences in reranking in animals that only change by 0.5 SD or less. Similarly, Durunna et al. (2011) found that the proportion of the steers that changed their efficiency rank did not differ greatly from those steers that did change their efficiency rank. They had a control group, however, and found that similar proportions of steers changed or maintained the same feed efficiency group as well. They fed steers a grower diet in the first trial, followed by a finisher diet in the second trial. In a separate study by Durunna et al. (2012), they found that only about half (41%)

of the heifers maintained their RFI rank from one feeding period to another while fed the same diet each period. The reranking of bulls suggests that diet (concentrate or forage based) affects the efficiency performance of bulls. Diets that change from high-energy to a low-energy diet can have changes in pH and affect the population of rumen microbes thus reducing intake (Calsamiglia et al., 2008).

In Figure 3.3, each animal was ranked numerically, increasing from 1-16 by RFIc rank and again for RFIg rank. The numerical values fro RFIc was plotted against the numerical values for RFIg to determine whether rankings remained the same. There was no significant r^2 value for either year.

It is important to recognize that the methodology for calculating RFI for bulls in confinement and on pasture had differences other than the measurement of intake. During the confinement study, bulls RFI ranks were based on a rank within a contemporary group of 42 and 33 bulls each year, respectively. While under grazing conditions, bulls were ranked for RFI with a much smaller contemporary group each year (16 bulls). In addition, reranking of bulls from one environment to another can potentially be influenced by various other factors such as the type of diet or an unrestricted diet. This could be attributed to an animal's limited ability to adjust to a new feed. There are limitations to adaptations concerning microbial populations. Guan et al. (2008) reported an association between the population of rumen microbes and the ability of an animal to utilize feed therefore potentially influencing intake. Other factors can influence phenotypic variation in RFI as indicated in Figure 2.1.

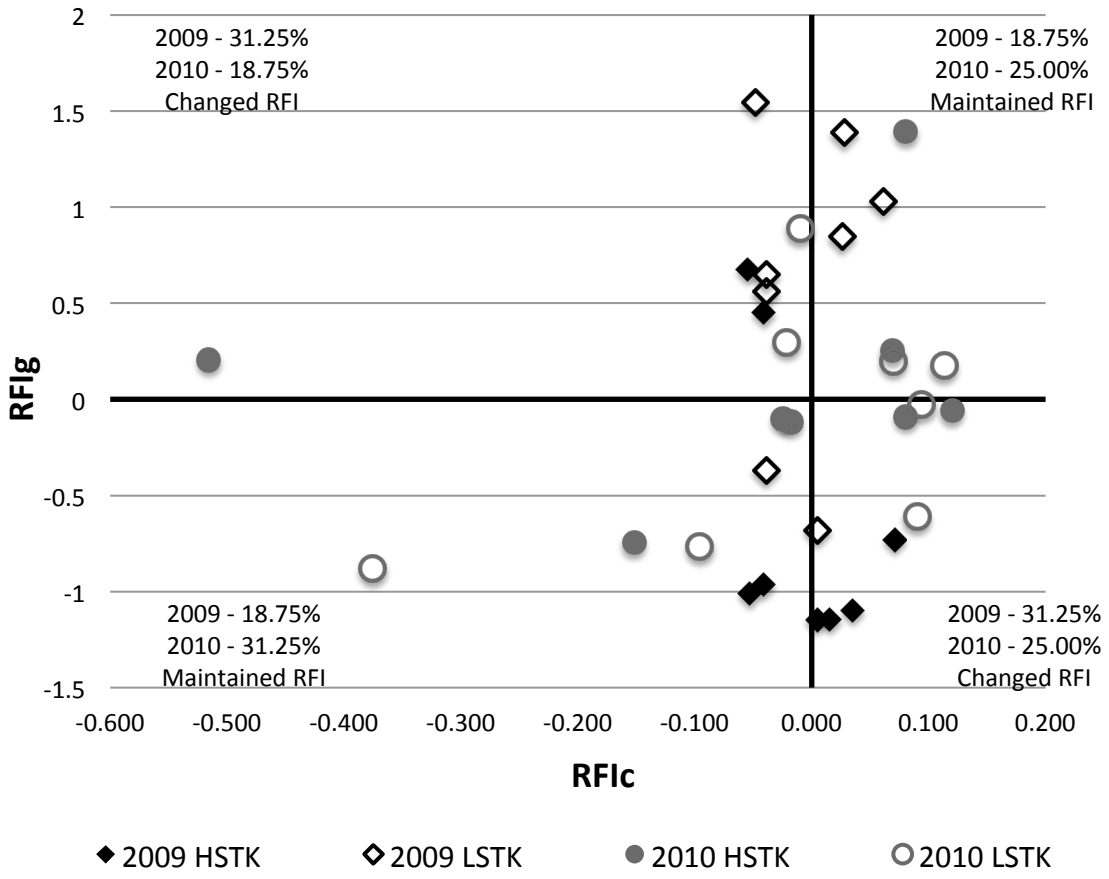

Figure 3.2. Comparison of residual feed intake determined from individual animals fed in confinement (RFIc) and subsequently determined under grazing conditions (RFIg) for 2009 (diamond) and 2010 (circle) data. The percent of bulls that maintained or changed their RFI class are represented in each quadrant.

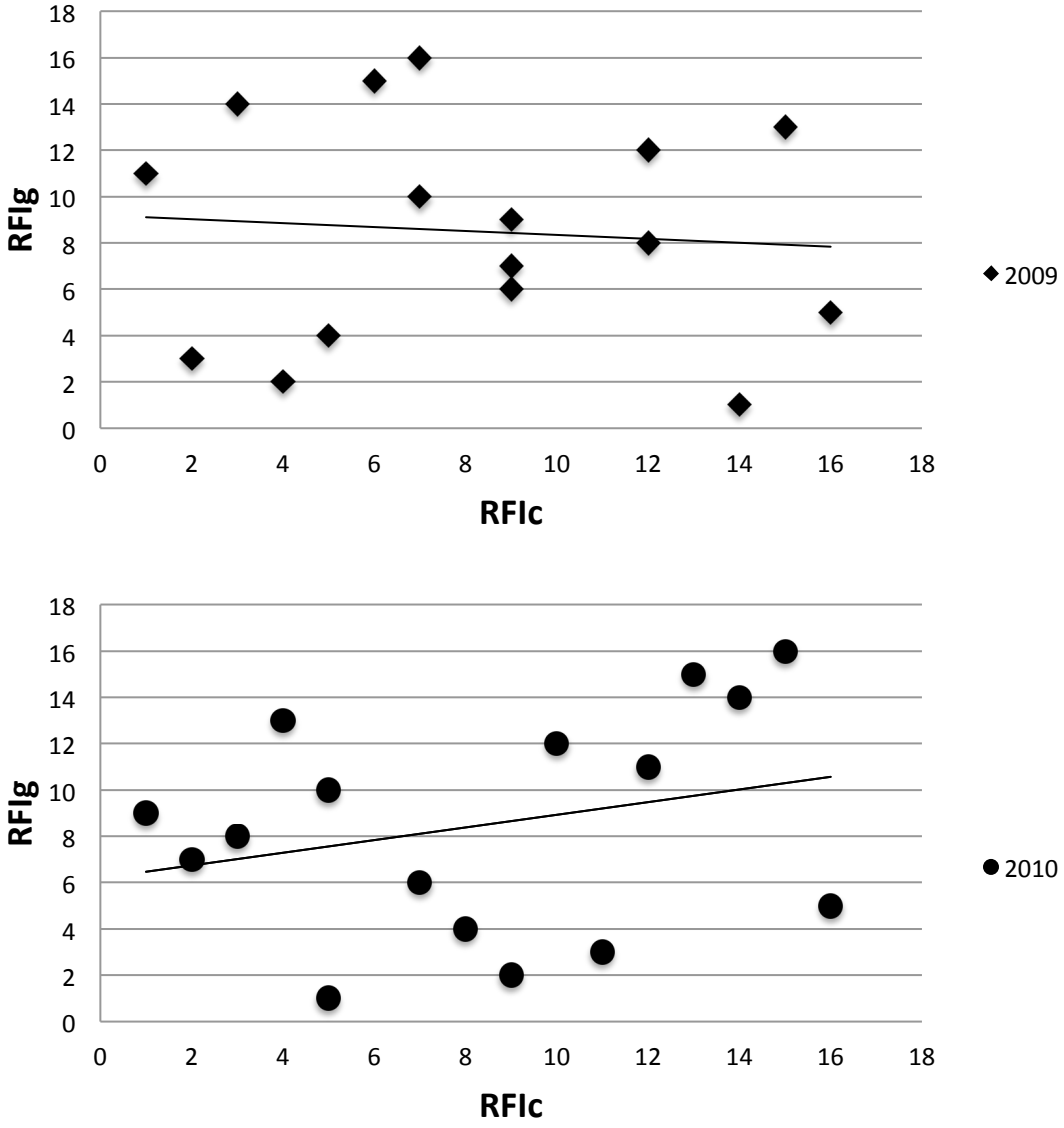

Figure 3.3. Relationship between predicted residual feed intake rank under confinement (RFIc) and forage grazing (RFIg) conditions for 2009 (r^2 = 0.00686) and 2010 (r^2 = 0.07593).

Ultrasound Measurements, Carcass Traits, and

Internal Organ and Gastrointestinal Tract Weights

Bull performance traits were analyzed however STK was an additional variable in Table 3.8. and was not in Table 3.6. There was an interaction (P=0.043) for final BW with inefficient bulls on LSTK being heavier. Heavier bulls also had an interaction with ADG where inefficient bulls had the greatest gain for all categories of bulls. In contrast, inefficient bulls on HSTK had the greatest DMI, yet however did not gain the most. As expected efficient bulls had a lower intake than inefficient bulls. However, adding STK as a variable, efficient bulls on LSTK still had the lowest intake compared to inefficient bulls on LSTK. As previously denoted in a prior section, efficient RFI animals consumed less than their counterparts. Bulls at a lower STK had more forage mass and were heavier at final BW, had a heavier ADG, and lower intakes than bulls at a high STK. Herd et al. (1998) found that predetermined efficient heifers remained heavier (7% heavier) as cows on pasture than the inefficient cows, while having similar subcutaneous fat stores and producing similar calves without increased levels of DMI.

Major internal organs were weighed and presented as a percent of shrunk BW (SBW). The weight of the gastrointestinal tract (P=0.093) tended (P>0.05) to be lighter for inefficient bulls on a LSTK and heavier for those at a HSTK. There were no significant ($P < 0.05$) differences between HRFI and LRFI in the weight of any of the organs. However, there was a tendency (P>0.05) for the weight of the liver as a percent SBW (P=0.072) and was heaviest for bulls on a LSTK treatment. The GIT was dissected and each compartment was weighed and reported as a percent SBW as well.

The small intestine as a percent of SBW was heavier for efficient bulls compared to inefficient bulls (P=0.09) at a HSTK. Richardson et al. (2001) reported weights for external (hide, head, hooves and tail) and internal (kidney, lung, liver, heart, spleen, gall bladder, bladder, neck, diaphragm and esophagus) organs and GIT. They found that organs and GIT weights were similar for low and high RFI steers fed a high concentrate diet. Basarab et al. (2003) reported a difference for liver (P<0.01), small and large intestine (P=0.09), and stomach and intestine (P<0.01) where low and medium RFI steers had lower weights than high RFI steers.

Real-time ultrasound measurements were taken on the final day of the grazing study and prior to transportation and slaughter. Ultrasound composition traits are denoted in Table 3.9. There were no interactions or main effects for RFIg or STK for any of the traits (uREA, uIMF, uBF, rump, and uKPH depth). These results disagree with other studies of growing animals because it has been generally observed that LRFI animals are leaner compared to their HRFI counterparts. Bulls were harvested directly off pasture without a feedlot residence. It is largely observed that efficient animals are leaner compared to their counterparts (Arthur et al., 2001a; Schenkel et al., 2004; Nkrumah et al., 2007; Lancaster et al., 2009a; Lancaster et al., 2009b; Hafla et al., 2012b). Lancaster et al. (2009b) and Hafla et al. (2012b) found, specifically, that for growing bulls on feed, more efficient growing bulls were leaner.

Table 3.8. Effects of residual feed intake of grazing (RFIg) classification at high/high or low stocking intensities (STK) on performance, carcass, rib composition, internal organ, and gastrointestinal tract (GIT) dissection traits of growing bulls in 2009

	Efficient RFIg		Inefficient RFIg			*P*-value		
	STK		STK					
Trait[1]	High	Low	High	Low	SEM	STK	RFIg	RFIgxSTK
No. of bulls	4	4	4	4				
Performance traits								
Initial BW, kg	423	359	359	370	48.688	0.568	0.522	0.364
Final BW, kg	395^{b}	400^{b}	398^{b}	428^{a}	9.171	0.222	**0.023**	**0.043**
ADG, kg/d	0.46^{b}	0.54^{b}	0.50^{b}	1.03^{a}	0.158	0.222	**0.023**	**0.043**
DMI, kg/d	6.54^{b}	5.83^{b}	7.87^{a}	6.51^{b}	0.381	0.121	**0.003**	0.218
F:G, kg/kg	14.1	15.8	17.1	7.76	7.246	0.406	0.585	0.247
Carcass traits								
Hot carcass wt[2], kg	210	210	206	219	8.401	0.416	0.660	0.373
Yield grade	1.19	0.55	0.81	0.44	0.289	0.170	0.322	0.586
Shrunk BW, kg	384^{ab}	379^{b}	385^{ab}	416^{a}	12.119	0.505	**0.003**	**0.005**
Fat, cm	0.31	0.18	0.21	0.16	0.060	0.192	0.222	0.457
cREA, cm^2	55.6	65.1	60.8	69.3	7.618	0.339	0.400	0.927
cBF, cm	0.32	0.16	0.15	0.15	0.079	0.345	0.185	0.239
cKPH, kg	3.77	3.09	3.03	3.16	0.962	0.846	0.384	0.297
cKPH depth, cm	12.1	10.9	10.9	10.4	1.186	0.609	0.219	0.589
Hump, kg	0.75	0.88	0.49	0.57	1.032	0.929	0.678	0.971
Rib composition traits								
Muscle	1.55	1.56	1.56	1.75	0.161	0.497	0.447	0.508
Total Fat, %	17.8	19.1	20.1	18.1	2.229	0.888	0.668	0.319
Rib EE, %	14.9	14.3	15.3	12.4	2.334	0.433	0.697	0.552
Rib water, %	65.1	65.3	65.1	67.6	1.578	0.400	0.382	0.385

Table 3.8. Continued

	Efficient RFIg		Inefficient RFIg			*P*-value		
	STK		STK					
Trait[1]	High	Low	High	Low	SEM	STK	RFIg	RFIgxSTK
Organ traits								
Liver, kg	3.85	4.67	3.41	4.81	0.569	0.139	0.752	0.548
Liver, % SBW	0.86[b]	1.27[a]	0.92[b]	1.09[ab]	0.101	0.126	0.333	**0.072**
Heart, kg	1.61	1.3	1.31	1.3	0.172	0.360	0.309	0.320
Heart, % SBW	0.37	0.35	0.35	0.32	0.028	0.416	0.347	0.762
Kidney, kg	0.77	0.76	0.56	0.83	0.152	0.408	0.590	0.289
Kidney, % SBW	0.18	0.2	0.16	0.2	0.036	0.333	0.746	0.729
GIT, kg	87.0	68.0	75.63	81.5	8.233	0.431	0.873	**0.093**
GIT, % SBW	19.9	18.1	20.3	20.2	1.260	0.454	0.252	0.401
GIT dissection traits								
Rumen, kg	7.13	5.91	6.32	6.46	0.516	0.330	0.760	0.141
Rumen, % SBW	1.63	1.59	1.69	1.60	0.090	0.452	0.628	0.769
Omasum, kg	1.57	1.56	1.67	1.80	0.256	0.815	0.438	0.754
Omasum, % SBW	0.36	0.41	0.45	0.44	0.045	0.591	0.141	0.388
Abomasum, kg	1.29	1.17	0.96	1.27	0.216	0.574	0.612	0.256
Abomasum, % SBW	0.29	0.25	0.31	0.31	0.047	0.672	0.322	0.625
Small Intestine, kg	3.19	3.28	3.50	3.68	0.379	0.697	0.283	0.885
Small intestine, % SBW	0.73[b]	0.89[ab]	0.94[a]	0.91[ab]	0.077	0.392	**0.090**	0.172
Large intestine, kg	2.44	1.91	2.11	2.30	0.330	0.596	0.904	0.218
Large intestine, % SBW	0.56	0.50	0.57	0.57	0.057	0.634	0.426	0.578

[a,b] Within a row, means without a common superscript differ ($P<0.05$).

[1] Initial BW = body weight at start of grazing study, ADG = average daily gain, EE = ether extract, %/sbw = percent of shrunk body weight, GIT = gastrointestinal tract

[2] Hot carcass was adjusted to include KPH and hump.

Table 3.9. Effects of residual feed intake on grazing (RFIg) classification at high or low stocking intensities (STK) on ultrasound composition traits of growing bulls in 2009

	Efficient RFIg		Inefficient RFIg			*P*-value		
	STK		STK					
Trait[1]	High	Low	High	Low	SEM	STK	RFIg	RFIgxSTK
No. of bulls	4	4	4	4				
Ultrasound composition traits								
uREA, cm^2	51.9	60.4	54.2	60.8	4.136	0.147	0.689	0.771
uIMF, cm	2.62	2.23	2.47	2.41	0.404	0.549	0.966	0.631
uBF, cm	0.28	0.24	0.21	0.23	0.038	0.698	0.195	0.289
Rump, cm	0.37	0.42	0.42	0.41	0.068	0.803	0.697	0.623
uKPH depth, cm	14.1	14.7	14.9	15.1	0.952	0.713	0.370	0.830

[1] REA = rib eye area, IMF = intramuscular fat, BF = back fat thickness, KPH = kidney, pelvic, and heart fat,

Pyrosequencing Analysis

Rumen fluid samples were sequentially processed and analyzed each year in the same research and testing lab for the genotyping of the bacteria DNA. However, since their database was consistently updated as new information was reported, all samples from both years were reanalyzed after the 2010 study though the most recent database. The results from both years are in Tables 3.10 and 3.11 for 2009 and Table 3.12 for 2010.

The bacterial DNA % identified in the rumen in 2009 reported interactions (RFIgxSTK) from hemicellulolytic (P=0.048), starch (P=0.025), and pectinolytic (P=0.057) degrading bacteria (Table 3.10). The inefficient bulls on a HSTK had the lowest percentage of hemicellulolytic degrading bacteria. These bulls also had the greatest DMI for all categories of bulls. Pastures at a HSTK had a greater % NDF than LSTK suggesting there was more hemicellulose in the grazed forage (Table 3.3). There was more hemicellulose available for consumption, suggesting that inefficient bulls do not host the proper bacteria for hemicellulose degradation. Although it was not statistically different, inefficient bulls on a LSTK numerically had the greater percentage of hemicellulolytic degrading bacteria than other categories of bulls. Efficient bulls at a HSTK had a much greater difference in percent of amylolytic bacteria in the rumen compared to inefficient bulls on HSTK suggesting that efficient animals can utilize starch from forage better in the rumen. Pectinolytic degrading bacteria were the greatest in the rumen of inefficient bulls at a LSTK versus a HSTK.

Inefficient bulls at a LSTK had a greater percentage for both amylolytic and pectinolytic bacteria (P=0.008 and P=0.051, respectively) in the large intestine (Table 3.11). The starch content of the grazed bermudagrass at the LSTK treatment in Table 3.4 showed a 1.8 %, DM of starch in period 1 and 3.2 %, DM in period 2, therefore over time the starch level was increasing. Bulls were harvested at the end of the grazing study when starch percentages could have possibility been greater. In future studies, a final sample of the grazed forage should be analyzed. As seen in the rumen, starch digestion of efficient and inefficient bulls on LSTK numerically had the same percentage of amylolytic bacteria. However, amylolytic bacteria in the large intestine of inefficient bulls on a LSTK remained high compared to efficient bulls whose amylolytic bacteria percentage drastically decreased. This suggests that efficient bulls utilize starch better in the rumen for the production of volatile fatty acids and allow for less to be passed out of the rumen. There were no other significant interactions (RFIgxSTK) in the large intestine for the other substrates.

Table 3.10. Bacterial DNA % identified in the rumen for low (efficient) or high (inefficient) residual feed intake (RFI) at both high or low stocking intensities (STK) in 2009

	Efficient RFIg		Inefficient RFIg			*P-value*		
	STK		STK					
Substrate[1]	High	Low	High	Low	SEM	STK	RFIg	RFIgxSTK
No. bulls	4	4	4	4				
Cellulose	5.16	6.65	6.19	6.35	0.77	0.432	0.469	0.199
Hemicellulose	17.5^{a}	16.6^{a}	13.8^{b}	18.0^{a}	1.37	0.272	0.317	**0.048**
Starch	30.8^{a}	26.2^{ab}	22.9^{b}	27.7^{ab}	2.19	0.943	0.105	**0.025**
Pectin	16.1^{ab}	14.6^{ab}	12.0^{b}	16.5^{a}	1.76	0.406	0.463	**0.057**
Sugar	19.2	16.7	16.6	17.6	1.65	0.639	0.535	0.221
Protein	31.1	32.9	31.5	31.9	1.47	0.504	0.795	0.569
Lipid	0.95	0.73	1.27	0.99	0.50	0.621	0.476	0.954
FC	17.6^{a}	16.7^{a}	13.8^{b}	18.0^{a}	1.39	0.287	0.312	**0.048**
NFC	41.6	38.0	34.7	40.4	3.08	0.725	0.385	0.093

^{a,b} Within a row, means without a common superscript differ ($P<0.05$).

[1] FC = Fiber carbohydrate; NFC = Non-fiber carbohydrate

Table 3.11. Bacterial DNA % identified in the large intestine for low (efficient) or high (inefficient) residual feed intake (RFI) at both high or low stocking intensities (STK) in 2009

	Efficient RFIg		Inefficient RFIg			*P-value*		
	STK		STK					
Substrate[1]	High	Low	High	Low	SEM	STK	RFIg	RFIgxSTK
No. bulls	4	4	4	4				
Cellulose	6.42	4.30	5.29	3.23	1.17	0.161	0.277	0.978
Hemicellulose	6.91	4.45	5.57	3.70	1.20	0.159	0.312	0.769
Starch	10.4^{b}	7.53^{b}	9.83^{b}	16.8^{a}	1.85	0.310	**0.017**	**0.008**
Pectin	2.65^{b}	2.43^{b}	3.35^{b}	11.7^{a}	2.35	0.169	**0.027**	**0.051**
Sugar	8.95	12.9	15.8	16.1	4.14	0.670	0.106	0.522
Protein	24.2	26.0	23.2	34.9	6.73	0.343	0.486	0.391
Lipid	0.49	0.65	1.03	1.26	0.48	0.662	0.172	0.935
FC	6.91	4.46	5.57	3.70	1.20	0.159	0.310	0.772
NFC	13.9^{ab}	15.7^{b}	20.7^{ab}	21.5^{a}	3.44	0.776	**0.014**	0.816

^{a,b} Within a row, means without a common superscript differ ($P<0.05$).

[1] FC = Fiber carbohydrate; NFC = Non-fiber carbohydrate

Table 3.12. Bacterial DNA % identified in the rumen for low (efficient) or high (inefficient) residual feed intake (RFI) at high or low stocking intensities (STK) in 2010

	Efficient RFIg		Inefficient RFIg			*P-value*		
	STK		STK					
Substrate[1]	High	Low	High	Low	SEM	STK	RFIg	RFIgxSTK
No. bulls	4	4	4	4				
Cellulose	3.51	4.18	2.76	4.89	0.66	0.212	0.974	0.240
Hemicellulose	32.1	28.3	35.7	27.5	5.91	0.424	0.822	0.714
Starch	42.4	43.8	45.7	37.8	5.65	0.673	0.786	0.366
Pectin	31.6	27.7	36.1	27.5	6.09	0.420	0.737	0.709
Sugar	15.2	21.2	15.7	17.0	2.42	0.276	0.471	0.369
Protein	37.0	34.7	39.2	37.5	5.71	0.765	0.673	0.954
Lipid	1.55	1.41	2.13	2.19	0.62	0.966	0.110	0.809
FC	32.2	28.5	35.8	27.6	5.91	0.427	0.825	0.713
NFC	58.6	57.9	62.2	52.5	6.56	0.598	0.850	0.346

[a,b] Within a row, means without a common superscript differ ($P<0.05$).
[1] FC = Fiber carbohydrate; NFC = Non-fiber carbohydrate

There was no interaction (RFIgxSTK) in the rumen for any substrates in 2010. Although RFI groups were not statistically similar within STK, bulls at a HSTK were numerically similar compared to those at a LSTK. Guan et al. (2008) reported that bacterial profiles in the rumen of efficient RFI steers were more similar among other efficient steers than those in the rumen of inefficient animals. This suggests that perhaps exclusive bacterial groups only inhabitant efficient steers. These results did not show whether efficient bulls on the same STK were similar. Therefore, there was the possibility for all efficient bulls being similar without an affect due to STK.

Within year, there were few similarities among RFI groups even though bulls were managed and maintained under the same conditions for each year. Variations in STK alone did not affect the substrates observed in the GIT, therefore increasing stocking intensity and decreasing the forage availability does not affect the microbial populations. Microbial populations for bulls of different RFI groups degraded substrates

differently, however not consistently the same substrates among years. Guan et al. (2008) reported an association between the population of rumen microbes and the ability of an animal to utilize feed therefore manipulating intake. Bacteria are easily influenced by internal environmental changes (pH and temperature) as well as external changes such as season and location (Stewart et al., 1997; Russell, 2002). The host animal may influence the ruminal bacterial populations (Li et al., 2009), but from our results the ruminal bacterial populations did not consistently influence the host. Li et al. (2012) reported that bacterial populations for the rumen content of 22 Hereford-Aberdeen Angus crossbred steers, raised under the same conditions, varied vastly as well.

The methodology for collecting rumen fluid (samples taken after harvest and aspirated from the rumen orally, respectively) differed for each year. In 2009, fluid from the rumen and large intestine were collected when the bulls were harvested allowing for a better mixture of fluid and solid digesta. Whereas in 2010, only ruminal fluid was collected via aspiration through a flexible tube guided down each bull's esophagus. This procedure limited the amount of solid digesta that was collected. Other studies have reported results from both the liquid and solid digesta of the rumen (Guan et al., 2008; Petri et al., 2012). A study by Li et al. (2012) compared rumen epithelial tissue associated bacteria to rumen content microbial bacteria. They found that there was a distinctive difference in the bacterial communities where rumen tissue bacterial populations were more similar than rumen content bacterial populations.

Protozoa can aid in the breakdown of feedstuffs allowing accessibility for the bacteria, but can also be a competition for food. The protozoa population can also be

negative to the bacterial populations since protozoa feed upon bacteria communities. There are conflicts in the literature regarding to the impact that protozoa have on the host. Becker and Hsiung (1929) found that protozoa provide no obvious benefit to the host animal, while others reported a reduction in both dietary and microbial protein that was available to the host animal (Ushida et al., 1986). Carberry et al. (2012) reported an abundance of *Entodinium* ssp. in animals offered a low forage high concentrate diet due to that starch concentration. In return these protozoa consumed starch and the amylolytic bacteria attached, therefore regulating starch fermentation in the rumen.

There was also a limited amount of bacteria selected per guild (Table 3.5). Hespell et al. (1997) reported that only 10-50% of rumen bacteria have been isolated and identified. There was considerable overlapping within guilds because most species are capable of fermenting more than one type of substrate (Yokoyama and Johnson, 1988). *Prevotella* that is predominant in rumen content of dairy cows (Tajima et al., 2000) can selectively utilize carbohydrates and proteins from digesta and therefore be present in four (hemicellulose, starch, pectin, and protein) of the seven guilds used in this study.

Conclusions

Results from this study suggest that previously RFI phenotyped Brahman bulls in confinement perform differently than when grazing on bermudagrass pasture. This is likely to occur because important differences exist in energy and nutrient availability, depending on site of digestion and absorption, and because of differences in feed selection and feeding behavior.

The approach used to calculate RFIc was more accurate since daily intake was measured for each bull, while currently intake under grazing is estimated to calculate RFIg. There are numerous factors that contribute to differences in RFI-c vs RFI-g. Switching diets and the pasture stocking intensity, may affect RFI ranks and contributes to reranking in bulls. The use of pyrosequencing is a valuable tool to identify and quantify bacterial populations to further understand their shifts in the rumen. Additional research is needed to complete the exploration of which biological mechanisms cause variation in intake and efficiency of cattle fed in confinement and subsequently under grazing conditions.

CHAPTER IV

RELATIONSHIPS BETWEEN PERFORMANCE AND RESIDUAL FEED INTAKE IN BONSMARA HEIFERS WHEN FED IN CONFINEMENT AND UNDER GRAZING CONDITIONS

Overview

Traditionally, cattle producers focus on output performance traits such as weaning weight and ADG. Advances in determining animal efficiency have increased research databases on individual animal performance and the reason that similar animals perform differently. The first objective of this study was to estimate the dry matter intake (DMI) of Bonsmara heifers grazing annual Ryegrass (*Lolium multiflorum*) pasture using the n-alkane technique, and to determine residual feed intake (RFI) while grazing. The second objective was to investigate the relationship between residual feed intake previously determined in confinement conditions (RFIc) and under grazing (RFIg).

Bonsmara heifers were previously RFI-phenotyped and classified as high RFI (HRFI) and referred to as inefficient or low RFI (LRFI) and referred to as efficient in a 70 d confinement feeding study. They were transported to Uvalde, TX and placed on annual Ryegrass pastures for a 60 d grazing study. Forage intake was estimated using the alkane ratio technique.

Daily DMI in confinement was greater ($P < 0.01$) for each year for inefficient heifers when fed in confinement. There were no differences between the RFIc group for initial BW, final BW, MBW and ADG during the 70-d confinement trial. There was a

significant difference ($P < 0.05$) in the DMI of Ryegrass each year for low and high RFIg animals where estimates of DMI (P=0.0168, P=<0.0001, P=<0.0001 each year, respectively) where estimates for inefficient animals were greater then efficient animals each year. There were no significant differences between the RFIg group each year for initial BW, final BW, MBW, ADG between low and high RFIg animals for a 70 d grazing period. Over three years 54, 70.8, and 58%, respectively, of heifers did not change RFI rank from confinement to grazing.

There were few significant differences in performance traits. More research is needed to fully understand why cattle under grazing conditions do not maintain the predetermined efficient or inefficient RFI group from confinement. However, being able to determine which animals cannot maintain their predetermined rank, can still allow producer to select against those individuals and keep less efficient animals out of the breeding herd.

Introduction

Grazing system producers have an assorted set of challenges greatly differing from feedlot operations, with specific interest in animal gain and animal efficiency. Dry matter intake (DMI) is a major component for determining gain and efficiency for cattle production operations. Predicting individual animal DMI is a challenge for grazing operations since we cannot physically measure the exact amount or type of feedstuffs those animals are consuming daily. More than 70% of a beef animal's lifetime will be spent on forage and 100% for cows and bulls (Rouquette et al., 2009), underlining the importance to understand DMI. Ruminants on pasture consume the majority of their nutrients through grazing available forages thus emphasizing why it is important to further understand grazing animal intake (Lippke, 2002). It is important to understand a grazing animal's forage intake to allow for appropriate management decisions to be made to maximize production for pastures grazed. The goal for quantifying grazing intake for what is consumed is to gain a measure of nutrient utilization.

The use of alkanes has proved to be an effective methodology available for estimating DMI of grazing animals. The use of n-alkanes as an indigestible marker to predict DMI was initially proposed by Mayes and Lamb (1984). The most commonly dosed alkanes are even-chained and not present in the forage (external marker). The ratio of the dosed alkane and an odd-chain alkane from the diet collected in the feces may be used to calculate both digestibility and fecal output; thus, enabling the estimation of DMI (Mayes et al., 1986). It has been widely accepted that alkanes may not be fully recovered (Dove and Mayes, 1996; Molina et al., 2004; Olivan et al., 2007); however,

for C_{32} and C_{33} there is 89% recovery (Mayes et al., 1986). Many researchers have successfully estimated intake using the n-alkane technique in sheep (Mayes et al., 1986; Dove et al., 2000) and dairy cattle (Dillion and Stakelum, 1989; Unal and Garnsworthy, 1999). Olivan et al. (2007) confirmed the reliability of the n-alkane technique, including the C_{32}:C_{33} technique, to be used in predicting the DMI of grazing cattle animals, but that individual variability of fecal recovery may influence individual estimated intake.

The concept residual feed intake (RFI) was first proposed by Koch et al. (1963) as a measurement for feed efficiency on an individual animal basis. Residual feed intake is calculated using a linear regression of DMI on average daily gain (ADG) and metabolic body weight (MBW). Currently, the *n*-alkane technique is an accepted methodology and is an effective method available for estimating DMI of grazing ruminants. An accurate measure of intake is important since DMI strongly controls RFI. By calculation, RFI is independent of growth traits such as ADG and body weight (BW) unlike the previously used feed conversation ratio (FCR) that correlates negatively with ADG and BW. This type of ratio is undesirable because it is genetically associated to growth traits, therefore selecting for these traits in replacement animals will ultimately result in increasingly larger mature animals. Larger animals have greater energy requirements, and since feed accounts for 65% of a producer's operational expense, therefore, larger animals are not economically beneficial (Herd and Bishop, 2000; Arthur et al., 2001b). Animals that mathematically result in a negative RFI are classified as efficient, and animals that show a positive RFI are classified as inefficient. Efficient

animals consume less feed than expected, while inefficient animals consume more than expected.

There is a lack of information that examines the effects of differing diets and simultaneously different environments on animal feed efficiency. Cattle under grazing conditions have a high roughage based diet in an uncontrolled environment, and cattle in confinement feeding operations are generally fed a high grain diet and are restricted. Recent studies investigating potential for reranking of feed efficiency measured as RFI for animals consuming different diets or animals in separate environments have found variable results (Durunna et al., 2011; Durunna et al., 2012).

The objectives of this study were to investigate previously calculated RFI ranks of Bonsmara heifers during a confinement fed period (RFIc) and subsequently under grazing conditions (RFIg) on annual Ryegrass [*Lolium multiflorum* Lam] pastures.

Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee of Texas A&M University (AUP #2007-198), prior to the commencement of the trials. Bonsmara heifers that originated from the Texas A&M Agrilife Research and Extension Center, at Uvalde, Texas were measured for performance and feed intake. Bonsmara is a tropically adapted *Bos taurus* breed that originated from South Africa and is a 62:19:19 ratio composite of Afrikaner, Hereford, and Shorthorn, respectively (Corbet et al., 2006). Over three consecutive years, Bonsmara heifers (n=62, n=53 and n=60, initial BW = 285 ± 37.1 kg; age = 281 ± 21.4 d) were individually fed ad libitum twice daily a forage-based total mixed ration (Table 4.1) in a Calan gate system (American Calan, Northwood, NH) located at O.D. Butler Jr. Animal Science Complex in College Station, TX.

Table 4.1. Summary of dietary composition for Bonsmara heifers during the post weaning confinement trial

Item	% (as fed)
Dietary composition,	
Chopped alfalfa	35
Pelleted alfalfa	15
Cottonseed hulls	21.5
Cracked corn	19.5
Molasses	7
Premix[1]	2
Chemical composition	
DM, %	90
ME, Mcal/kg DM	1.99
CP, % of DM	13.0
NDF, % of DM	44.8

[1]Premix contained cracked corn, salt, vitamin E at 44,000 IU/kg of product, and a trace mineral mix which contained a minimum of 19% Zn, 7.0% Mn, 4.5% Cu, 4,000 mg/kg of Fe, 2,300 mg/kg of Se, and 500 mg/kg of Co

At the end of each post weaning confinement trial, heifers were ranked by RFI for confinement (RFIc). The lowest RFI rankings, the efficient animals (n = 12 per yr), and highest RFI rankings, the inefficient animals (n = 12 per yr), were returned to the Texas A&M AgriLife Research Center at Uvalde. Uvalde is in semi-arid southwest Texas (29°21′N 99°79′W) with a mean temperature of 17.3°C, 15.6°C, and 19.76, respectively for each spring in 2009, 2010 and 2011. Each year heifers (initial BW= 357.5±33.2, 361.0±29.1, and 345.9±30.4 kg) were placed on irrigated annual Ryegrass pasture for 58, 54, and 56 d respectively. Animals were weighed weekly during which seven 10 d intake measurement trials were conducted, two trials in 2009 and three trials in both 2010 and 2011 using the n-alkane ration method (Dove and Mayes, 2006). Estimates of forage DMI were calculated daily and averaged within trials.

Forage and Supplement

Ryegrass forage samples were hand-plucked at random locations throughout the pasture that the animals were grazing to represent the animals' diet selection. Forage was hand harvested to ground level from quadrates (2 quadrats per trial) for measurements of forage mass. Access to a commercial mineral supplement and fresh water was available ad libitum for the duration of each trial (Table 4.2.). Forage samples that were selected to represent the grazed regions were collected daily beginning 2 d prior to the first day of dosing heifers with n-alkanes.

Table 4.2. Composition of mineral supplement on pasture

Mineral	Concentration
Calcium (Min), %	12.0
Calcium (Max), %	14.0
Phosphorous (Min), %	7.5
Salt (Min), %	40.0
Salt (Max), %	45.0
Magnesium (Min), %	0.5
Potassium (Min), %	0.2
Copper (Min), ppm	20.0
Copper (Max), ppm	40.0
Selenium (Min), ppm	10.0
Zinc (Min), ppm	1825.0
Vitamin A	None added

Preparation and Administration of Marker

Alkane boluses were prepared at the Texas A&M AgriLife Research Center in Uvalde by dissolving 20 g dotriacontane (C_{32}) in 1 L heptane. Each gelatin capsules was filled approximately half full with cellulose powder and 10 mL of the C_{32} solution. The heptane was allowed to evaporate from each capsule before they were sealed. Each animal was administered one capsule with a balling gun twice daily at 0800 and 1700 to provide 400 mg of C_{32} daily for 9 d.

Feces Collection Procedures

Starting on day 6 of dosing, fecal samples were collected by hand immediately following defecation or through rectal palpation at 0700 h and 1600 h daily for 5 days. A study by (Aguiar et al., 2012) determined that collections could be reduced to two per day due to the low variability of the alkane excreted in the feces and was in accordance with Malossini et al. (1996). There were 2 collection periods in 2009 and 3 in 2010 and

2011. Samples were placed in a -20°C freezer after collection for 24 h for further analysis.

Forage and Fecal Chemical Analyses

After forage samples were collected, they were frozen for storage purposes, then placed in an oven and dried at 60°C. Prior to extraction for alkane determination and subsequent gas chromatography, both forage and fecal dried samples were ground using a cyclone mill equipped with a 1 mm screen. A sample of corn gluten was also dried and prepared as above. Daily forage samples for the week were combined to represent each treatment per period. Forage samples and corn gluten were sent to an independent laboratory (Cumberland Valley Analytical Services Inc., Hagerstown, MD 21742) for chemical analysis. For forages, dry matter (DM) was a two-step process. The first step was a partial DM of a whole unground, sample if less than >85% DM, performed according to Goering and Van Soest (1970). For the second step, the oven temperature was modified to 105°C. The second step determines the laboratory DM for a ground sample and is multiplied by the partial DM to determine a total DM, according to the National Forage Testing Association (2002). Crude protein and non-sequential ADF analyses were performed according to AOAC (2002; method 2001.11 and 973.18; respectively). The NDF was determined according to Van Soest et al. (1991). Lignin analysis was performed according to Goering and Van Soest (1970) using 72% sulfuric acid with modifications. Ash was performed according to AOAC (2002, method 942.05).

Alkane Determination

Fecal samples from each morning and each evening were analyzed separately per day and for each animal, to account for diurnal variation. N-alkane determination in the fecal and forage samples was performed using a gas chromatography system (Agilent 6890N, Santa Clara, CA, USA) with auto sampler and computer program (Chemstation). A Supelco Special Order SPB-1, fused silica capillary column, 30 x 0.75mm ID x 1.00 μm was used. The settings for the injector were set to add 1.0 μl of sample in a split ratio of 4.3:1 and wash with heptane, once pre-injection and twice post-injection. The injector temperature was set at 300°C. Within each analysis, 5 standard samples were included for calibration. The oven temperature was set at 285°C and held for 12 min, and the detector heater was set at 320°C using a gradient run. The initial temperature was set at 210°C, then ramped to 285°C at 20°C/minute and was held for 5.5 minutes. The temperature was then amplified to 310°C at 25°C/min and held for 2 min.

Intake Calculations

Intake calculations were based on a 24-h passage rate of forage and n-alkanes dosed with corn gluten (19.6% CP, 33.2% NDF, and 9.3% ADF). According to Dove and Mayes (1996), when calculating intake, the intake of the feed supplement carrying can be disregarded because it is a small proportion of the daily diet. Mayes et al. (1986) suggested that DMI can be estimated for grazing animals by using C_{31} or C_{33} with adjustment for forage C_{32}. For this study, four methods of intake calculations were performed: C_{31} and C_{33} with or without adjustments for C_{32} (C_{31}, C_{33}, C_{31_0}, and C_{33_0},

respectively). The first two calculations accounted for C_{32} in the forage intake equation below:

DM intake = ((Fecal C_{31}/(Fecal C_{32}-Forage C_{32})×Dose value)/Forage C_{31})/1000

The second two calculations assumed that forage C_{32} was insignificant and therefore not accounted for in the forage intake equation below:

DM intake = (((Fecal C_{31} /Fecal C_{32}) × Dose value)/Forage C_{31})/1000

Calculation of Residual Feed Intake and Statistical Analysis

Residual feed intake (RFIc and RFIg) was calculated using a linear regression of DMI on ADG and metabolic BW ($MBW^{0.75}$):

$y = \beta0 + \beta1(ADG) + \beta2(MBW) + RFI$

where y is DMI, β0 is the regression intercept, β1 is the partial regression of daily intake on ADG, and β2 is the partial regression of daily intake on BW expressed as metabolic BW (MBW).

Animals were sorted by RFI under confinement (RFIc) and classified as low, medium or high RFI based on ± 0.5 SD from mean RFIc within each years. The linear regressions were obtained with PROC REG (SAS Institute Inc., Cary, NC). The comparison between previous determinations of efficiency via RFIc with a subsequent determination of efficiency via RFI under grazing conditions (i.e. RFIg) was performed using PROC REG.

The effects of RFIc and RFIg, and their interaction, on animal performance were analyzed using PROC REG.

Comparison of RFIc and RFIg

The RFIc and RFIg values were computed using a multiple linear regression as shown in the below equation (Arthur et al., 2004).

$$\text{Actual DMI} = \text{ADG} + (\text{Mean BW})^{0.75} + \text{RFI}$$

where DMI of the period, kg/d; ADG of the period, kg/d, and RFI is residual feed intake, kg/d.

Results and Discussion

Comparison of RFIc and RFIg

Relationships between RFIc and RFIg and performance traits are shown in Table 4.3 Estimates of DMI were calculated daily and averaged within trials. Daily DMI on feed was greater ($P < 0.01$) for each year for inefficient heifers when fed in confinement. There were no differences between the RFIc group for initial BW, final BW, MBW and ADG during the 70 d confinement trial.

There was a significant difference ($P < 0.05$) in the DMI of Ryegrass each year for low and high RFIg animals where estimates of DMI (P=0.0168, P=<0.0001, P=<0.0001 each year, respectively) where estimates for inefficient animals were greater then efficient animals each year. There were no significant differences between the RFIg group each year for initial BW, final BW, MBW, ADG between low and high RFIg animals for a 70d grazing period.

Majority of research on cattle feed efficiency using the RFI technique has been done in confinement where individual animal intake can be measured. Due to the complexity of estimating intake for animals under grazing conditions results have from confinement trials have only been assumed to be applicable to pasture. Herd et al. (1998) had used predetermined RFI groups from a pelleted ration and subsequently reranked again while under grazing conditions, where intake was estimated using n-alkanes. Results from this study found no differences in RFI groups or DMI of forage consumed by grazing animals. However, they failed to adjust for differences in recovery

because their recovery values were from previous studies. Failure to adjust for differences in recovery can result in inaccurate estimates of intake (Dicker et al., 1996).

Our results disagree with Meyer et al. (2008) who found that Hereford heifers that were fed unprocessed flakes of square-baled alfalfa-grass mixed hay ad libitum using a GrowSafe system were phenotyped as either low, medium, or high RFI, had no significant difference in DMI or BW change between RFI groups when animals grazed tall fescue (*Lolium arundinacea Schreb*) pasture. Meyer et al. (2008) calculated DMI using a rising plate meter of pre- and post-forage yield to calculate pasture disappearance during the grazing season. It is important to recognize that error in the rising plate meter method of estimating forage biomass may have attributed to the lack of detectable differences of DMI between the divergent RFI groups. Furthermore, Dittmar (2008) reported that Brahman heifers previously ranked as low or high RFI under dry lot conditions showed no correlation with ADG, DMI, BW, or F:G when they successively grazed irrigated tall fescue and annual Ryegrass. In a backgrounding study by Herd et al. (2005), previously determined RFI divergent selected efficient steers, were heavier at the end of the period 418 vs. 409 kg; $P = 0.07$) and grew faster (0.66 vs. 0.64 kg/d; $P < 0.05$) than inefficient steers. The literature does not agree in that some studies show differences in divergent RFI groups for gain and rate of growth.

Table 4.3. Relationship between RFIc and RFIg for performance traits for all years

	RFIc				RFIg			
Traits[1]	High	Low	SEM	*P*-value	High	Low	SEM	*P*-value
				2009				
No. heifers	6	6			6	6		
Initial BW, kg	310	302	9.70	0.5470	350	364	10.0	0.3299
Final BW, kg	409	399	11.4	0.5478	400	416	10.6	0.2739
MBW, kg	82.5	80.9	1.77	0.5411	85.1	87.7	1.72	0.2907
ADG, kg/d	1.41	1.39	0.07	0.8345	0.89	0.93	0.05	0.5378
DMI, kg/d	10.0[a]	7.92[b]	0.26	**<0.0001**	9.34[a]	8.50[b]	0.24	**0.0168**
F:G, kg/kg	7.17[a]	5.79[b]	0.22	**0.0002**	10.9	9.42	0.58	0.0794
				2010				
No. heifers	6	6			6	6		
Initial BW, kg	282.60	280.42	7.38	0.8362	360.05	361.90	8.57	0.8801
Final BW, kg	367.88	364.82	9.52	0.8224	409.33	412.74	8.79	0.7869
MBW, kg	76.55	76.07	1.44	0.8141	86.82	87.26	1.46	0.8318
ADG, kg/d	1.22	1.21	0.07	0.9184	0.91	0.94	0.04	0.6264
DMI, kg/d	10.62[a]	8.68[b]	0.23	**<0.0001**	5.43[a]	4.92[b]	0.07	**<0.0001**
F:G, kg/kg	8.94[a]	7.56[b]	0.47	**0.0496**	6.11	5.34	0.29	0.0692
				2011				
No. heifers	6	6			6	6		
Initial BW, kg	270.23	272.41	10.37	0.8832	348.10	343.79	8.96	0.7370
Final BW, kg	332.98	335.26	9.31	0.8639	380.37	371.83	9.37	0.5257
MBW, kg	72.28	72.72	1.73	0.8576	83.35	82.19	1.55	0.6027
ADG, kg/d	0.90	0.90	0.05	0.9830	0.58	0.50	0.05	0.3227
DMI, kg/d	10.48[a]	7.55[b]	0.34	**<0.0001**	6.07[a]	5.32[b]	0.08	**<.0001**
F:G, kg/kg	12.28[a]	8.74[b]	0.86	**0.0083**	11.51	12.45	1.46	0.6535

[a,b] Within a row, means without a common superscript differ ($P<0.05$).

[1]ADG = Average daily gain for 70d confinement and 60d forage grazing, MBW = mid test metabolic body weight.

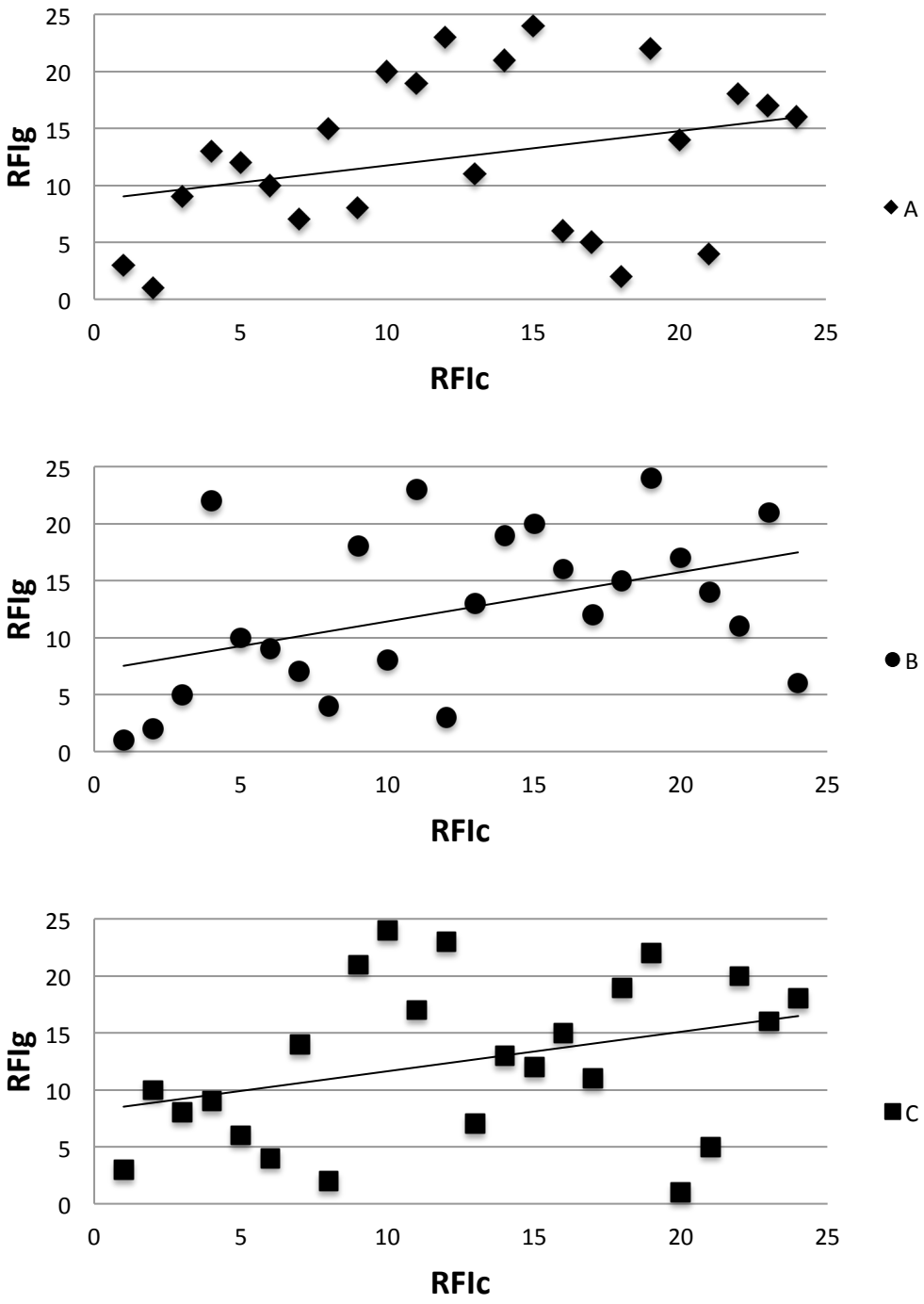

Figure 4.1. Relationship between residual feed intake predicted numerical rank under confinement (RFIc) and under grazing (RFIg) conditions using n-alkane method to predict DMI for 2009 (r^2=0.09105), 2010 (r^2=0.18753), and 2011 (r^2=0.11978).

In Figure 4.1, each animal's numerical ranking (1-24) was placed in ascending order by RFIc rank and again for RFIg rank. RFIc was plotted against RFIg to determine whether rankings remained the same. There was no significant r^2 value for any of the 3 years.

For each of the three years, RFIc was plotted against RFIg in order to better recognize the differences in rank change from confinement fed and under grazing conditions. In 2009, 6 heifers that were ranked as high RFIc maintained high RFIg, and 7 that were ranked as low RFIc remained low RFIg (Figure 4.2). The other 11 heifers switched from high RFIc to low RFIg or from low RFIc to high RFIg. In 2010, 9 heifers that were ranked as high RFIc maintained high RFIg, and 8 that were ranked as low RFIc remained low RFIg. The other 6 heifers switched from high RFIc to low RFIg or from low RFIc to high RFIg. In 2011, 7 heifers that were ranked as high RFIc maintained high RFIg, and 7 that were ranked as low RFIc remained low RFIg. The other 12 heifers switched from high RFIc to low RFIg or from low RFIc to high RFIg.

All three years were collectively plotted to graphically demonstrate change in RFI rank when animals where fed in confinement and under grazing conditions (Figure 4.2). Over three years 54%, 70.8%, and 58% of heifers did not change rank, respectively.

For all three years, the majority of heifers maintained their previous RFI ranking. On the contrary, Durunna et al. (2011) found that the proportion of the steers that changed their efficiency rank did not differ greatly from those steers that maintained their efficiency rank. They had a control group, and found that similar proportions of

steers changed or maintained the same feed efficiency group as well. Different from this study, steers were fed a high-energy diet first, followed by a low energy diet. In a separate study by Durunna et al. (2012), only about half (41%)of the heifers maintained their RFI rank from one feeding period to another. Low (0.33) to moderate (0.62) repeatability has been reported causing some concern about the usefulness of RFI. (Kelly et al., 2010; Durunna et al., 2011)

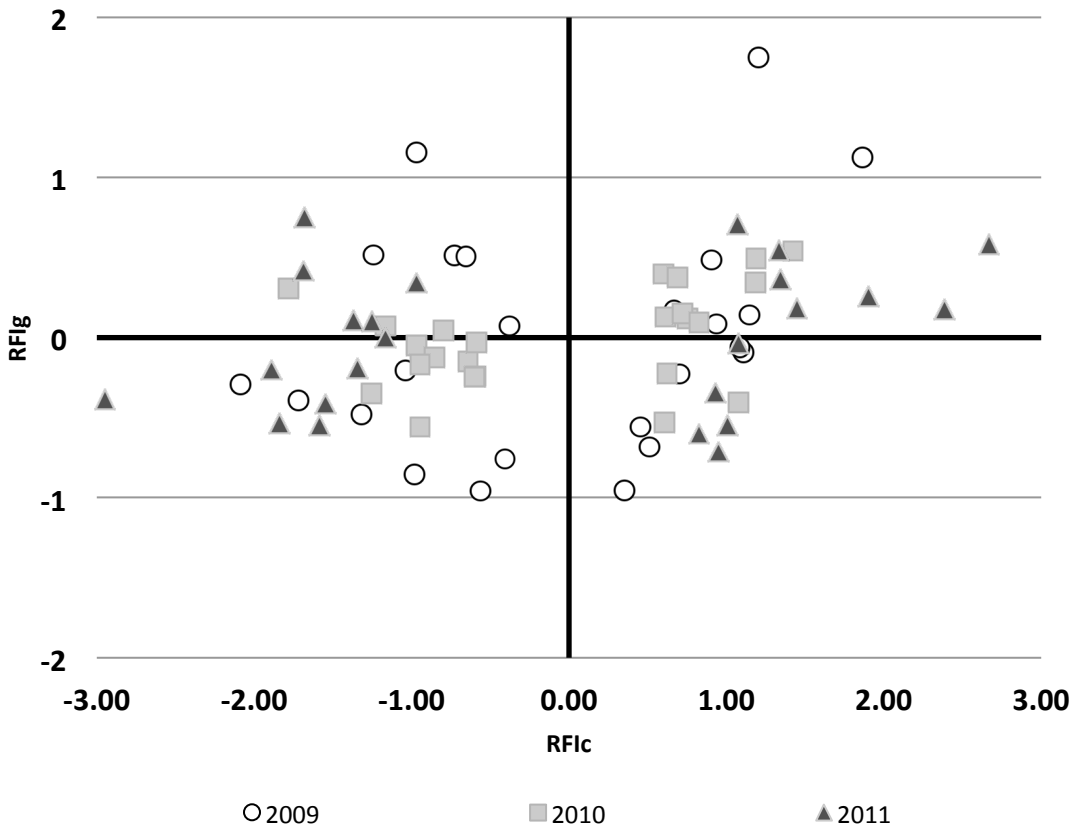

Figure 4.2. Comparison of residual feed intake rank from animals fed in confinement and subsequently grazed forage for 2009 (circle), 2010 (square), and 2011 (triangle).

The reranking of heifers suggests that diet and environment affect the efficiency performance of heifers. Diets that change from high-energy to a low-energy diet can have changes in pH and affect the population of rumen microbes thus reducing intake (Calsamiglia et al., 2008). Guan et al. (2008) reported an association between the population of rumen microbes and the ability of an animal to utilize feed therefore manipulating intake.

It is important to recognize that the methodology for calculating RFI for heifers in confinement and on pasture has differences other than the measurement of intake. During the confinement period, heifer RFI rankings were based on a rank within a contemporary group of 62, 53, and 60 head each year, respectively. While under grazing conditions, heifers were ranked for RFI with a relatively smaller contemporary group of 24 heifers each year. In addition, reranking of heifers from one environment to another can potentially be influenced by various other factors. This could potentially be attributed to an animal's limited ability to adjust to a new feed. There are limitations to adaptations concerning microbial populations. Other factors can influence phenotypic variation in RFI as indicated in Figure 4.2.

Conclusions

In conclusion there were few significant differences in performance traits over all. As expected inefficient animals had a larger DMI than efficient animals both for confinement fed and under grazing conditions. There were significant differences in the DMI each year for low and high RFIc animals and RFIg animals. Although there were significant differences, animals did not necessarily remain in the same RFI group. Over three years 46%, 29.2%, and 42% changed RFI groups respectively where as they were efficient or inefficient in confinement they performed as the opposite under grazing conditions. Since the majority maintained their rank, it offers an opportunity for selecting heifers that can maintain their efficiency though diet and environmental changes.

The collection processes used to obtain the data to calculate RFIc and RFIg each have varying differences. The alkane predicted intake from grazing is only an estimate unlike in confinement in which daily intake is closely quantified. Switching diets and stage of animal maturity affect RFI ranks and contributes to reranking in Bonsmara heifers.

This data indicated that just over half of the heifers maintained their RFI class regardless of diet. Further research is needed to complete the exploration of which biological mechanisms cause variation in intake and efficiency of cattle fed in confinement and subsequently under grazing conditions.

CHAPTER V

SUMMARY

The understanding of cattle efficiency and ways to manipulate it will eventually bring greater profits to the industry. Previous research has been conducted on cattle under both confinement and grazing conditions. Brahman and Bonsmara cattle are both sub tropically adapted breeds. In the first study, no performance traits were consistent among RFI class or years. In the second study, only DMI was different for RFI classes across 3 years. It is possible that the same classifications cannot be used interchangeably for cattle in confinement as well as for cattle under grazing conditions.

It is important to understand the processes responsible for observed variation in feed efficiency for 3 reasons. First of all, knowledge of the physiological basis for variation in efficiency can assist in predicting possible correlated responses to selection. This at least gives an indication of where future research should be focused. Secondly, knowledge of physiological basis of variation in feed efficiency it may be possible to identify traits that are easy and less expensive to measure than feed intake and efficiency. Finally understanding the physiological basis of variation in feed efficiency might suggest alternative, non-genetic methods, which might be used to manipulate the metabolism of cattle and therefore improve feed efficiency (Archer et al., 1999).

LITERATURE CITED

Aguiar, A. D. 2010. Predicting forage nutritive value using an in vitro gas production technique and dry matter intake of grazing animals using n-alkanes. Master's Thesis, Texas A&M University, College Station.

Aguiar, A. D., T. D. A. Forbes, L. O. Tedeschi, J. Rouquette, F. M. , and R. R. D. 2012. Evaluating the statistical variation in estimating forage dry matter intake of grazing brahman bulls using n-alkanes. J Agric Sci. 151: 124-140.

Aguiar, A. D., L. O. Tedeschi, F. M. Rouquette, K. McCuistion, J. A. Ortega-Santos, R. Anderson, D. DeLaney, and S. Moore. 2011. Determination of nutritive value of forages in south texas using an in vitro gas production technique. Grass and Forage Science. 66: 526-540.

Allen, V. G., C. Batello, E. J. Berretta, J. Hodgson, M. Kothmann, X. Li, J. McIvor, J. Milne, C. Morris, A. Peeters, M. Sanderson, F. The, and C. Grazing Terminology. 2011. An international terminology for grazing lands and grazing animals. Grass and Forage Science. 66: 2-28.

Archer, J. A., E. C. Richardson, R. M. Herd, and P. F. Arthur. 1999. Potential for selection to improve efficiency of feed use in beef cattle: A review. Aust. J. Agric. Res. 50: 147-162.

Arthur, P. F., J. A. Archer, and R. M. Herd. 2004. Feed intake and efficiency in beef cattle: Overview of recent australian research and challenges for the future. Aust. J. Exp. Agric. 44: 361-369.

Arthur, P. F., J. A. Archer, D. J. Johnston, R. M. Herd, E. C. Richardson, and P. F. Parnell. 2001a. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in angus cattle. J Anim Sci. 79: 2805-2811.

Arthur, P. F., G. Renand, and D. Krauss. 2001b. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young charolais bulls. Livestock Prod. Sci. 68: 131-139.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing cattle. Can J Anim Sci.

Becker, E. R., and T. S. Hsiung. 1929. The method by which ruminants acquire their fauna of infusoria and remarks concerning experiments on the host specificity of these protozoa. Proc. Natl. Acad. Sci. U. S. A. 15: 684 – 690.

Beever, D. E., and F. L. Mould. 2000. Forage evaluation for efficient ruminant livestock production. In: D. I. Givens, E. Owen, R. F. E. Axford and H. M. Omed (eds.) Forage evaluation in ruminant nutrition. 15-42. CABI Publishing, Wallingford.

Body, D. R., and N. D. Grace. 1981. The possible use of long chain (c 19 -c 32) fatty acids in herbage as an indigestible faecal marker. J Agric Sci. 97: 743-745.

Brelin, B., and E. Brannang. 1982. Phenotypic and genetic variation in feed efficiency of growing cattle and their relationship with growth rate, carcass traits and metabolic efficiency. Swed. J. Agric. Res. 12: 29-34.

Brulc, J. M., D. A. Antonopoulos, M. E. Miller, M. K. Wilson, A. C. Yannarell, E. A. Dinsdale, R. E. Edwards, E. D. Frank, J. B. Emerson, P. Wacklin, P. M. Coutinho, B. Henrissat, K. E. Nelson, and B. A. White. 2009. Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. In: Proc. Natl. Acad. Sci. U. S. A. 106: 1948-1953.

Calsamiglia, S., P. W. Cardozo, A. Ferret, and A. Bach. 2008. Changes in rumen microbial fermentation are due to a combined effect of type of diet and ph. J. Anim. Sci. 86: 702-711.

Carberry, C. A., D. A. Kenny, S. Han, M. S. McCabe, and S. M. Waters. 2012. Effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. Appl Environ Microbiol. 78: 4949-4958.

Corbet, N. J., R. K. Shepherd, H. M. Burrow, J. van der Westhuizen, P. E. Strydom, and D. J. Bosman. 2006. Evaluation of bonsmara and belmont red cattle breeds in south africa. 1. Productive performance. Aust. J. Exp. Agric. 46: 199-212.

Crews, D. H., Jr. 2005. Genetics of efficient feed utilization and national cattle evaluation: A review. Genet. Mol. Res. 4 (2): 153-165.

Crowley, J. J., M. McGee, D. A. Kenny, D. H. Crews, Jr., R. D. Evans, and D. P. Berry. 2010. Phenotypic and genetic parameters for different measures of feed efficiency in different breeds of irish performance-tested beef bulls. J. Anim. Sci. 88: 885-894.

Dehority, B. A. 2003. Rumen microbiology N. U. Press. Thrumpton, Nottingham, United Kingdom.372.

Dicker, R. W., R. M. Herd, and V. H. Oddy. 1996. Alkanes and controlled release devices for estimating intake of ryegrass by cattle. Proc Nutr Soc Aust. 20: 107-107.

Dillion, P., and G. Stakelum. 1989. Herbage and dosed alkanes as a grass measurement technique for dairy cows. Irish J. Agric. Res. 28: 104.

Dittmar, R. O. 2008. Determining biological sources of variation in residual feed intake in brahman heifers during confinement feeding and on pasture. M.S. Thesis, Texas A&M Univ., College Station.

Dove, H., M. Freer, and J. Z. Foot. 1988. Alkance capsules for measuring pasture intake. Proc. Nutr. Soc. Aust. 13: 131.

Dove, H., M. Freer, and J. Z. Foot. 2000. The nutrition of grazing ewes during pregnancy and lactation: A comparison of alkane-based and chromium/in vitro-based estimates of herbage intake. J. Agric. Res. 51: 765-777.

Dove, H., and R. Mayes. 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: A review. Aust. J. Agric. Res. 42: 913-952.

Dove, H., and R. W. Mayes. 1996. Plant wax components: A new approach to estimating intake and diet composition in herbivores. J. Nutr. 126 (1): 13-26.

Dove, H., and R. W. Mayes. 2006. Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. Nature Protocols. 1: 1680-1697.

Dowd, S. E., T. R. Callaway, R. D. Wolcott, Y. Sun, T. McKeehan, R. G. Hagevoort, and T. S. Edrington. 2008a. Evaluation of the bacterial diversity in the feces of cattle using 16s rdna bacterial tag-encoded flx amplicon pyrosequencing (btefap). BMC microbiology. 8: 125.

Dowd, S. E., Y. Sun, R. D. Wolcott, A. Domingo, and J. A. Carroll. 2008b. Bacterial tag-encoded flx amplicon pyrosequencing (btefap) for microbiome studies: Bacterial diversity in the ileum of newly weaned salmonella-infected pigs. Foodborne Pathog. Dis. 5: 459-472.

Dowd, S. E., R. D. Wolcott, Y. Sun, T. McKeehan, E. Smith, and D. Rhoads. 2008c. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded flx amplicon pyrosequencing (btefap). P. LoS One. 3: 1-7.

Durunna, O. N., M. G. Colazo, D. J. Ambrose, D. McCartney, V. S. Baron, and J. A. Basarab. 2012. Evidence of residual feed intake reranking in crossbred replacement heifers. J. Anim. Sci. 90: 734-741.

Durunna, O. N., F. D. N. Mujibi, L. Goonewardene, E. K. Okine, J. A. Basarab, Z. Wang, and S. S. Moore. 2011. Feed efficiency differences and reranking in beef steers fed grower and finisher diets. J. Anim. Sci. 89: 158-167.

Edwards, J. E., N. R. McEwan, A. J. Travis, and R. J. Wallace. 2004. 16s rdna library-based analysis of ruminal bacterial diversity. Antonie van Leewenhoek. 86: 263-281.

Ellis, K. J., R. H. Laby, and R. G. Burns. 1981. Continuous controlled-release administration of chromic oxide to sheep,. Proc. Nutr. Soc. Aust. 6: 145.

Ferreira, L. M. M., U. Garcia, M. A. M. Rodrigues, R. Celaya, A. Dias-Da-Silva, and K. Osoro. 2007. Estimation of feed intake and apparent digestibility of equines and cattle grazing on heathland vegetation communities using the n-alkane markers. Livestock Science. 110: 46-56.

Ferrell, C. L., and T. G. Jenkins. 1985. Cow type and the nutritional environment - nutritional aspects. J. Anim. Sci. 61: 725-741.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook. No. 379. ARS-USDA, Washington, D.C.

Guan, L. L., J. D. Nkrumah, J. A. Basarab, and S. S. Moore. 2008. Linkage of microbial ecology to phenotype: Correlation of rumen microbial ecology to cattle's feed efficiency. FEMS (Fed Eur Microbiol Soc) Microbiol Ecol. 288: 85-91.

Hafla, A. N., G. E. Carstens, T. D. A. Forbes, L. O. Tedeschi, J. C. Bailey, and J. T. Walter. 2012a. Relationships between postweaning residual feed intake in heifers and forage utilization, body composition, feeding behavior, physical activity and heart rate of mid-gestation beef females. J. Anim. Sci. 90: 3937-3944.

Hafla, A. N., P. A. Lancaster, G. E. Carstens, D. W. Forrest, J. T. Fox, T. D. A. Forbes, M. E. Davis, R. D. Randel, and H. J. W. 2012b. Relationships between feed efficiency, scrotal circumference, and semen quality traits in yearling bulls. J. Anim. Sci. 90: 3937-3944.

Herd, R. M., J. A. Archer, and P. F. Arthur. 2003. Reducing the cost of beef production through genetic improvement in residual feed intake: Opportunity and challenges to application. J. Anim. Sci. 81: E9-E17.

Herd, R. M., J. A. Archer, and P. F. Arthur. 2005. When pastures limit growth rate of steers those bred for low residual feed intake grow faster. In: Proc. Adv. Anim. Breed. Gen. 16: 330-333.

Herd, R. M., and P. F. Arthur. 2009. Physiological basis for residual feed intake. J. Anim. Sci. 87: E64-E71.

Herd, R. M., and S. C. Bishop. 2000. Genetic variation in residual feed intake and its association with other production traits in british hereford cattle. Livestock Prod. Sci. 63: 111-119.

Herd, R. M., V. H. Oddy, and E. C. Richardson. 2004. Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Aust. J. Exp. Agric. 44: 423-430.

Herd, R. M., E. C. Richardson, R. S. Hegarty, R. Woodgate, J. A. Archer, and P. F. Arthur. 1998. Pasture intake by high versus low net feed efficient angus cows. Anim. Prod. Aust. 22: 137-140.

Hernandez-Sanabria, E., L. L. Guan, L. A. Goonewardene, M. Li, D. F. Mujibi, P. Stothard, S. S. Moore, and M. C. Leon-Quintero. 2010. Correlation of particular bacterial pcr-denaturing gradient gel electrophoresis patterns with bovine ruminal fermentation parameters and feed efficiency traits. Appl Environ Microbiol. 76: 6338-6350.

Hespell, R. B., D. E. Aiken, and B. A. Dehority. 1997. Bacteria, fungi, and protozoa in the rumen. . In: R. I. Mackie and B. A. White (eds.) Gastrointestinal mircorbiology. p 59-141. Chapman and Hall, New York.

Hungate, R. E. 1966. The rumen and its microbes.Academic Press, New York, N.Y.

Hungate, R. E., M. P. Bryant, and R. A. Mah. 1964. The rumen bacteria and protozoa. Annual Review of Microbiology. 18: 131-166.

Illius, A. W. 1998. Advances and retreats in specifying the constraints on intake in grazing ruminants. In: Proceedings of XVIII International Grassland Congress, Calgary, Canada. III: 39–44.

Kelly, A. K., M. McGee, D. H. Crews, Jr., T. Sweeney, T. M. Boland, and D. A. Kenny. 2010. Repeatability of feed efficiency, carcass ultrasound, feeding behavior, and blood metabolic variables in finishing heifers divergently selected for residual feed intake. J. Anim. Sci. 88: 3214-3225.

Klosterm, E. W. 1972. Beef-cattle size for maximum efficiency. J. Anim. Sci. 34: 875-880.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22: 486-494.

Koots, K. R., J. P. Gibson, and J. W. Wilton. 1994. Analyses of published genetic parameter estimates for beef production traits. 2. Phenotypic and genetic correlations. Animal breeding abstracts. Anim. Breed. Abstr. 62: 825-853.

Kotb, A. R., and T. D. Luckey. 1972. Markers in nutrition. Nutrition Abstracts and Reviews. 42: 813-845.

Krause, D. O., and J. B. Russell. 1996. How many ruminal bacteria are there? J. Dairy. Sci. 79: 1467-1475.

Krueger, W. K., H. Gutierrez-Banuelos, G. E. Carstens, B. R. Mind, W. E. Pinchak, R. R. Gomez, R. C. Anderson, N. A. Krueger, and T. D. A. Forbes. 2010. Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation and carcass and non-carcass traits in steers fed a high-grain diet. Animal Feed Science and Technology. 159: 1-9.

Lancaster, P. A., G. E. Carstens, D. H. Crews, Jr., T. H. Welsh, Jr., T. D. A. Forbes, D. W. Forrest, L. O. Tedeschi, R. D. Randel, and F. M. Rouquette. 2009a. Phenotypic and genetic relationships of residual feed intake with performance and ultrasound carcass traits in brangus heifers. J. Anim. Sci. 87: 3887-3896.

Lancaster, P. A., G. E. Carstens, F. R. B. Ribeiro, L. O. Tedeschi, and D. H. Crews, Jr. 2009b. Characterization of feed efficiency traits and relationships with feeding behavior and ultrasound carcass traits in growing bulls. J. Anim. Sci. 87: 1528-1539.

Lawrence, P., D. A. Kenny, B. Earley, and M. McGee. 2012. Grazed grass herbage intake and performance of beef heifers with predetermined phenotypic residual feed intake classification. Animal. 6: 1648-1661.

Li, M., G. B. Penner, E. Hernandez-Sanabria, M. Oba, and L. L. Guan. 2009. Effect of sampling location and time, and host animal on assessment of bacterial diversity and fermentation parameters in the bovine rumen. J. Appl. Microbiol. 107: 1924-1934.

Li, M., M. Zhou, E. Adamowicz, J. A. Basarab, and L. L. Guan. 2012. Characterization of bovine ruminal epithelial bacterial communities using 16s rrna sequencing, pcr-dgge, and qrt-pcr analysis. Vet Microbiol. 155: 72-80.

Lippke, H. 2002. Forage and grazing lands: Estimation of forage intake by ruminants on pasture. Crop Science. 42: 869-872.

MacRae, J. C. 1974. The use of intestinal markers to measure digestive function in ruminants In: Proc. Nutr. Soc. 33: 147-154.

Malossini, F., S. Bovolenta, E. Piasentier, C. Piras, and F. Martilloti. 1996. Comparison of n-alkanes and chromium oxide methods for estimating herbage intake by grazing dairy cows. Animal Feed Science and Technology. 61: 155-165.

Mayes, R. W., and C. S. Lamb. 1984. The possible use of n-alkanes in herbage as indigestible fecal markers. In: Proc. Nutr. Soc. 43: 39A.

Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986. The use of dosed and herbage n-alkanes as marker for the determination of herbage intake. J Agric Sci. 107: 161-170.

McBee, R. H. 1953. Manometric method for the evaluation of microbial activity of rumen with application to utilization of cellulose and hemicelluloses. Applied microbiology. 1: 106-110.

Meyer, A. M., M. S. Kerley, and R. L. Kallenbach. 2008. The effect of residual feed intake classification on forage intake by grazing beef cows. J. Anim. Sci. 86: 2670-2679.

Molina, D. O., I. Matamoros, and A. N. Pell. 2004. Accuracy of estimates of herbage intake of lactating cows using alkanes: Comparison of two types of capsules. Animal Feed Science and Technology. 114: 241-260.

Nkrumah, J. D., J. A. Basarab, M. A. Price, E. K. Okine, A. Ammoura, S. Guercio, C. Hansen, C. Li, B. Benkel, B. Murdoch, and S. S. Moore. 2004. Different measures of energetic efficiency and their phenotypic relationships with growth, feed intake, and ultrasound and carcass merit in hybrid cattle. J. Anim. Sci. 82: 2451-2459.

Nkrumah, J. D., J. A. Basarab, Z. Wang, C. Li, M. A. Price, E. K. Okine, D. H. Crews, and S. S. Moore. 2007. Genetic and phenotypic relationships of feed intake and

measures of efficiency with growth and carcass merit of beef cattle. J. Anim. Sci. 85: 2711-2720.

Nocker, A., M. Burr, and A. Camper. 2007a. Genotypic microbial community profiling: A critical technical review. Microb Ecol. 54: 276-289.

Nocker, A., M. Burr, and A. K. Camper. 2007b. Genotypic microbial community profiling: A critical technical review. Microb Ecol. 54: 276-289.

Olivan, M., L. M. M. Ferreira, R. Celaya, and K. Osoro. 2007. Accuracy of the n-alkane technique for intake estimates in beef cattle using different sampling procedures and feeding levels. Livestock Science. 106: 28-40.

Petri, R. M., R. J. Forster, W. Yang, J. J. McKinnon, and T. A. McAllister. 2012. Characterization of rumen bacterial diversity and fermentation parameters in concentrate fed cattle with and without forage. J Appl Microbiol. 112: 1152-1162.

Pieper, R., C. W. Cook, and L. E. Harris. 1959. Effect of intensity of grazing upon nutritive content of the diet. J. Anim. Sci. 18: 1031-1037.

Pitta, D. W., E. Pinchak, S. E. Dowd, J. Osterstock, V. Gontcharova, E. Youn, K. Dorton, I. Yoon, B. R. Min, J. D. Fulford, T. A. Wickersham, and D. P. Malinowski. 2010. Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. Microb Ecol. 59: 511-522.

Quanbek, K., and R. J. Johnson. 2009. Livestock, dairy, and poultry outlook: Livestock inventories respond to decreased demand. Economic Research Service http://webarchives.cdlib.org/sw16m34098/http://www.ers.usda.gov/Publications/ldp/2009/08Aug/ldpm182.pdf Accessed October 5, 2012.

Ribeiro, F. R. B., and L. O. Tedeschi. 2012. Using real-time ultrasound and carcass measurements to estimate total internal fat in beef cattle over different breed types and managements. J. Anim. Sci. 90: 3259-3265.

Richardson, E. C., and R. M. Herd. 2004. Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Aust. J. Exp. Agric. 44: 431-440.

Richardson, E. C., R. M. Herd, V. H. Oddy, J. M. Thompson, J. A. Archer, and P. F. Arthur. 2001. Body composition and implications for heat production of angus steer progeny of parents selected for and against residual feed intake. Aust. J. Exp. Agric. 41: 1065-1072.

Roesch, L. F., R. R. Fulthorpe, A. Riva, G. Casella, A. K. Hadwin, A. D. Kent, S. H. Daroub, F. A. Camargo, W. G. Farmerie, and E. W. Triplett. 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME Journal. 1: 283-290.

Romney, D. L., and M. Gill. 2000. Intake of forages. In: D. I. Givens, E. Owen, R. F. E. Axford and H. M. Omed (eds.) Forage evaluation in ruminant nutrition 43-62. CABI Publishing, Wallingford, Oxon, New York.

Rouquette, F. M., Jr., L. A. Redmon, G. E. Aiken, G. M. Hill, L. E. Sollenberger, and J. Andrae. 2009. ASAS centennial paper: Future needs of research and extension in forage utilization. J. Anim. Sci. 87: 438-446.

Russell, J. B. 2002. Rumen microbiology and its role in ruminant nutrition. (Ed.) Cornell Univ., Ithaca, New York.

Russell, J. B., and H. J. Strobel. 1989. Effect of ionophores on ruminal fermentation. Appl Environ Microbiol. 55: 1-6.

Saro, C., M. J. Ranilla, and M. D. Carro. 2012. Postprandial changes of fiber-degrading microbes in the rumen of sheep fed diets varying in type of forage as monitored by real-time pcr and arisa. J. Anim. Sci. In Press.

Schenkel, F. S., S. P. Miller, and J. W. Wilton. 2004. Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Can J Anim Sci. 84: 177-185.

Smit, H. J., H. Z. Taweel, B. M. Tas, S. Tamminga, and A. Elgersma. 2005. Comparison of techniques for estimating herbage intake of grazing dairy cows. J. Dairy. Sci. 88: 1827-1836.

Tajima, K., R. I. Aminov, T. Nagamine, K. Ogata, M. Nakamura, H. Matsui, and Y. Benno. 1999. Rumen bacterial diversity as determined by sequence analysis of 16s rdna libraries. FEMS Microbiology Ecology. 29: 159-169.

Tajima, K., S. Arai, K. Ogata, T. Nagamine, H. Matsui, M. Nakamura, R. I. Aminov, and Y. Benno. 2000. Rumen bacterial community transition during adaptation to high-grain diet. Anaerobe. 6: 273-284.

Taylor, C. S. S., G. B. Young, and H. G. Turner. 1981. Genetic control of equilibrium maintenance efficiency in cattle. Anim. Prod. 33: 179-194.

Tedeschi, L. O., T. R. Callaway, J. Muir, and R. C. Anderson. 2011. Potential environmental benefits of feed additives and other strategies for ruminant production. Proceedings of Brazilian Animal Science Society. 40: 291-309.

Tedeschi, L. O., P. J. Kononoff, K. Karges, and M. L. Gibson. 2009. Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co)products. J. Dairy. Sci. 92: 401-413.

Unal, Y., and P. C. Garnsworthy. 1999. Estimation of intake and digestibility of forage-based diets in group-fed dairy cows using alkane as markers. J Agric Sci. 133: 419-425.

USDA. 2007. Census of Agriculture - United States data. http://www.agcensus.usda.gov/Publications/2007/Full_Report/usv1.pdf Accessed October 1 2012.

USDA-NRI. 2007. Land use status and trends 2007. http://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/technical/nra/nri/?cid=stelprdb1083124 Accessed April 4 2013.

Ushida, K., J. P. Jouany, and P. Thivend. 1986. Role of rumen protozoa in nitrogen digestion in sheep given two isonitrogenous diets. Br. J. Nutr. 56: 407-419.

Van Soest, P. J. 1982. Nutritional ecology of the ruminant. 2nd ed. Cornell Univ., Ithaca, New York

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy. Sci. 74: 3586-3597.

Varel, V. H., and B. A. Dehority. 1989. Ruminal cellulolytic bacteria and protozoa from bison, cattle-bison hybrids, and cattle fed three alfalfa-corn diets. Applied Environmental Microbiology. 55: 148-153.

Waghorn, G. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—progress and challenges. Animal Feed Science and Technology. 147: 116-139.

Weimer, P. P., G. C. Waghorn, C. L. Odt, and D. R. Mertens. 1999. Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. 122-134.

Wilkins, R. J. 2000. Forages and their role in animal systems. In: D. I. Givens, E. Owen, R. F. E. Axford and H. M. Omed (eds.) Forage evaluation in ruminant nutrition 1-14. CABI Publishing, Wallingford, Oxon, New York.

Yokoyama, M. T., and K. A. Johnson. 1988. Microbiology of the rumen and intestine. In: D. C. Church (ed.) The ruminant animal: Digestive physiology and nutrition. p 125-144. Prentice Hall, Englewood Cliffs, N.J.

Zhou, M., E. Hernandez-Sanabria, and L. L. Guan. 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Appl Environ Microbiol. 75: 6524-6533.

APPENDIX

A-1. Performance traits for bulls in confinement in 2009 and 2010

Year	ID	RFIc	Initial BWkg	Final BWkg	Metabolic BW kg	ADG kg/d	DMI kg/d
2009	8107	0.028	386	489	437	1.46	7.35
	8123	-0.039	312	394	353	1.17	5.93
	8129	-0.041	321	390	355	0.97	5.86
	8136	0.005	376	472	424	1.37	7.10
	8151	0.035	378	479	429	1.45	7.21
	8154	-0.039	291	345	318	0.77	5.18
	8158	-0.053	304	369	336	0.93	5.55
	8164	-0.039	292	364	328	1.03	5.48
	8170	-0.048	339	413	376	1.06	6.21
	8176	-0.041	326	400	363	1.06	6.02
	8184	-0.055	278	355	317	1.11	5.35
	8194	0.005	236	289	262	0.77	4.35
	8601	0.071	211	277	244	0.95	4.17
	8603	0.026	230	281	255	0.73	4.23
	8605	0.015	253	334	294	1.16	5.02
	8616	0.061	207	271	239	0.91	4.08
2010	8618	-0.516	415	478	447	0.89	11.4
	8623	0.113	361	445	403	1.21	11.0
	9114	0.090	335	445	390	1.56	10.6
	9119	0.120	358	432	395	1.05	10.9
	9122	0.080	327	399	363	1.02	10.1
	9128	0.080	348	408	378	0.86	10.3
	9145	0.094	328	419	374	1.30	10.3
	9147	-0.010	262	328	295	0.95	8.02
	9153	0.069	325	415	370	1.29	10.2
	9157	-0.018	268	338	303	1.01	8.16
	9160	0.070	327	404	365	1.10	10.0
	9162	-0.376	347	484	416	1.96	10.8
	9193	-0.096	249	324	286	1.07	7.87
	9197	-0.025	271	363	317	1.32	8.65
	9199	-0.021	261	356	308	1.36	8.36
	9211	-0.152	271	334	303	0.91	7.86

A-2. Performance traits for bulls under grazing conditions in 2009 and 2010

Year	ID	RFIg	Stock	Pasture	Initial BWkg	Final BWkg	Metabolic BW kg	ADG kg/d	DMI kg/d
2009	8107	1.386	High	1B	474	494	103.2	0.34	9.40
	8123	-0.368	High	3B6-7	385	413	89.3	0.47	6.74
	8129	-0.965	Low	3B8	390	420	90.2	0.52	6.09
	8136	-0.680	High	3B6-7	461	476	100.7	0.27	7.37
	8151	-1.096	Low	2B	477	486	102.8	0.16	7.27
	8154	0.561	High	1B	346	373	82.6	0.47	7.36
	8158	-1.010	Low	3B8	368	418	88.3	0.87	5.25
	8164	0.648	High	3B6-7	357	387	84.7	0.52	7.45
	8170	1.547	High	1B	409	440	93.5	0.53	8.73
	8176	0.452	Low	2B	389	451	92.8	1.08	6.49
	8184	0.674	Low	2B	351	392	84.6	0.71	7.08
	8194	-1.149	Low	3B8	288	333	74.0	0.78	4.62
	8601	-0.732	Low	2B	284	314	71.9	0.52	5.46
	8603	0.847	High	3B6-7	291	326	73.6	0.60	6.96
	8605	-1.145	Low	3B8	344	389	83.8	0.77	5.11
	8616	1.029	High	1B	277	315	71.3	0.66	6.91
2010	8618	0.205	Low	2B	490	540	108.1	0.80	6.75
	8623	0.176	High	1B	450	451	97.8	0.02	5.25
	9114	-0.613	High	3B6-7	443	442	96.5	-0.01	4.38
	9119	-0.060	Low	3B8	438	492	100.1	0.87	6.38
	9122	1.393	Low	2B	411	447	94.2	0.58	7.23
	9128	-0.093	Low	2B	413	463	95.7	0.80	6.12
	9145	-0.029	High	1B	419	433	93.8	0.23	5.26
	9147	0.888	High	3B6-7	344	363	81.5	0.31	5.97
	9153	0.253	Low	3B8	411	447	94.2	0.58	6.09
	9157	-0.120	Low	2B	340	404	84.6	1.03	6.15
	9160	0.198	High	3B6-7	397	397	88.9	-0.01	4.99
	9162	-0.880	High	1B	463	478	101.1	0.24	4.62
	9193	-0.768	High	3B6-7	328	345	78.6	0.26	4.16
	9197	-0.101	Low	3B8	372	426	89.3	0.87	6.05
	9199	0.296	High	1B	353	372	83.1	0.31	5.42
	9211	-0.745	Low	3B8	346	408	85.6	0.99	5.49

A-3. Performance traits for bulls under grazing conditions in 2009 and 2010

Traits[1]																
Animal ID	8107	8123	8129	8136	8151	8154	8158	8164	8170	8176	8184	8194	8601	8603	8605	8616
Pasture	1B	3B67	3B8	3B67	2B	1B	3B8	3B67	1B	2B	2B	3B8	2B	3B67	3B8	1B
Stock	High	High	Low	High	Low	High	Low	High	High	Low	Low	Low	Low	High	Low	High
RFIc	0.028	0.039	0.041	0.005	0.035	0.039	0.053	0.039	0.048	0.041	0.055	0.005	0.071	0.026	0.015	0.061
RFIg	1.386	0.368	0.965	0.680	1.096	0.561	1.010	0.648	1.547	0.452	0.674	1.149	0.732	0.847	1.145	1.029
Performance traits																
Initial BW, kg	475	386	390	461	477	346	368	357	409	389	351	288	284	291	344	277
Final BW, kg	495	413	420	476	486	373	418	387	440	451	392	333	314	326	389	315
ADG, kg/d	0.34	0.47	0.52	0.27	0.16	0.47	0.87	0.52	0.53	1.08	0.71	0.78	0.52	0.60	0.77	0.66
Carcass Traits																
Hot carcass, lb	557	490	493	530	551	398	487	460	521	501	460	339	365	357	419	333
Hot carcass, kg	253	222	224	240	250	181	221	209	236	227	209	154	166	162	190	151
Adj. hot carcass, kg	256	227	230	248	252	182	226	214	239	230	211	157	168	165	196	153
YG	0.19	1.31	0.48	0.84	0.66	0.91	0.28	0.98	0.66	0.94	-0.07	0.16	0.87	0.70	0.99	1.51
Shrunk BW, kg	477	406	411	465	457	358	398	380	430	428	379	326	292	318	368	299
Fat, in	0.05	0.20	0.10	0.07	0.10	0.10	0.05	0.10	0.05	0.07	0.05	0.03	0.02	0.02	0.07	0.02
Fat, cm	0.12	0.20	0.10	0.10	0.10	0.10	0.07	0.15	0.08	0.09	0.05	0.05	0.05	0.05	0.10	0.05
REA in^2	12.0	9.30	10.8	10.1	10.1	8.40	11.3	9.60	10.0	9.00	11.5	11.0	8.20	9.20	9.30	6.00
KPH, kg	3.29	4.77	3.54	4.72	2.35	1.39	4.09	3.85	2.96	2.56	1.90	3.63	1.93	3.27	4.58	1.74
KPH depth, cm	13.2	12.0	8.82	12.6	13.9	10.1	10.1	10.1	12.0	12.6	10.1	8.8	10.7	8.8	10.7	11.3
Hump, kg	0.00	0.00	3.05	2.87	0.00	0.00	1.30	1.83	0.00	0.00	0.00	0.00	0.00	0.00	1.80	0.00
Rib composition traits																
Muscle	1.79	1.79	1.68	1.73	1.93	1.34	1.59	1.48	2.24	2.02	1.50	1.29	1.35	1.16	1.31	1.13
Lean	52.5	51.3	52.3	54.4	52.9	50.6	52.3	51.2	55.7	49.2	57.5	52.2	52.1	48.5	48.7	49.8
Total Fat	19.4	22.1	22.2	15.7	18.9	18.5	20.1	22.8	16.7	20.2	15.7	17.4	18.5	22.2	17.8	18.9
Rib EE, %	15.5	19.4	15.1	11.3	14.4	17.6	13.9	18.5	16.4	15.6	9.15	11.2	16.3	11.0	14.2	12.3
Rib water, %	65.0	62.1	64.6	67.5	65.6	63.9	64.9	62.9	64.6	65.4	69.8	68.0	64.3	67.9	64.4	66.5
Organ traits																
Liver, kg	4.12	3.58	4.46	4.11	6.52	3.05	4.64	3.54	3.76	5.71	3.90	3.74	3.96	3.08	4.68	2.88
Heart, kg	1.62	1.20	1.35	2.02	1.45	1.33	1.32	1.14	1.52	1.45	1.14	1.18	1.12	1.22	1.37	1.05
Kidney, kg	0.08	0.68	0.76	0.86	1.01	0.57	0.65	0.62	0.91	1.01	0.65	0.63	0.65	0.62	0.86	0.57
GIT, kg	88.3	76.6	64.6	97.4	87.2	76.7	68.8	72.8	82.7	86.0	77.0	62.8	46.2	65.7	78.2	67.6

A-3. Continued

Traits																
GIT dissection																
Rumen, kg	6.97	6.45	5.88	7.80	6.63	6.90	6.55	6.29	7.03	6.72	6.19	5.34	4.95	5.40	6.13	5.32
Omasum, kg	1.83	1.25	2.03	1.89	1.90	1.65	1.69	1.63	1.94	2.32	1.27	1.22	1.26	1.50	1.25	1.48
Abomasum, kg	1.31	0.94	1.40	1.64	1.13	1.71	0.89	1.12	1.16	1.36	1.17	0.79	0.70	0.77	0.85	0.95
Small intestine, kg	4.47	2.50	3.17	3.88	4.01	3.27	3.00	3.13	3.74	4.02	3.34	3.46	2.85	3.07	3.21	3.29
Large intestine, kg	2.74	2.03	1.94	2.84	2.87	2.31	1.80	1.85	1.97	2.57	2.03	1.62	1.25	1.71	1.99	2.10

[1] Initial BW = body weight at start of grazing study, ADG = average daily gain, EE = ether extract, %/sbw = percent of shrunk body weight, GIT = gastrointestinal tract

A-4. 2009 Brahman bulls ultrasound measurements

Traits[1]																
Animal ID	8107	8123	8129	8136	8151	8154	8158	8164	8170	8176	8184	8194	8601	8603	8605	8616
uREA, cm2	10.5	8.5	9.3	9.4	10.7	7.5	9.7	8.4	9.9	8.4	10.5	9.1	7.6	7.5	8.7	5.7
uIMF, cm	1.78	2.76	1.71	2.06	2.34	2.2	2.38	2.97	1.94	3	1.8	2.42	2.07	2.48	2.69	3.86
uBF, cm	0.11	0.13	0.09	0.11	0.11	0.09	0.07	0.09	0.07	0.11	0.07	0.06	0.11	0.05	0.11	0.07
Rump, cm	0.19	0.14	0.2	0.18	0.14	0.16	0.14	0.18	0.21	0.14	0.18	0.11	0.14	0.13	0.23	0.11
KPH depth, cm	17.1	15.1	15.5	12.5	14.6	14.7	13.8	14	15.6	15	14.8	15.1	14	13.4	15.1	15.2

[1] REA = rib eye area, IMF = intramuscular fat, BF = back fat thickness, KPH = kidney, pelvic, and hear

A-5. Performance traits for heifers in confinement in 2009, 2010 and 2011

Year	ID	RFIc	Initial BWkg	Final BWkg	Metabolic BW kg	ADG kg/d	DMI kg/d
2009	8004	0.90	275.6	362.2	75.5	1.24	9.0
	8018	-0.73	264.9	351.4	73.5	1.24	7.2
	8042	0.67	312.6	388.9	81.1	1.09	9.0
	8060	1.86	267.5	370.5	75.5	1.47	10.4
	8067	-0.66	269.6	359.5	74.7	1.28	7.5
	8089	1.21	302.8	445.2	85.1	2.03	11.8
	8102	-1.04	346.5	469.1	90.7	1.75	9.6
	8103	-1.32	306.6	418.8	83.1	1.60	8.1
	8112	-0.98	295.7	399.6	80.5	1.48	8.0
	8118	-1.72	305.7	409.5	82.2	1.48	7.5
	8122	-0.97	301.7	398.3	80.9	1.38	7.9
	8132	-1.24	332.7	417.3	85.2	1.21	7.7
	8151	0.35	329.9	409.1	84.3	1.13	9.1
	8801	0.94	366.8	474.5	92.9	1.54	11.3
	8810	1.11	357.2	465.7	91.4	1.55	11.3
	8824	0.45	373.4	469.7	93.0	1.38	10.5
	8827	-2.09	326.9	421.3	85.1	1.35	7.2
	8828	-0.38	301.7	361.8	77.7	0.86	7.2
	8834	0.70	259.0	344.0	72.4	1.21	8.5
	8843	1.09	284.5	379.0	77.7	1.35	9.6
	8845	0.51	316.0	415.5	83.6	1.42	9.7
	8854	-0.56	301.1	405.3	81.5	1.49	8.6
	8863	1.15	274.2	382.2	77.1	1.54	10.0
	8869	-0.41	265.7	376.2	75.8	1.58	8.5
2010	9004	0.60	300.7	408.6	81.7	1.54	11.44
	9005	-0.64	326.3	394.8	82.7	0.98	9.78
	9009	0.62	322.5	400.1	82.9	1.11	11.23
	9011	0.61	286.0	387.1	78.6	1.44	11.01
	9014	-1.78	298.3	387.2	79.7	1.27	8.23
	9015	-0.95	309.7	425.1	83.9	1.65	9.73
	9017	-1.26	306.9	400.5	81.6	1.34	9.47
	9023	1.42	274.9	357.3	75.0	1.18	10.71
	9026	-0.59	268.5	344.7	73.3	1.09	8.55
	9028	-0.97	297.5	381.3	79.1	1.20	9.24
	9035	0.75	284.3	391.5	78.8	1.53	11.08
	9038	0.63	265.5	348.7	73.4	1.19	9.88
	9042	0.69	260.0	327.6	71.0	0.97	9.39
	9084	-0.85	233.8	339.9	69.7	1.52	8.23
	9113	-0.80	250.3	311.7	68.6	0.88	7.56
	9114	1.19	315.9	400.3	82.3	1.21	11.83
	9122	1.08	296.6	358.9	77.0	0.89	10.64

A-5. Continued

Year	ID	RFIc	Initial BWkg	Final BWkg	Metabolic BW kg	ADG kg/d	DMI kg/d
2010	9125	-0.59	260.8	306.5	69.1	0.65	7.42
	9132	-0.60	290.7	375.6	78.0	1.21	9.40
	9192	-0.95	266.2	348.5	73.4	1.18	8.32
	9200	-1.17	256.0	362.0	73.7	1.52	8.21
	9209	0.72	271.8	371.5	76.0	1.42	10.49
	9215	1.19	257.6	340.9	71.9	1.19	10.10
	9226	0.83	255.4	322.0	70.0	0.95	9.62
2011	5	0.95	244.98	311.37	68.11	0.95	9.35
	9	1.45	305.79	367.28	78.57	0.88	11.64
	13	-1.69	310.70	370.64	79.30	0.86	8.60
	20	1.08	362.55	404.94	86.70	0.61	12.32
	28	-1.89	320.47	367.84	79.90	0.68	8.22
	33	-1.84	246.02	300.78	67.24	0.78	6.13
	46	-1.37	244.56	318.72	68.75	1.06	7.32
	48	1.90	284.73	339.59	74.26	0.78	11.16
	54	-1.55	282.89	344.71	74.56	0.88	7.92
	70	2.67	320.48	369.31	80.03	0.70	12.84
	71	1.00	261.28	322.85	70.65	0.88	9.75
	79	-1.17	290.93	349.91	75.73	0.84	8.45
	85	-2.95	294.33	379.00	78.60	1.21	7.77
	87	0.93	228.37	284.87	64.12	0.81	8.37
	95	-1.35	285.47	347.09	75.00	0.88	8.20
	98	1.07	228.06	310.74	66.50	1.18	9.54
	106	1.34	258.31	313.94	69.57	0.79	9.76
	108	-1.68	244.55	326.97	69.50	1.18	7.33
	109	-1.58	246.68	321.56	69.20	1.07	7.20
	133	-0.97	239.02	279.55	64.62	0.58	6.21
	210	1.34	274.48	341.22	73.49	0.95	10.72
	219	2.39	248.01	335.40	70.58	1.25	11.71
	225	0.82	225.75	294.24	64.75	0.98	8.65
	233	-1.25	263.32	316.38	70.25	0.76	7.23

A-6. Performance traits for heifers under grazing conditions in 2009, 2010 and 2011

Year	ID	RFIg	Initial BWkg	Final BWkg	Metabolic BW kg	ADG kg/d	DMI kg/d
2009	8004	0.49	329.25	369.61	80.82	0.72	8.77
	8018	0.51	306.12	364.17	78.33	1.04	8.72
	8042	0.17	347.39	388.66	84.03	0.74	8.75
	8060	1.13	326.08	368.25	80.43	0.75	9.39
	8067	0.51	314.74	367.80	79.40	0.95	8.77
	8089	1.75	402.72	448.98	93.74	0.83	11.25
	8102	-0.21	410.43	473.47	96.39	1.13	9.68
	8103	-0.48	370.52	420.86	88.72	0.90	8.60
	8112	-0.86	351.47	383.22	83.91	0.57	7.63
	8118	-0.39	363.27	429.48	88.83	1.18	8.84
	8122	1.16	382.77	434.47	90.88	0.92	10.45
	8132	0.52	363.72	435.83	89.40	1.29	9.85
	8151	-0.95	344.67	401.36	84.88	1.01	7.83
	8801	0.08	413.61	452.61	94.94	0.70	9.63
	8810	-0.09	411.79	459.41	95.35	0.85	9.57
	8824	-0.56	409.07	464.40	95.54	0.99	9.19
	8827	-0.29	365.99	436.73	89.67	1.26	9.05
	8828	0.07	324.72	376.42	81.02	0.92	8.47
	8834	-0.23	315.19	363.72	79.08	0.87	7.96
	8843	-0.06	336.05	390.93	83.25	0.98	8.56
	8845	-0.68	357.37	396.37	85.54	0.70	8.01
	8854	-0.96	360.54	408.16	86.81	0.85	7.93
	8863	0.14	340.59	390.48	83.60	0.89	8.75
	8869	-0.76	331.97	379.14	81.88	0.84	7.67
2010	9004	0.40	385.03	448.53	92.24	1.18	5.66
	9005	-0.15	407.26	463.04	95.27	1.03	5.25
	9009	0.13	394.56	443.08	92.58	0.90	5.48
	9011	-0.53	368.25	405.90	87.27	0.70	4.72
	9014	0.31	375.51	431.29	90.01	1.03	5.54
	9015	-0.56	412.24	453.51	94.90	0.76	4.91
	9017	-0.35	385.94	440.36	91.64	1.01	4.94
	9023	0.54	367.35	418.59	88.26	0.95	5.75
	9026	-0.03	339.23	385.03	83.01	0.85	5.04
	9028	-0.05	378.68	438.10	90.85	1.10	5.19
	9035	0.12	367.35	423.13	88.64	1.03	5.31
	9038	-0.22	347.39	390.02	84.14	0.79	4.90
	9042	0.38	336.51	375.51	81.96	0.72	5.46
	9084	-0.12	319.73	376.87	80.62	1.06	4.82
	9113	0.04	304.76	361.90	78.01	1.06	4.90
	9114	0.49	396.83	439.00	92.43	0.78	5.88
	9122	-0.41	347.39	399.09	84.92	0.96	4.70

A-6. Continued

Year	ID	RFIg	Initial BWkg	Final BWkg	Metabolic BW kg	ADG kg/d	DMI kg/d
2010	9125	-0.24	321.54	377.78	80.86	1.04	4.71
	9132	-0.24	370.52	430.39	89.52	1.11	4.95
	9192	-0.17	344.67	392.74	84.14	0.89	4.93
	9200	0.08	359.18	409.98	86.84	0.94	5.24
	9209	0.15	352.38	394.56	84.96	0.78	5.31
	9215	0.35	365.53	402.72	86.77	0.69	5.58
	9226	0.09	315.65	363.72	79.12	0.89	5.04
2011	5	-0.72	320.62	344.66	77.89	0.43	4.87
	9	0.18	383.21	398.17	87.88	0.27	5.93
	13	0.42	363.71	385.93	85.19	0.40	6.24
	20	-0.03	429.92	453.95	96.39	0.43	5.86
	28	-0.20	374.59	419.49	88.95	0.80	5.45
	33	-0.54	313.37	324.25	75.45	0.19	5.44
	46	0.11	331.51	371.42	81.17	0.71	5.82
	48	0.26	360.53	385.48	84.88	0.45	5.95
	54	-0.41	339.22	390.46	83.48	0.92	5.27
	70	0.58	379.13	411.78	88.68	0.58	6.20
	71	-0.55	339.67	359.63	80.86	0.36	5.08
	79	0.00	367.34	385.93	85.49	0.33	5.76
	85	-0.39	369.15	399.99	86.84	0.55	5.39
	87	-0.35	297.95	321.08	73.79	0.41	5.12
	95	-0.19	345.57	380.94	83.21	0.63	5.23
	98	0.71	327.88	365.07	80.31	0.66	6.63
	106	0.37	332.87	375.04	81.60	0.75	6.00
	108	0.75	350.56	397.72	85.07	0.84	6.24
	109	-0.55	319.26	347.83	78.05	0.51	5.03
	133	0.34	304.75	335.59	75.69	0.55	6.01
	210	0.54	361.89	397.72	86.03	0.64	6.22
	219	0.18	356.45	385.48	84.53	0.52	5.96
	225	-0.60	308.83	333.78	75.89	0.45	5.29
	233	0.10	324.71	355.09	79.16	0.54	5.68