UNDERSTANDING BEEF CATTLE EFFICIENCY: I) UNDERSTANDING PHYSIOLOGICAL AND DIGESTIVE FACTORS AFFECTING RESIDUAL FEED INTAKE AND II) TANNIN SUPPLEMENTATION: EFFECTS ON ANIMAL PERFORMANCE, FERMENTATION, AND CARCASS TRAITS

A Dissertation

by

WIMBERLEY KAY KRUEGER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2009

Major Subject: Nutrition

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ABSTRACT

Understanding Beef Cattle Efficiency: I) Understanding Physiological and Digestive Factors Affecting Residual Feed Intake and II) Tannin Supplementation: Effects on Animal Performance, Fermentation, and Carcass Traits. (August 2009)

Wimberley Kay Krueger, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Gordon E. Carstens

Objectives of this study were 1) to characterize the relationship between beef cattle efficiency, namely residual feed intake (**RFI**), and digestive, microbial, and fermentation parameters in growing beef calves and 2) to examine the effects of added dietary hydrolysable or condensed tannin on animal performance and efficiency, fermentation and carcass and non-carcass traits. To accomplish the first objective, multiple RFI studies were conducted and in all studies RFI was calculated as the difference between actual and expected dry matter intake (**DMI**) based on average gaily gain (**ADG**) and body weight$^{0.75}$(**BW**). A total of 187 head selected out of a population of 600 head of growing beef calves were evaluated for diet and nutrient digestibility, ruminal and fecal volatile fatty acids (**VFA**) concentrations and methane producing activity (**MPA**). Low RFI calves consumed less DMI and had lower feed conversion ratios (**FCR**) as compared to high RFI calves. Low RFI calves also had higher diet and nutrient digestibilities compared to high RFI calves. Residual feed intake was negatively correlated with diet and nutrient digestibilities such that more efficient animals had

higher digestibilities. Low RFI calves tended to have lower ruminal propionate and higher acetate:propionate ratios when fed a high-forage diet. Calves with divergent RFI did not have different gross microbial populations as evidenced by the Firmicute:Bacteriodetes ratio, but low RFI calves tended to have higher fecal *Prevotella* spp. and lower fecal Spirochaetes and ruminal Cyanobacteria. The importance of these subtle shifts in microbial ecology is not evident at this time and more research is needed to fully elucidate the interaction of host and microbes to fully grasp the importance of minor microbial deviations. No differences in 3 h MPA were detected in low vs. high RFI calves but low RFI calves had higher fecal MPA when sampled at 24 h; however, calculated methane emissions were lower for low RFI calves. Tannin supplementation had no effect on animal performance and efficiency, ruminal fermentation VFA concentrations, MPA, or ammonia concentrations in finishing beef steers. There was also no detrimental effect of tannins on carcass traits; however, hydrolysable tannin supplementation resulted in increased empty rumen mass. Results from these studies indicate that diet and nutrient digestibility are affected by RFI such that more efficient calves had higher DMD, microbial ecology is responsive to RFI such that minor microbial shifts were observed, and tannin supplementation, at the current inclusion rate, had no effect on animal and carcass performance.

DEDICATION

I wish to dedicate this work to the wonderful animals that share my life

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I owe my success to God, who has blessed me in countless ways and provided me with strength, courage, and the will to go on. Through Him, all things are possible.

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NOMENCLATURE

A:P	Acetate : propionate ratio
Ac	Acetate
ADF	Acid detergent fiber
ADFD	Acid detergent fiber digestibility
ADG	Average daily gain
ADIA	Acid detergent insoluble ash
AIA	Acid insoluble ash
APA	Ammonia producing activity
BES	α-Bromoethanesulfonic acid
bTEFAP	Bacterial tag-encoded FLX 16s rDNA amplicon pyrosequencing
BW	Body weight
By	Butyrate
CFU	Colony forming unit
CH_4	Methane
CP	Crude protein
CPD	Crude protein apparent digestibility
CT	Condensed tannin
DGGE	Denaturing gradient gel electrophoresis
DM	Dry matter
DMD	Dry matter digestibility

DMI	Dry matter intake
DMI-Xm	Dry matter intake as a multiple of maintenance
f	Fecal
FBW	Final body weight
FCR	Feed conversion ratio (kg feed/kg gain)
FT	Fat thickness
G:F	Gain : feed (kg gain/kg feed)
GE	Gross energy
GIT	Gastrointestinal tract
HCW	Hot carcass weight
HT	Hydrolysable tannin
IBW	Initial body weight
IU	International unit
KPH	Kidney, heart, and pelvic
LW	Live weight
M	Maintenance
ME	Metabolizable energy
MPA	Methane producing activity
NDF	Neutral detergent fiber
NDFD	Neutral detergent fiber digestibility
NH_3	Ammonia
OM	Organic matter

PCR	Polymerase chain reaction
ppm	Part per million
Pr	Propionate
r	Ruminal
PRP	Proline-rich salivary protein
REA	Ribeye area
RFI	Residual feed intake
SF_6	Sulfahexaflouride
spp.	Species
trt	Treatment
VFA	Volatile fatty acid
YG	Yield grade

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Understanding Physiological and Digestive Factors Affecting Residual Feed Intake

Introduction

The goal of animal agriculture is to maximize the value of product outputs relative to the costs of inputs. Since the primary input costs for beef production systems are feed, animal efficiency has been a key driver of animal production for years. Production efficiency can be improved either by increasing product output or by decreasing inputs. It has long been recognized that it is more beneficial to maintain efficient animals. Mather et al. (1959) estimated the feed saved in raising 20 dairy heifers with above average efficiency vs. 20 heifers with below average efficiency would save enough feed to maintain two more cows and produce 310 kg more butterfat in a complete milking period. More recently, Crews (2005) estimated that it costs $38.00 less to feed an efficient bull for 150 d compared to an inefficient bull. That translates into significant input costs that can be saved by focusing on animal efficiency.

Residual feed intake (**RFI**) is a feed efficiency trait that can be used to select efficient cattle. Koch et al. (1963) reported RFI to be a superior measure of feed efficiency due to its independence of component traits, BW and ADG. Selection for improved feed efficiency based on RFI will not alter mature cow size unlike using feed

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This dissertation follows the style of Journal of Animal Science.

conversion ratio (FCR; feed:gain), which is highly correlated with growth traits, such that selection using FCR increases cow mature BW and cow maintenance (Herd and Bishop, 2000). Residual feed intake is the difference between actual feed intake and expected feed intake based on energy requirements for growth rate and body size. In a given contemporary group, some animals with similar BW and ADG will consume less feed than expected (more efficient; negative RFI) while others will consume more feed than expected (less efficient; positive RFI). Residual feed intake is a moderately heritable trait ($h^2 \sim 0.3$ to 0.4) and several studies have demonstrated that sufficient genetic variation does exist in RFI among the beef population to merit genetic selection (Archer et al., 1999; Arthur et al., 2001b, c; Herd and Bishop, 2000).

Genetic variation in rumen dilution rate appears to exist in sheep (Kahn, 1996; Orskov et al., 1971; Smuts et al., 1995; Thompson et al., 1989) and cattle (Orskov et al., 1988). Smuts et al. (1995) reported that the retention time of digesta in the rumen was heritable in sheep ($h^2 \sim 0.45$ to 0.6). Messiner et al. (1996) observed large inter-animal variation in amino acid and glucose availability in the duodenum of corn-fed steers. These studies suggest that this variation was associated with inherent differences in rumen retention time but not with feed intake. Kahn et al. (2000) found that differences in the supply of amino acids was associated with variation in efficiency of microbial production in sheep genetically selected for divergence in fleece weights. There is also evidence of genetic differences in starch digestion in low vs. high RFI animals (Channon et al., 2004). There is ample evidence to believe that there is room for genetic selection when it comes to animal efficiency and diet digestibility and utilization.

Biological Sources of Variation in RFI

Herd et al. (2004) summarized the biological basis for phenotypic variation in RFI in beef cattle (Figure 1.1); digestion accounted for 14% of the biologic variation in RFI, 9% to heat increment, 5% to retained energy, 5% to activity, with the largest proportion (67%) allocated to "other processes" like protein turnover, mitochondrial proton leakage, ion pumping, etc.

Genetic variation in maintenance energy requirements of cattle has been shown to exist (Carstens et al., 1987; Hotovy et al., 1991). Body composition also affects an animal's energy requirement; variation in composition of gain and resulting body composition can influence the efficiency of nutrient utilization (Herd and Arthur, 2009). In beef steers selected for divergent RFI, Richardson et al. (2001) reported that carcass chemical composition was correlated with RFI such that steers from low RFI parents had less empty-body fat and more empty-body protein than steers from high RFI parents. They concluded that variation in carcass chemical composition accounted for 5% of the variation in DMI while the balance was due to heat production. Basarab et al. (2003) reported that steers with high RFI had 6.4% more empty body fat than steers with low RFI fed a finishing ration.

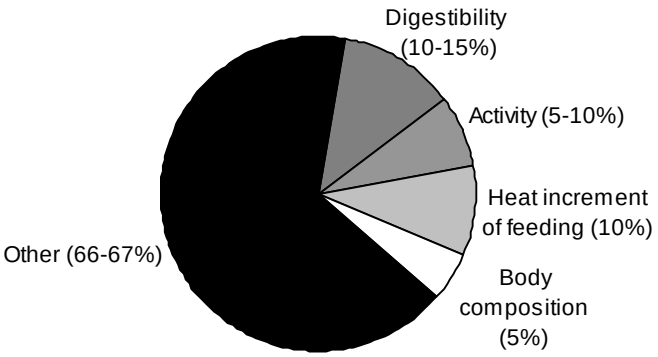

Figure 1.1. Contribution of various mechanisms to the variation in residual feed intake (Herd et al., 2004).

Variation in maintenance energy requirements may be caused by variation in visceral organ mass. Burrin et al. (1990) reported that the weights of liver, kidney, stomach, and small intestine respond to level of energy intake. Visceral organ mass accounts for approximately 6 to 10% of BW but accounts for 40 to 50% of whole body O_2 consumption (Burrin et al., 1990). During nutrient restriction, metabolic activity of the liver is decreased primarily by reductions in liver size (Burrin et al., 1990), and results in a decrease in the relative contribution of the liver to whole-body O_2 consumption (Burrin et al., 2007). Studies that have examined the association between RFI and proportional weights of visceral organs have provided inconsistent results. Basarab et al. (2003) reported that low RFI steers had 7.8% lower ($P < 0.05$) liver mass compared to the high RFI steers (6.06 vs. 6.57 kg), and 7.6% less stomach and intestines combined. White (2006) reported that Branvieh crossbred calves of differing RFI had similar liver and gastrointestinal weight. Ribeiro et al. (2007) also reported similar liver weight in low and high RFI calves but high RFI calves had heavier gastrointestinal tract weights compared to low RFI calves. Richardson et al. (2001) reported similar gastrointestinal tract and internal organ weight for steer progeny of parents selected for RFI.

Variation in heat production can also occur due to variation in activity. Luting et al. (1991) determined that 79% of the genetic variation in RFI in chickens was related to differences in activity. Hens with low RFI were less active compared to hens with high RFI. Richardson et al. (2000) reported that approximately 10% of the observed variation in RFI was explained by daily pedometer count such that low RFI calves were

less active than high RFI calves. Arthur et al. (2001a) calculated that approximately 5% of the variation in RFI was accounted for by daily distance walked and time spent standing and ruminating.

Differences in thermoregulation may also contribute to observed variation in RFI. Luiting et al. (1994) reported that hens with low RFI were less active, had less nude body area, and were slightly better feathered suggesting that ability to conserve body heat in low RFI chickens may explain variations in RFI. Schaefer et al. (2005) reported that cows with low RFI had 9% lower average dorsal temperatures as compared to high RFI cows. However, Brown (2006) observed no differences between high and low RFI steers in hair density, fiber, and curvature as well as dorsal infrared digital thermal images.

Collectively, results from these studies demonstrate that animal variation in RFI is the result of differences in a multitude of biological processes that contribute to observed phenotype differences. In experiments with cattle that differ by 6 to 7% in feed intake following a single generation of divergent selection for RFI, measuring the biological processes (i.e. digestibility, visceral organ mass, energy expenditure) that contribute to observed differences in DMI between divergent selection lines is a challenge (Herd et al., 1997; Herd et al., 2004; Richardson et al., 1998).

Feed Efficiency and Diet Digestibility

Diet digestibility is a function of dietary gross energy intake and fecal energy losses (NRC, 1996). Factors that can influence digestibility include level of intake, passage rate, environmental conditions, breed differences, and diet characteristics, to

name a few. The DMI of calves with high (> 0.5 SD) RFI is typically 15 to 20% greater than the DMI of calves with low (< 0.5 SD) RFI (Basarab et al., 2003; Brown, 2006; Kolath et al., 2006; Nkrumah et al., 2004; White, 2006). The increase in DMI of calves with high RFI may affect apparent dry matter digestibility (**DMD**) leading to the possibility that the difference in DMD observed in divergent RFI calves is due to a level of intake effect.

In ruminants, it is generally recognized that as DMI increases DMD decreases (NRC, 2001), primarily due to a reduction in the amount of time digesta spends in the rumen (Staples et al., 1984). Robertson and Van Soest (1975) found a decrease of 5% units in DMD when feeding level of a mixed diet was increased from maintenance to 2X maintenance. In dairy cattle fed a total mixed ration, for each multiple of maintenance increase in DMI there was a 4% unit decrease in diet digestibility (Tyrrell and Moe, 1975). However, diets high in fiber are less affected by the depression in DMD when feeding level increases (Colucci et al., 1982). This is primarily because cell solubles and N digestibility account for the majority of depression in digestibility as DMI increases. Galyean et al. (1979) fed a feedlot diet to dairy-beef crossbred steers at maintenance, 1.33X, 1.67X, and 2X maintenance. There was an overall decrease in DMD as DMI increased; however, there was no difference in DMD at the 1X and 1.33X maintenance levels or at the 1.67X and 2X maintenance levels. This would indicate that if the difference in the feeding level is negligible, (i.e. 1.67X vs. 2X) there is not a significant effect of DMI on DMD.

Channon et al. (2004) presented evidence to suggest differences in starch digestibility in divergent RFI steers fed a high energy feedlot diet. Angus and Angus-cross steer progeny of parent lines selected for either low or high RFI were used. The authors used fecal pH and fecal DM as a proxy for lower gut starch fermentation. When starch is fermented in the hindgut, fecal pH (Degregorio et al., 1982) and fecal DM are likely to be decreased leading to diarrhea (Huber, 1976). Steers with low RFI (from efficient parents) had higher fecal pH and fecal DM compared to high RFI steers (from inefficient parents) suggesting that the calves from more efficient parents fermented more starch in the rumen. This provides evidence of genetic differences in starch digestion. Channon et al. (2004) suggested that measuring fecal starch would have been useful to quantify as fecal starch has been shown to be closely associated with total tract starch digestibility [$R^2 = 0.95$; (Zinn, 1994)]. In cattle fed a high roughage pelleted ration (70 alfalfa hay:30 wheat mixture), Richardson et al. (1996) reported a 1% unit difference in DMD between steers with low compared to high RFI. They estimated that this difference in DMD equated to a 2.3% reduction in DMI in steers weighing 450 kg gaining 1.3 kg/d. Nkrumah et al. (2006) reported a tendency ($P = 0.1$) for low RFI Contentinal x British crossbred steers fed a high energy feedlot ration to have 6% higher DMD compared to high RFI steers. There was also a tendency ($P = 0.09$) for low RFI steers to have 7% higher apparent CP digestibility. Numerically, calves with low RFI had higher NDF and ADF digestibility compared to calves with high RFI but this difference did not approach significance due to the low fiber concentration of the feedlot diet. In monogastrics, differences in DMD are minimal and not important sources of

variation in RFI [chickens (Katle, 1991; Luiting et al., 1994) and pigs (De Haer et al., 1993)].

Feed Efficiency and Microbial Ecology

There has been recent interest in the association between the gut microbiome and obesity in mice and humans (Ley et al., 2005; Turnbaugh et al., 2009). These studies have revealed the importance of the interaction between the host and the microbiome. Ley et al. (2005) reported that obese mice (*ob* / *ob)* had phylum level alterations in the cecum microflora characterized by decreased Bacteriodetes and increased Firmicutes. They also speculated that there was an increased ability to harvest energy from poorly fermentable polysaccharides. This was confirmed by Turnbaugh et al. (2006). They demonstrated increased acetate and butyrate in *ob* / *ob* mice as well as decreased fecal energy concentration indicating that a microbiome dominated by Firmicutes (or low proportions of Bacteriodetes), a signature feature of obesity, are better able to extract energy from the diet leading to increased adiposity. Obese humans, similar to mice, also have an increased proportion of Firmicutes and decreased Bacteriodetes, which is responsive to weight loss; as weight loss increases, the proportion of Bacteriodetes increases (Ley et al., 2006). These studies demonstrate that obesity may have a microbial component. Additionally, Turnbaugh et al. (2009) reported that obese humans have decreased proportions of Bacteriodetes, increased Actinobacteria, and similar proportions of Firmicutes compared to lean humans. Natural selection for a microbiome low in Bacteriodetes, as is the case in obesity, results in decreased level of bacterial diversity and altered representation of bacterial genes and metabolic pathways as the

level of functional diversity in a microbiome is significantly linked ($R^2 = 0.81$) to the proportion of Bacteriodetes (Turnbaugh et al., 2009). Collectively, these data indicate that there is an undeniable interrelationship between host genetics, diet, environment, and the microbiome in energy utilization.

Consequently, there is also interest in understanding the relationship between RFI and microbial ecology and resulting VFA profiles to explain possible biological sources of variation in RFI. Guan et al. (2008) reported that Angus, crossbred, and Charolais calves, fed a high corn finishing ration, had distinctive ruminal fluid microbial populations based on RFI. Not only were differences noted between RFI groups, but low RFI steers were more similar to each other based on Dice similarity index (D_{sc} = 91% similarity) than high RFI steers (D_{sc} = 71% similarity). When additional data was collected on ruminal digesta from Charolais and Hereford-Angus crossbred steers that were raised at a different location but under similar conditions, denaturing gradient gel electrophoresis (**DGGE**) bacterial clustering based on RFI group was observed in the rumen digesta samples; clustering based upon RFI group was also observed in the rumen fluid samples. Additionally, when the digesta DGGE profiles were compared, two clusters formed, based on specific breed. To examine the effect of breed type in the initial rumen fluid samples, breed information was included; when correlated to the DGGE profiles, no direct correlation was observed. When the DGGE profiles of only the Angus calves were compared, clear separation patterns were observed for low and high RFI steers. This gives evidence of a host-microbe interaction such that the microbes present depend upon the host genetic make-up as all of the calves in the above

study were fed the same diet and raised in a similar environment under the same management conditions in both experiments (i.e. rumen fluid sampled steers and rumen digesta sampled steers). Guan et al. (2008) also demonstrated that divergent RFI calves had different VFA profiles. Low RFI calves had increased total VFA ($P = 0.06$), acetate ($P = 0.07$), butyrate ($P < 0.001$), and valerate ($P = 0.01$) concentrations. The authors interpreted these data to indicate a causal relationship between microbial ecology and resulting VFA profile in calves of divergent RFI. Methane was not measured in the previous study but the increased butyrate production is not only a measure of bacterial fermentation and rumen epithelial absorption (Brockman, 1993), but can serve as an alternative H^+ sink in the rumen to dispose of reducing equivalents (Mathison et al., 1998) and possibly decrease ruminal methane production.

Dry matter intake alone may also play a role in microbial diversity. McEwan et al. (2005) used day-length sensitive Soay sheep to demonstrate a change in microbial population in response to a photoperiod. Sheep that were allowed 8 h light consumed 42% less DMI as compared to sheep exposed to 16 h light (729 vs. 1277 ± 54 g DMI). Denaturing gradient gel electrophoresis analysis showed that there was a distinctive cluster difference in low vs. high intake sheep as well as an accompanying alteration in VFA profile. Sheep exposed to 8 h light had decreased total VFA, acetate, propionate, butyrate, valerate and caproate concentrations compared to sheep exposed to 16 h light. This provides indirect evidence of an effect of level of DMI on the microbiome but the authors offer tentative reasons why this may not be the case: 1) circulating hormones in low vs. high DMI sheep, due to photoperiod, may cross the rumen epithelia and

influence the microbial population and serve to differentially modify ruminal bacteria, 2) available substrate is at a lower limit for a particular population of bacteria in the low DMI sheep that is not limited in the high DMI sheep, and 3) high DMI sheep have a component of the diet that has a differential outflow rate compared to low DMI sheep serving to alter the high DMI sheep's microbiome. Interestingly, there was minimal diversity in the ciliate population in low vs. high DMI sheep.

Inter-animal variation in ruminal production of methane may also result in differences in metabolizable energy that contribute to variation in RFI. Variation in DMI explains a large proportion of the variation in methane production (Blaxter and Clapperton, 1965; Ellis et al., 2007). Diet digestibility plays a role in determining total methane emissions (Iqbal et al., 2008; Johnson and Johnson, 1995). Nkrumah et al. (2006) reported that calves fed a feedlot ration differed by 6% units in DMD between low and high RFI calves and low RFI calves lost less methane as a proportion of GE than high RFI calves (3.19 vs. 4.28 ± 0.3% of GE lost as CH_4). Hegarty et al. (2007) also noted that low RFI calves lost 25% less CH_4 as compared to high RFI calves fed a high barley feedlot ration; however, this was only significant if RFI was calculated over the 15 d CH_4 sampling period vs. the 70 d RFI. The authors attribute this lack of significance to altered DMI during the intensive cattle handling procedures needed to use the SF_6 tracer method to quantify methane production. Together with the intake modification of the SF_6 method and a high daily variation in methane production (Vlaming et al., 2008), it may be pertinent to delay methane measures in calves of divergent RFI until after a 70 d test or other methods should be employed (i.e. whole

animal calorimetry). Results from this study illustrate the challenges to quantify animal variation in methane production. Studies have not been conducted to examine the association between RFI and methane production and VFA profile concurrently. Theoretically, a low RFI calf would be expected to produce less methane (due to both level of intake and increased diet digestibility) and have an altered VFA profile, perhaps having increased propionate. Literature is lacking relating the efficiency of microbial growth and animal efficiency; however, Kahn et al. (2000) demonstrated that increased microbial growth was associated with genetic selection for increased fleece weight in sheep. Variation in the efficiency of microbial growth may also explain biological differences in RFI in ruminant animals.

Mitigating Methane Production in the Ruminant

Methane production in the ruminant is a necessary process to maintain homeorhesis in the rumen. It is a way to avoid hydrogen accumulation and subsequent inhibition of microbial dehydrogenases. Methane production in the rumen serves to decrease the partial pressure of H^+ in the rumen (Van Nevel and Demeyer, 1996) and stoichiometrically equates to:

$$CO_2 + 8\ H \rightarrow CH_4 + 2\ H_20$$

Methanogenic bacteria are very efficient at scavenging all available H_2 in the rumen thus allowing normal fermentation to continue (Mathison et al., 1998). If NADH were allowed to accumulate in the rumen and no alternative way to dispose of reducing equivalents were available, more reduced products such as lactate and ethanol would accumulate thus decreasing organic matter digestion and microbial growth (Wolin et al.,

1997). An alternative pathway for the disposal of reducing equivalents is through acetogenesis (Van Nevel and Demeyer, 1996) and stoichiometrically equates to:

$$2\ CO_2 + 8\ H \rightarrow CH_3COOH + 2\ H_20$$

Acetogenesis is present in the hindgut of termites and mammals and is an important H^+ sink in hindgut fermentation (Ljungdhal, 1986). However, H_2-oxidizing acetogenic bacteria are present in the rumen of sheep and cattle; they are easily outcompeted for H_2 as they grow fermentatively on organic substrates and will not actively consume hydrogen until preferred substrates have been exhausted (Van Nevel and Demeyer, 1996).

Several ways to mitigate methane production in the ruminant have been proposed; they include: alternative electron acceptors, halogenated methane analogues, use of ionophores, ruminal defaunation, addition of dietary unsaturated fatty acids, modification of feeding practices, selection to improve feed efficiency, vaccination, probiotics, and tannin supplementation.

Alternative electron acceptors include the organic acids fumarate and malate, inorganic sulfur, and nitrates/nitrites. Fumarate is a metabolic precursor to propionate and may provide alternative hydrogen sinks to decrease methane in vitro (Asanuma et al., 1999; Lopez et al., 1999) and in vivo (Bayaru et al., 2001); however, results are conflicting (Beauchemin and McGinn, 2006). Mathison et al. (1998) note that stoichiometrically, it is not economically feasible to include enough fumarate in the diet to channel electrons completely away from methanogens but may be important by having a stimulatory effect on certain microbial species. Malate is stimulatory to

Selenomonas ruminantium (Linehan et al., 1978) and malate supplementation in vitro increased propionate and decreased methane (Martin and Streeter, 1995). Inorganic sulfur can act as an electron acceptor by:

$$SO_4^{-2} + 8\ e^- + 10\ H^+ \rightarrow H_2S + 4\ H_20$$

Sulfate reducers are capable of using hydrogen at lower partial pressures than methanogens, easily outcompeting methanogens for electrons; however, the maximum tolerable level of sulfur in the diet is low, limiting its use as a potential methane reducer (Van Nevel and Demeyer, 1996). Nitrate reducers serve as terminal electron sinks by:

$$NO_3^- + 8\ e^- + 10\ H^+ \rightarrow NH_4 + 3\ H_20$$

Nitrates easily outcompete methanogens for terminal electrons resulting in reduced methane emissions when nitrate is fed (Takahashi and Young, 1991). However, absorbed nitrates can interfere with oxygen transport; nitrate supplementation would need to be highly controlled.

α-Bromoethanesulfonic acid (**BES**) has been one of the most widely studied halogenated methane analogs. α-Bromoethanesulfonic acid is a potent inhibitor of methanogenesis because it is structurally similar to the co-factor mercaptoethanesulfonic acid (HS-coenzyme M) used by methanogens (Taylor et al., 1974). However, some mutant strains of methanogens are resistant to BES (Smith and Mah, 1981). Additionally, there is an adaptation to prolonged administration of BES severely limiting its use as a methane inhibitor (Mathison et al., 1998).

Ionophores, such as monensin and lasalocid interfere with Gram positive bacteria while Gram negative bacteria are less sensitive due to the additional protection of the

outer membrane of the cell wall. These ionophores interfere with ion transport across the cell wall and disrupt transmembrane ion gradients (Van Nevel and Demeyer, 1996) resulting in a shift in microbial population towards less sensitive microbes that tend to produce propionate by diverting electrons from methane. The use of ionophores also raises the question of feeding antibiotics to cattle for prophylactic purposes and their possible subsequent effect on human health (Moss et al., 2000).

Ruminal defaunation may decrease the energy loss as a proportion of gross energy from 7.9 to 5.5% (Kreuzer, 1986). Newbold et al. (1995) estimated that from 9 to 25% of methane production originates from ciliate-associated methanogenic bacteria. Additionally, some rumen ciliates have endosymbiotic methanogens that account for 1 to 2% of the host ciliate volume (Finlay et al., 1994). The authors calculated that protozoa present at concentrations of 5×10^5 per mL could account for up to 37% of the total methane production in the rumen of sheep. Methanogens commonly colonize the outside of ciliates resulting in a symbiotic relationship such that the ciliates provide hydrogen substrate to the methanogens and the methanogens decrease the partial pressure of hydrogen locally to help maintain ciliate enzyme reactions. This methanogen-ciliate association has been observed in cattle (Vogels et al., 1980) as well as sheep (Stumm et al., 1982). Defaunation may serve to decrease methanogens but the ability to keep animals ciliate-free will severely limit its application as a way to decrease methane production.

Addition of dietary unsaturated fatty acids decrease methane production both by offering an alternative electron sink and direct toxic effects on methanogens (Henderson,

1973). Fatty acid supplementation often decrease the number of ciliate protozoa (Broudiscou et al., 1990; Czerkawski and Breckenridge, 1975), possibly leading to decreased methane emissions. Johnson and Johnson (1995) stated that dietary fat sources fed to sheep and cattle decrease methane emissions; however, it is a result of decreased substrate fermentation rather than a direct effect of diverting electrons to hydrogenate unsaturated fatty acids or toxic effects of fatty acids on methanogens. This was also reported by Beauchemin and McGinn (2006). Unsaturated dietary lipids may serve to decrease methane production but added fat must not be so high as to decrease fiber digestion.

Modifications of feeding practices can alter methane production by ruminants. Concentrate diets vs. forage diets will decrease methane production (Russell, 1998; Van Kessel and Russell, 1996). Diet characteristics also affect methane production. Mature dry forages tend to produce more methane than young dry forages; short chopped or pelleted diets produce less methane compared to long chop; leguminous forages produce less methane compared to grass forages, and silage produces more methane compared to dry conserved forages (Mathison et al., 1998). Increased feeding frequency (i.e. more meals throughout the day) decreases methane production and increases propionate production (Sutton et al., 1986).

Increasing the level of animal productivity can also mitigate methane emissions by ruminants. Beef calves gaining faster on a high quality diet will produce less methane than beef calves gaining slower on the same diet or on diets of poorer quality per unit of feed intake (Mathison et al., 1998). Additionally, increasing milk production

in dairy cows will reduce the amount of methane produced per kg of milk. Kirchgessner et al. (1995) suggested that doubling of yearly milk production from 5,000 to 10,000 kg of milk per year would only increase methane production by 5% (i.e. from 110 to 115 kg per year), but reduce methane per unit of milk by 48%.

Vaccination against microbes involved in lactic acidosis was reported (Shu et al., 1999). Immunized cattle had higher feed intakes, lower concentrations of lactate and lower numbers of *Streptococcus bovis* and *Lactobacillus* spp. demonstrating that ruminal microbial ecology can be altered in response to vaccination. Gnanasampanthan (1993) demonstrated that ruminal protozoa could be controlled by immunization. The author did note that inducing a salivary antibody response is critical for immunological control of protozoa in the rumen. Decreasing the number of protozoa by vaccination may be an effective strategy to reduce methane production in ruminant animals.

One of the most common probiotics used as a microbial feed additive is *Aspergillus oryaze*. The effects of *Aspergillus oryaze* on rumen fermentation and animal production are wide ranging. *Aspergillus oryaze* has been shown to reduce methane by 50% in a RUSITEC system (Frumholtz et al., 1989). The decrease in methane was directly related to the decrease in the protozoal population. However, addition of *Saccharomyces cerevisiae* to an in vitro system initially reduced methane production by 10% but this effect was transient (Mutsvangwa et al., 1992). Due to the variable responses seen with different probiotics, more research is needed to determine if a yeast extract will have a long-term effect on methanogenesis as well as the economics of feeding a probiotic.

A number of reports have demonstrated that enteric methane production in ruminants is reduced when supplemented with tannins (Hess et al., 2006; Min et al., 2006; Puchala et al., 2005). However, these studies were conducted with forage-fed animals where up to 12% of GE may be lost as methane. Methane loss on a high corn finishing ration is generally assumed to be approximately 3% of GE according to Johnson and Johnson (1995). Woodward et al. (2001) demonstrated that the feeding of *Lotus corniculatus* (2.6% CT_{DM}) and *Lotus pedunculatus* (8% CT_{DM}) decreased methane production in sheep and late lactation dairy cows. Sheep fed *L. corniculatus* lost 3.9% GE as methane vs. the control sheep fed cut perennial ryegrass that lost 6.2% GE as methane. Dairy cows fed *L. pedunculatus* silage lost 27 g CH_4/kg DMI vs. control cows fed perennial ryegrass silage that lost 35.7 g CH_4/kg DMI. This may be due to both a direct effect on methanogens (Field et al., 1989) as well as altered fiber fermentation (Newbold and Rode, 2006).

Tavendale et al. (2005) found that two strains of methanogens, *Methanobrevibacter ruminantium* YLM-1 and DSM1093 were sensitive to polymeric CT (> 12 mean degree of polymerization based on thiolysis) such that polymeric CT was bacteriostatic for strain YLM-1 and toxic for strain DSM1093, even after prolonged culture periods. In vitro methane production was also 30% lower in *L. pedunculatus* tubes vs. *Medicago sativa* incubations (8.8 vs. 12.5 mL CH_4; 0.05 g CT per in vitro incubation vial) resulting in decreased hydrogen accumulation in the CT treated vials. Additionally, pH was numerically lower in *Lotus* vs. *Medicago* treatment (6.21 vs. 6.48); *Lotus* supplemented vials also had lower propionate, butyrate, valerate, isobutyrate and

isovalerate concentrations compared to *Medicago* supplemented vials. The results of these trials indicate that condensed tannins have a direct bacteriostatic effect on methanogens as well as an indirect effect of decreased pH on methanogenesis as evidenced by no further increase in reduced organic acids (propionate and butyrate).

Ruminal methanogens lose their ability to take up H_2 at low pH (Russell, 1998). Van Kessel and Russell (1996) reported that at pH of less than 6.0, methanogens were not able to produce methane and concluded that diets that were able to decrease rumen pH to below 6 may be a viable strategy to reduce methane emissions from ruminants. At low pH, there is an accumulation of H_2 and no effect on propionate indicating that low pH drives a decrease in methnaogenesis and not just an increase in H_2 competition via increased propionate production (Russell, 1998). Methane production was found to be positively correlated with acetate:propionate ratio ($r^2 = 0.80$) which is dependent on pH; as pH decreases, the acetate:propionate ratio decreases resulting in decreased methane production (Russell, 1998).

Hess at al. (2003) decreased methane production by 50% in RUSTIEC in vitro systems inoculated with 15 g *Calliandra calothyrsus*/L (4.1 g CT/L) vs. control systems. Alterations in VFA profile were also observed. Acetate was increased while propionate, butyrate, and isobutyrate were decreased relative to control systems that contained *Brachiaria dictyoneura*, devoid of CT. The acetate:propionate ratio and pH was similar for CT treated and control systems (7.13 vs. 7.11, 2.9 vs. 2.7, respectively). Condensed tannin systems had 23% lower organic matter degradation as compared to control systems (174 vs. 227 mg OM/g supplied). Decreased OM degradation would also lead

to decreased methane production; however, there was still a 35% reduction in methane per unit of OM apparently degraded indicating that CT supplementation reduced methane production not only via reduced fermentation activity but through direct action on methanogens.

Tannin Supplementation: Effects on Animal Performance, Fermentation, and Carcass Traits

Introduction

Tannins are a complex group of polyphenolic compounds that plants have evolved to avoid animal consumption (Foley et al., 1999). Tannins are commonly referred to as plant secondary compounds (as well as oxalates, terpenes, saponins, to name a few) and may have positive or negative effects on animal production. Originally, the term "tannin" was applied to any substance that was able to tan leather; however, currently, it is generally used to denote any naturally occurring substance of high molecular weight and contains a large number of phenolic hydroxylic groups to enable it to form effective cross-links with proteins (Swain, 1979). Tannins are classified into two categories: hydrolysable and condensed (**HT** and **CT**, respectively). Hydrolysable tannin consist of a carbohydrate core with phenolic carboxylic acids bound by ester linkage and CT consist of oligomers of flavon-3-ols and related flavanol residues (Mueller-Harvey and McAllan, 1992). Tannins are ubiquitous in nature and are widely found in many forages, fodders, and agroindustrial wastes.

Tannin Effect on Growth and Composition of Gain

Elevated dietary tannins can result in decreased DMI, growth and damage to the gastrointestinal tract (Hervas et al., 2003; Mcleod, 1974; Robbins et al., 1991). In rats, Mitjavila et al. (1977) demonstrated that tannin supplementation reduced intestinal permeability; this could lead to decreased nutrient absorption and lowered DMI as well. Reduced DMI is thought to be caused by the astringent taste and decreased palatability possibly resulting in food avoidance (Kumar and Singh, 1984). Many mammals, especially browsers, are able to produce proline-rich salivary proteins (**PRP**) that are able to bind to dietary tannins to inactivate them (Austin et al., 1989). It is the binding of PRP and tannins that produce the astringent taste (Prinz and Lucas, 2000) and subsequent food avoidance. Cattle and sheep are devoid of PRP (Makkar, 2003) so the decrease in DMI due to the astringent taste mechanism associated with tannins may not occur in cattle and sheep. However, other proteins are present in the saliva of cattle fed tannin-rich diets which have a high affinity for tannins but are not rich in proline; these salivary proteins tend to form soluble tannin-protein complexes (Makkar, 2003).

Additionally, the reduction in DMI due to tannins may be a learned avoidance related to gastric upset (Provenza et al., 1990; Villalba and Provenza, 2001). This was demonstrated in goats fed blackbrush (*Coleogyne ramosissima*); when new growth blackbrush (high in CT) was fed, intake decreased over time indicating post-ingestive distress (Provenza et al., 1990). Villalba and Provenza (2001) demonstrated that lambs can sense the negative impacts that CT ingestion has and learn to consume polyethylene glycol (complexes with tannins to inactivate them) in conjunction with the tannins, to

ameliorate the malaise caused by tannin ingestion. When tannins were not offered in the diet, polyethylene glycol consumption was decreased indicating a self-medicating ability of the lambs. There is definite evidence of preference of consuming diets low in tannin when given a choice. In goats offered basal diet and added CT diet in two individual feed pans, goats routinely selected the basal diet over the added CT diet and when they did consume the CT diet they consumed less of the tannin added diet (Provenza et al., 1990). Frutos et al. (2000) intra-ruminally dosed sheep with Quebracho CT (0.75 g/ kg LW) for 60 d and found no gross or histological evidence of toxicity; however, in sheep fed Quebracho CT at 3 g/kg LW, DMI was decreased and were weak and depressed by d 5, and were harvested on d 8. Ewes had striking digestive tract lesions throughout and altered blood chemistry (Hervas et al., 2003).

Tannins can cause a decrease in ADG due to lowered DMI and toxicity in sheep (Hervas et al., 2003). Frutos et al. (2004) demonstrated no effect of chestnut tannin on lamb carcass traits when fed approximately 0.84 g tannin/kg LW. Additionally, there was no effect of HT supplementation on ADG, feed efficiency, and length of finishing period. Individual weights of offal (blood, skin, fat depots, and parts of the GIT) did not differ in weight between control and chestnut treated finished lambs. Chemical composition and energy concentration of the empty body weight was not different between control and HT treated lambs. Maxson et al. (1973) demonstrated decreased DM, CP, and TDN digestibility in steers fed a high corn finishing ration (0.51 $\%_{DM}$ tannin) or a high tannin sorghum finishing ration (2.15$\%_{DM}$ tannin). Average daily gain was decreased ($P < 0.05$) in tannin supplemented steers and F:G was numerically higher.

Additionally, dressing percent, hot carcass weight, and yield grade was reduced by tannin supplementation.

McBrayer et al. (1983) fed heifers varying levels of peanut skins (0.4, 2.2, or 3.9% $tannin_{DM}$) were fed for 100 d. Heifers fed the tannin supplemented diet had lower ADG, DMI, and higher F:G. Diet DM and CP digestibility was also reduced with peanut skin supplementation. However, in steers supplemented with peanut skins (0.6, 1.7, or 2.7% $tannin_{DM}$) for 84 d, there was no detrimental effect of added tannin on ADG, F:G, dressing percent, marbling score, yield grade, quality grade, and back fat thickness. However, DM digestibility was reduced for the added peanut skin diets. Additionally, heifers grazing ryegrass pasture that were supplemented with corn plus peanut skins (0.91 kg corn, 0.91 kg peanut skins) for 112 d had higher ADG compared to corn (1.82 kg corn) supplemented heifers.

Chickens fed high-tannin sorghum also showed no effect of tannins on carcass traits or yield of organs (Kumar et al., 2005). In rats fed graded levels of high tannin sorghum (3.76% CT), Larrain et al. (2007), observed no detrimental effects of tannin supplementation on BW, ADG, G:F, and average daily feed intake when fed for only two weeks. When rats were fed for ten weeks, no difference in G:F between control and tannin diets; however, the 35% high tannin sorghum diet resulted in heavier ($P = 0.05$) rats at d 70, higher ADG and intake during the first two weeks of the study; because G:F was not different at any time point, greater feed and energy intake seems to be driving higher ADG. In finishing pigs, Cousins et al. (1981) demonstrated increased feed intake and F:G and similar ADG when fed a high tannin sorghum diet (3.4% $tannin_{DM}$)

compared to pigs fed corn. Collectively, these studies indicate that the response to tannin supplementation is varied and depends on type of tannin, rate of supplementation, and species and in many cases suggest that tannin supplementation at low rates do not have a detrimental effect on economically important carcass and non-carcass traits.

Tannin Effect on Microbial Populations

Tannins have many and diverse effects on microbial populations both in vitro and in vivo. Henis et al. (1964) found an antimicrobial effect of carob pod tannin extract on *Cellvibrio fulvus* (a celluloytic bacterium) in vitro; tannin addition also resulted in morphological changes indicating tannin effect on this bacterium. Alteration in gut microbial population was demonstrated in rats, fed CT at 20 mg/kg diet. There was a shift in fecal microbial population favoring *Enterbacteriaceae* and the *Bacteroides* species (Smith and Mackie, 2004). Decreased celluloytic and proteolytic activity was also observed by Tagari et al. (1965) with carob pod extract in an artificial rumen indicating some effect on the microbial population. McSweeney et al. (2000) fed sheep a diet of 30% *Calliandra calothyrsus* and the population of *Rumminococcus* spp. and *Fibrobacter* spp. were decreased but fungi, protoza and proteolytic bacteria were less affected. When polyethylene glycol was added to neutralize the tannin effect, bacterial populations were restored. Additionally, post feeding concentrations of branch chained VFA were decreased in *Calliandra* supplemented sheep possibly due to protein binding by the *Calliandra*.

Sotohy et al. (1997) reported that the number of total bacteria in the rumen of goats decreased when they were fed *Acacia nilotica*, a tannin-rich plant. The decrease in

bacterial population was directly proportional to the amount of *Acacia nilotica* fed. Condensed tannins extracted from Sainfoin (*Onobrychis viciifolia)* inhibited growth and cell-associated proteolytic activity of *Butyrivibrio fibrisolvens* A38 and *Streptococcus bovis* 45S1 with little effect on *Prevotella ruminicola* $B_1$4 and *Ruminobacter amylophilus* WP225.

Tannin degrading bacteria have been described. Brooker et al. (1994) isolated a tannin degrading bacterium from feral goats grazing *Acacia aneura*. This bacterium was also isolated from feral camels but not from domestic goats or sheep in the area. The specialized bacterium, similar to *Streptococcus bovis*, was determined to be a new species of *Streptococcus* and was named *Streptococcus caprinus*. In vitro, the bacterium grows in a medium with at least 2.5% (wt/vol) tannic acid or condensed tannin. This would be equivalent to an animal consuming 1 kg of plant material containing 12.5% condensed tannin. *S. caprinus* was found to be at low concentrations in the rumen at 2 x 10^6 cfu/mL. Odenyo and Osuji (1998) isolated three strains of tannin degrading bacteria from sheep, goat, and antelope in East Africa. Animals had been grazing *Acacia aneura*, *Acacia angustissima*, and *Calliandra callothyrsus*. All three isolates (EAT2, ES3, and EG19) were able to grow in media that contained up to 8 g of condensed tannin/L. Thirty grams of hydrolysable tannin/L in a medium would be equivalent to 15% tannin in the diet of a ruminant (Nelson et al., 1995). Isolates were able to degrade up to 15 g tannic acid/L and tolerated 30 to 70 g of tannic acid/L, which is equivalent to 15 to 35% tannin in feed. The tannin degrading specialty of the isolates is evident as the content of

tannins in feedstuffs (i.e. sorghum, *Vicia faba*, sal and rapeseed meals, carob, and forage legumes) range from less than 1% to 50% tannin on a DM basis (Giner-Chavez, 1996).

Nelson et al. (1998) isolated tannin degrading bacteria from Sardinian sheep, Honduran and Columbian goats, white-tailed deer from upstate New York, and Rocky Mountain elk from Oregon. Six isolates were identified; four of the six were members of the genus *Streptococcus* and were most closely related to *S. bovis* and *S. gallolyticus* [new research found *S. caprinus* to be *S. gallolyticus*; (Sly et al., 1997)]. One isolate fell into the Firmicute phylum and the sixth isolate was a member of the family *Enterobacteriaceae*. The bacteria were isolated from different geographical locations indicating that presence of tannin degrading bacteria is not restricted by climate, geography, or host animal as long as tanniniferous forage is consumed. Tannin degrading bacteria could not be isolated from cows on low tannin diets. However, Goel et al. (2007) isolated tannin degrading bacteria from the feces of goats that were not fed tannin-rich diets. A 24 h enrichment with 1% tannic acid was performed which may have facilitated their identification; an enrichment was not noted in Nelson et al. (1998).The lack of tannin substrate in low tannin diets may indicate that tannin-degrading bacteria are very specialized and are only needed at low levels to detoxify tannins. This is quite similar to the detoxifying effect of *Synergistes jonesii* in leucaena (mimosine) toxicity when animals graze *Leucaena leucocepaala* (Allison et al., 1992). In animal populations that are not adapted to *Leucaena*, *Synergistes jonesii* is not present so it is not surprising that tannin degrading bacteria were not isolated from cattle consuming low tannin diets.

Wiryawan et al. (1999) demonstrated that rumen fluid from tannin adapted animals transferred to naïve animals serves as inoculum resulting in tannin degrading bacteria colonization and decreased negative effects of high dietary tannins much like rumen fluid transfer from *Leucaena* adapted ruminants in Hawaii served as inoculum for Australian ruminants suffering from mimosine toxicity (Jones and Megarrity, 1986). Brooker et al. (1995) further demonstrated that not only could rumen fluid from tannin adapted goats be used to inoculate sheep, but that *S. gallolyticus* established a stable population of 10^4 to 10^5 cfu/mL of rumen fluid provided the sheep were fed a tannin-rich *Acacia* diet. When sheep were turned out onto low tannin-pasture, *S. gallolyticus* declined to 10^3 to 10^4 cfu/mL. When sheep were returned to a high tannin diet, *S. gallolyticus* was reestablished at 10^5 cfu/mL rumen fluid. Additionally, sheep inoculated with either rumen fluid from tannin-adapted goats or pure cultures of *S. gallolyticus* had higher nitrogen and dry matter digestibility when fed *Acacia* than un-inoculated sheep or sheep inoculated with *S. bovis*, a tannin-sensitive bacterium.

McSweeney et al. (1999) isolated 15 bacterial genotypes from sheep and goats fed *Calliandra calothyrus*. All isolates were able to grow in either the presence of tannic acid or condensed tannin. Only two were able to grow in both tannic acid and condensed tannin fortified agar plates. The strains isolated were proteolytic, but the authors were unable to demonstrate degradation of calliandra-protein complexes or fermentation of in situ complexed calliandra protein.

Condensed tannin from *Lotus corniculatus* decreased the rate of proteolysis and inhibited the growth of proteolytic rumen micro-organisms (Min et al., 2005a).

Condensed tannin was added in vitro at 0, 50, 100, 200, 400 and 600 μg CT/mL. At the 200 μg/mL treatment, bacterial growth was significantly reduced. At the 50 and 100 μg/mL treatment, there was a transient, synergistic effect of the CT such that increased microbial activity was observed in some strains. Various studies have also shown that low-level CT supplementation (100 – 200 μg/mL) increases microbial growth and enzyme activity (Bae et al., 1993; Jones et al., 1994; Oh and Hoff, 1986). Mole and Waterman (1985) suggest this could be due to structural changes in the substrate protein due to its protein-CT interaction allowing easier access of proteolytic enzymes to their substrate. Additionally, dietary condensed tannins (less than 2 – 3% $diet_{DM}$) have been shown to have benefit in ruminants as they serve to protect high quality dietary protein from microbial attack in the rumen making the protein available for small intestinal absorption (Barry and Blaney, 1987).

Tannin Degradation in the Rumen, Microbial Tannin Toxicity, and Tannase Activity

Hydrolysable tannins are usually degraded via enzymatic degradation by ruminal microflora with considerably less degradation of condensed tannins (Goel et al., 2005). Many times, ruminal bacteria are able to grow on HT but not CT leading to a lack of information on CT toxicity to rumen microbes. Condensed tannins have a more deleterious effect on digestibility than HT whereas HT cause varied toxicoses due to ruminal degradation (Kumar, 1991). Hydrolysable tannins degrade to gallic acid, pyrogallol, phloroglucinol, and finally, to acetate and butyrate (Bhat et al., 1998). Condensed tannins undergo limited ruminal action; CT degrade to quercetin,

phloroglucinol, 3-hydroxy-5-oxohexanoate and finally to acetate and butyrate (Bhat et al., 1998).

Three mechanisms of tannin toxicity in the rumen bacterial population have been suggested: 1) altered enzyme activity, 2) altered biological membranes, and 3) metal ion depravation. Altered enzyme activity via enzyme inhibition is the most common mechanism. Reed (1995) demonstrated that cell wall associated enzymes are less affected by tannins compared to extracellular enzymes. Decreased activity in ruminal urease, carboxymethylcellulase, protease, glutamate dehydrogenase, and alanine aminotransferase in response to tannin treatment was reported by Makkar et al. (1988). Additionally, tannins have been shown to inhibit pectinases, cellulases, β-galactosidases and proteases (Bell et al., 1965; Smart et al., 1961). In rats, tannins decrease activity of trypsin and α-amylase but not lipase, indicating that tannins have little affinity for lipase (Horigome et al., 1988). Tannins also have the ability to alter membrane function by inhibiting entry of substrates via decreased membrane permeability; this results from the formation of tannin complexes with cell wall protein (Goel et al., 2005). Scalbert (1991) reported that tannins exert their antimicrobial properties by iron depletion thus reducing the activity of metalloenzymes in the microbial cells.

Skene and Brooker (1995) isolated the anaerobe *Selenomonas ruminantium* ruminantium from feral goats browsing tannin-rich *Acacia* sp. The bacterium was the first rumen bacteria shown to have tannin acylhydrolase (tannase) activity. When the bacterium was cultured with tannic acid, a hydrolysable tannin, gallate accumulated in the media, indicating that the bacterium had the ability to cleave hydrolysable tannin but

not to further utilize the end products for energy. Goel et al. (2007) isolated bacterial strains that produced tannase and gallate decarboxylase from the feces of goats on a low tannin diet suggesting that the tannin-degrading bacteria are part of the normal microflora of the feral goats and that the tannin-degrading enzymes are inducible with the addition of tannin acid in vitro.

Tannase catalyze the hydrolysis reaction of the ester bonds present in hydrolysable tannins (Aguilar et al., 2007). Condensed tannins are not acted upon by classical anaerobic tannase but require oxygen for their degradation (Contreras-Domínguez et al., 2006); however negligible amounts of CT are acted upon by rumen microbes. Tannase (tannin acyl hydrolase) is produced by various fungi, yeast, and bacteria. No ruminal fungi or yeast have been identified that have tannase; however, numerous anaerobic bacteria have been isolated (Goel et al., 2007; Osawa et al., 1995a; Osawa et al., 1995b; Skene and Brooker, 1995). The fungus *Aspergillus niger* van Tieghem with tannase activity was identified from the feces of hill cattle fed largely *Quercus incana* (oak) leaves (Bhat et al., 1996). The authors note that feces were directly taken from the rectum and deposited into sterilized jars and the possibility of feed or fodder contamination with this fungus was examined. The authors conclude that the fungus was isolated because it is part of the normal flora in these hill cattle and not from contamination. The ability to commercially synthesize and harvest tannase may lead to detannification of high tannin feedstuffs rendering them more useful to any given animal.

Tannin Effect on Ruminal Fermentation

It is reported in the rat, that grape seed extract CT effectively decreased cecal pH and increased total cecal VFA concentration; specifically it increased acetate and decreased propionate and butyrate (Tebib et al., 1996). However, the physiologic differences in rats may limit inferences concerning cattle. Sheep supplemented with *Elaeis guineense* (18 g/kg DM CT; average 0.11 mg tannin/kg LW) showed a decrease in rumen pH sustained 5 h post ingestion and increased ammonia concentrations; rumen pH began to return to pre-feeding levels after 5 h (Osakwe et al., 2004). Increased total VFA was also observed. Makkar et al. (1995) showed lowered VFA production in vitro when tannins were added (0.8 mg/mL) to the medium with CT decreasing VFA production to greater extent that HT. There was also a substantial decrease in the proportions of acetate and propionate. Growing beef cattle fed a forage based diet supplemented with either 0, 1, or 2% DM with Quebracho tannin, showed no effect on total VFA concentration (Beauchemin et al., 2007), but the molar proportion of acetate was decreased as was the acetate:propionate and ruminal ammonia. Waghorn and Shelton (1997) fed 0.68 g CT/kg LW to sheep for 32 d; no effect of CT was observed on VFA concentrations or ammonia in these sheep.

Conclusion

Collectively, these studies indicate a lack of knowledge and understanding of the biological mechanisms that affect animal efficiency. Many biological processes interact together to explain RFI. Diet digestibility may help to explain variation in observed RFI as well as energy losses via methane emissions, microbial populations, and VFA profile in growing beef calves of divergent RFI. It is evident in the literature that RFI can be a tool to improve the efficiency of beef production; however, more research is needed to better understand the mechanisms that contribute to RFI as well as how to modify them. Additionally, tannin supplementation may be a management strategy to improve animal efficiency by altering VFA production or reducing energy losses via methane production in beef cattle. Thus, the objectives of this dissertation were to examine the relationship between RFI and physiological and digestive parameters in growing beef calves as well as to characterize the effects of dietary tannins on live animal performance and efficiency, fermentative characteristics, and subsequent carcass traits in finishing beef calves.

CHAPTER II

RELATIONSHIPS BETWEEN RESIDUAL FEED INTAKE AND APPARENT NUTRIENT DIGESTIBILITY, IN VITRO METHANE PRODUCING ACTIVITY, AND VFA CONCENTRATIONS IN GROWING BEEF ANIMALS

Introduction

The goal of animal agriculture has been to maximize the value of product outputs relative to the costs of inputs. Since the primary input costs for beef product systems are feed, animal efficiency has been a key driver of animal production for years. Arthur et al. (2004) estimated that 65% of the total feed requirements are used to maintain the breeding herd; small steps to increase efficiency may decrease inputs, increase outputs, or both. It has long been recognized that it is more beneficial to maintain efficient animals as Mather et al. (1959) estimated the feed saved in raising 20 dairy heifers with above average efficiency vs. 20 heifers with below average efficiency would save enough feed to maintain two more cows and produce 310 kg more butterfat in a complete milking period. More recently, Crews (2005) estimated that it costs $38.00 less to feed an efficient bull for 150 d compared to an inefficient bull. That translates into a substantial economic advantage to try and improve feed efficiency. A potential way to increase efficiency is to use residual feed intake (**RFI**) as a tool in cattle selection. Koch et al. (1963) reported RFI to be a superior measure of feed efficiency due to its independence of component traits BW and ADG. RFI is a moderately heritable trait (h ~ 0.30 to 0.40) (Arthur et al., 1997; Arthur et al., 2001c; Schenkel et al.,

2004). Selection for improved feed efficiency based on RFI will not alter mature cow size unlike using feed conversion ratio (**FCR**; feed:gain), which is correlated with growth traits, such that selection using FCR would increase cow mature BW and cow maintenance (Herd and Bishop, 2000). Residual feed intake is calculated as the residual from the linear regression of DMI on mid-test $BW^{0.75}$. Herd et al. (2004) estimated that approximately 14% of the biological variation in RFI was associated with differences in digestion and its processes based upon Richardson et al. (1996) who reported differences in dry matter digestibility in growing animals of divergent RFI . Recent studies with cattle fed high-grain diets suggested that inter-animal variation in RFI may be due to differences in digestibility (Channon et al., 2004; Richardson et al., 1996). However, few studies have examined the effects of RFI on fermentation parameters and nutrient digestibility in growing calves fed a high-roughage diet. The objectives of these studies were to quantify differences in nutrient digestibility, examine ruminal and fecal VFA profiles, and estimate in vitro methane producing activity in growing calves with divergent phenotypes for RFI.

Materials and Methods

Animals and Management

All experimental procedures were approved by the Animal Care and Use Committee for Livestock at Texas A&M University. Two studies were carried out to evaluate the relationships between RFI and diet digestibility and fermentation parameters in growing beef animals. Study 1 used Santa Gertrudis steers (n = 57) from King Ranch (Kingsville, TX), and study 2 used Brangus heifers (n = 468) from Camp

Cooley Ranch (Franklin, TX). Study 2 consisted of 4 trials conducted in 4 consecutive years. All tests were conducted at the O.D. Butler Jr. Animal Science Teaching and Extension Center, College Station, TX. The steers in study 1 were 9 to 11 mo of age and had initial BW of 289.8 ± 33.6 kg and the heifers in study 2 were 231.4 ± 11.5 d of age and weighed 271.4 ± 26.1 kg upon arrival. Upon arrival, calves were allotted by BW to Calan-gate pens (6 hd/pen) and were adapted to the diet and trained to eat from the Calan gate for 24 to 28 d. In both studies, calves were fed twice daily, sufficient to allow ad libitum intake, and had free access to water. All calves were fed for 70 d a high-roughage diet consisting of chopped alfalfa, alfalfa pellets, cottonseed hulls, dry rolled corn, molasses and premix (Table 2.1); the percentage of alfalfa pellets and corn varied slightly between studies. Nutrient concentration in each ration is given in Table 2.1. In both studies, body weight and orts were measured weekly. Residual feed intake was calculated as the residual of linear regression of DMI on mid-test $BW^{0.75}$ and ADG [i.e. the difference between actual DMI and expected DMI to meet growth and maintenance energy requirements (Koch et al., 1963)].

In study 1, data collected during the 70-d trial was used to calculate RFI, and the 16 steers with the lowest and 16 steers with the highest RFI identified for subsequent measurement of diet digestibility. In study 2, data collected from the first 56-d of the trial were used to calculate RFI and the 20 heifers with the lowest and 20 heifers with the highest RFI identified for subsequent measurement of diet digestibility.

Table 2.1. Ingredient and chemical composition of the diets fed in studies 1 and 2

Item	Study 1 Value, %	Study 2 Value, %
Feed Ingredient[1]		
Chopped alfalfa	35.00	35.00
Pelleted alfalfa	19.00	15.00
Dry rolled corn	15.50	20.95
Cottonseed hulls	21.50	21.50
Molasses	7.00	7.00
Premix[2]	2.00	---
Salt	---	0.40
Vitamin E[3]	---	0.14
Trace mineral[4]	---	0.02
Chemical composition[5]		
DM, %	87.1	87.9
ME[6], Mcal/kg$_{DM}$	2.13	1.98
CP, %$_{DM}$	11.2	12.7
NDF, %$_{DM}$	41.4	45.6
ADF, %$_{DM}$	32.0	32.3
P, %$_{DM}$	0.27	0.25
Ca, %$_{DM}$	0.98	0.86

[1] Expressed on an as-fed basis.
[2] 1.66 g/kg monensin, 0.55 g/kg tylosin, 675 mg/kg Cu, 1050 mg/kg Mn, 2850 mg/kg Zn, 15 mg/kg Se, 35 mg/kg I, 7.5 mg/kg Co, 132,300 IU/kg vitamin A, and 3308 IU/kg vitamin E.
[3] Vitamin E contained 44,000 IU/kg product.
[4] Trace mineral contained minimum 19.0% Zn, 7.0% Mn, 4.5% Cu, 4,000 ppm Fe, 2,300 ppm I, 1,000 ppm Se, and 500 ppm Co.
[5] Study 2 represents the average of four yr.
[6] Metabolizable energy content computed using Cornell Net Carbohydrate and Protein System.

Estimates of Digestibility

Study 1 calves were selected based on d 70 RFI while study 2 calves were selected based on d 56 RFI. The steers used in study 1 had RFI that were ± 0.55 SD from the mean RFI of 0.0 ± 0.85 kg/d, whereas the heifers in study 2 had RFI that were ± 1.1 SD from the mean RFI of 0.0 ± 0.70 SD.

In both studies, feed ingredients were sampled daily, and orts were weighed and sampled daily during the fecal collection period. Feces were collected for 7 consecutive d at 0700 daily and frozen. In study 1, fecal samples were collected d 70 to 76 and in study 2 fecal samples were collected on d 62 to 68 of the feed intake measurement period. In study 1 and study 2 (yr 1, 2, and 3), feed, feces, and orts were freeze dried and ground in a Wiley Mill to pass a 1mm screen. For yr 4 of study 2, feed, feces, and orts were dried at 105 ˚C to a stable weight and ground to pass a 1 mm screen. Daily fecal and ort samples were composited by weight to generate 1 fecal and ort sample for each calf. Individual feed ingredient samples were also composited by weight resulting in 1 sample for each feed ingredient used in the experimental diets. A weighted average of each feed ingredient was used to calculate diet internal marker concentrations. Acid insoluble ash (**AIA**) was used as an internal marker to estimate digestibility coefficients in study 1 and acid detergent insoluble ash (**ADIA**) was used, in addition to AIA, in study 2.

VFA, Methane, and pH Analysis

In study 2 (yr 2, 3, and 4), rumen fluid and feces were collected for VFA, methane producing activity (**MPA**), and pH analysis. Rumen fluid was collected via

stomach tube, before the morning feeding, into 50 mL serum vials that were filled to capacity, capped immediately and stored at ambient temperature until analysis later that day (less than 2 h). In vitro MPA of ruminal and fecal samples were determined by in vitro incubation of 5 mL rumen fluid or 2 g feces, mixed with 5 or 8 mL, respectively, anaerobic dilution solution (Bryant and Burkey, 1953) containing 60 mM sodium formate and 0.2 g finely ground alfalfa (to pass a 4 mm screen). The tubes were capped and incubated at 39 ˚C under a hydrogen:carbon dioxide (50:50) atmosphere. At the end of the incubation period, methane concentration was determined by gas chromatography according to Allison et al. (1992). For VFA analysis, 1 mL or 1 g of rumen fluid or feces were diluted 1:10 with water (pH = 7.0) and pH was recorded, samples were centrifuged and the supernatant frozen (-20 °C) for subsequent VFA analysis. Volatile fatty acids were analyzed via gas chromatography (Agilent 6890N, Santa Clara, CA, USA) with a 007 series bonded phase fused silica capillary column (25m x 0.25mm x 0.25 μm) with a flame ionizing detector with the following parameters: 1 μl injection, injector temperature = 240 ˚C, oven temperature = 80˚C for 1 min, ramp to 120 ˚C hold for 5 min, ramp to 165 ˚C hold for 2 min, detector temperature = 260 ˚C.

Chemical Analysis and Calculations

Acid insoluble ash was determined according to Van Keulen and Young (1977) using 2 N HCl digestion and ashing. Acid detergent insoluble ash was analyzed according to Van Soest et al. (1991) using the ADF procedure and subsequent ashing. Neutral detergent fiber and ADF were determined using an ANKOM Fiber Analyzer F200 (ANKOM Technology Corporation, Fairport, NY.) according to manufacturer's

protocols with the exception that sodium sulfite was not added for the NDF procedure (Ankom, 2006a, b). Nitrogen was determined using a LECO FP2000 nitrogen analyzer and 6.25 used as a conversion factor to calculate CP (LECO Corporation, St. Joseph, MI). Mineral analysis was determined by an independent laboratory using ICP analysis of a nitric acid digest. Metabolizable energy concentrations of the test diets were computed from the chemical analysis using the Cornell Net Carbohydrate and Protein System (Version 5.0, Cornell University, Ithaca, NY). Methane emission was calculated according to Blaxter and Clapperton (1965) as modified by Wilkerson et al. (1995). Methane production was also calculated from the VFA analysis (Wolin, 1960).

Statistics

Least squares procedures using PROC MIXED of SAS (SAS Inst., Cary, NC) were used to examine the effects of RFI group on nutrient digestibilities (study 1), VFA concentrations, in vitro MPA, and pH with a model that included the random effect of year (study 2). Differences in RFI group were determined by F-tests using Type III sums of squares. PROC CORR was used to examine the phenotypic correlations between RFI and response variables with study 2 including the partial option to account for the random effect of year. Dry matter intake (and the two-way interaction) was tested as a covariate in all analyses. When insignificant as a covariate, it was dropped from the model. Significance was declared at $P < 0.05$.

A two-step approach was used to determine if individual animal variation in apparent diet digestibility and ruminal fermentation parameters affected the derivation of expected DMI. First, stepwise regression analysis was performed (PROC REG of SAS)

to determine, in addition to the base model of BW and ADG, what additional parameters were meaningful in determining expected DMI. Secondly, from the significant parameters in stepwise analysis, traits were added to the base model, and the resulting change in coefficient of determination used to determine their relative importance to account for additional variation in DMI.

To compare various methods of combining DMI data from multiple trials to compute RFI, 3 models were evaluated:

(1) $Y_j = \beta_0 + \beta_1 MBW_j + \beta_2 ADG_j + \beta_x X_{jk} + \varepsilon_j$,

where: Y_j is the DMI of the *j*th heifer, X_{jk} = the kth fermentation parameter for the jth heifer, β_0 is the regression intercept, β_1 is the regression coefficient on MBW, β_2 is the regression coefficient on ADG, β_x is ther regression coefficient on fermentation parameter X, and ε_j is the random trial and uncontrolled error for the jth heifer;

(2) $Y_{ij} = \beta_0 + \beta_1 MBW_{ij} + \beta_2 ADG_{ij} + \beta_3 T_i + \beta_x X_{ijk} + \varepsilon_{ij}$,

where: Y_{ij} is the DMI of the jth heifer in the ith trial, T_i is the fixed effect of ith trial, Xi_{jk} is the the kth fermentation parameter for the jth heifer in the ith trial, β_0 is the regression intercept, β_1 is the regression coefficient on MBW, β_2 is the regression coefficient on ADG, β_3 = regression coefficient on trial, β_x is the regression coefficient on fermentation parameter X, and ε_{ij} is the random uncontrolled error and error associated with fixed interactions of independent variables and trial for the jth heifer in the ith trial;

(3) $Y_{ij} = \beta_0 + \beta_1 MBW_{ij} + \beta_2 ADG_{ij} + \beta_3 \tau_i + (\beta_4 MBW_j * \tau_i) + (\beta_5 ADGj * \tau_i) + \beta_{x1} X_{ijk} + (\beta_{x2} X_{jk} * \tau_i) + \varepsilon_{ij}$,

where: Y_{ij} is the DMI of the jth heifer in the ith trial, τ_i is the random effect of the ith trial, X_{ijk} is the kth fermentation parameter for the jth heifer in the ith trial, β_0 is the regression intercept, β_1 is the regression coefficient on MBW, β_2 is the regression coefficient on ADG, β_3 is the regression coefficient on random trial, β_4 is the regression coefficient on the random interaction of MBW and trial, β_5 is the regression coefficient on the random interaction of ADG and trial, β_{x1} is the regression coefficient on the kth fermentation parameter, β_{x2} is the regression coefficient on the random interaction of kth fermentation parameter and ith trial, and ε_{ij} is the uncontrolled error for the jth heifer in the ith trial.

Model 1, which did not include trial as an independent variable, was used as the base model from which to compare R^2 of models 2 and 3, which included the effect of trial to combine DMI data from multiple trials. Model 2 included trial as a fixed effect, while model 3 included trial and trial X MBW and trial X ADG interactions as random effects to account for potential variation in mean DMI and the differential relationships of DMI with MBW and ADG due to trial. The likelihood ratio test (**LRT**) was used to compare the reduced model (base model, **BM**) and the full model (BM + fermentation parameters) for models 1 and 2 according to Tempelman et al.(2001). Model 3 reduced and full models were evaluated using the ΔAIC according to Motulsky and Christopoulos (2004) and Conner et al. (2004) . Differences between models 1 and 2

was evaluated using the LRT while differences between models 1 and 3 and 2 and 3 were evaluated using ΔAIC.

Results and Discussion

Descriptive statistics of the traits measurement from study 1 are presented in Table 2.2. All animals (n = 57) in the study averaged 1.25 kg/d (range 0.65 to 1.86 kg/d) for ADG, 10.07 kg/d (range 7.66 to 13.62 kg/d) for DMI, and 8.20 kg DM/kg of gain (range 5.27 to 12.70 kg DM/kg gain) for FCR. Mean phenotypic RFI was 0.00 kg/d and ranged from -2.13 (most efficient) to 2.68 kg/d (least efficient). The least-squares means for performance and feed efficiency traits of steers with divergent RFI used in study 1 are presented in Table 2.3. Steers with low RFI consumed 19% less DMI ($P < 0.001$) and had 12% lower FCR than steers with high RFI. Animal performance (initial BW, final BW, and ADG) were similar amongst both phenotype groups.

Digestibility estimates are presented in Table 2.4. There was a tendency ($P = 0.10$) for low RFI steers to have 6% higher DMD compared to high RFI steers. Additionally, there was a tendency ($P = 0.13$) for low RFI steers to have higher NDF digestibility compared to high RFI steers. There was no difference ($P = 0.21$) in apparent CP digestibility in steers with divergent RFI, but low RFI steers had

Table 2.2. Descriptive statistics (mean ± SD) for traits measured during the two 70-d experimental trials in growing beef animals

Item[1]	Study 1 n = 57	Study 2 n = 468
Initial BW, kg	291.1 ± 33.8	271.4 ± 26.1
Final BW, kg	395.4 ± 39.0	341.5 ± 29.7
ADG, kg/d	1.26 ± 0.21	1.01 ± 0.15
DMI, kg/d	10.1 ± 1.30	9.51 ± 1.02
FCR, kg feed/kg gain	8.20 ± 1.20	9.55 ± 1.27
RFI, kg/d	0.00 ± 0.89	0.00 ± 0.71

[1] ADG = average daily gain, DMI = dry matter intake, FCR = feed conversion ratio, RFI = residual feed intake.

Table 2.3. Descriptive statistics for selected low and high RFI digestibility steers in Study 1 (Santa Gertrudis steers)

Item[1]	Low RFI n = 16	High RFI n = 16	SD	*P* -value
Initial BW, kg	304.9	308.5	27.2	0.693
Final BW, kg	397.7	407.3	32.4	0.328
ADG, kg/d	1.19	1.28	0.21	0.192
DMI, kg/d	9.05	11.17	1.44	< 0.001
DMI - Xm	1.84	2.24	0.03	< 0.001
FCR	7.75	8.81	1.14	0.004
RFI, kg/d	-0.929	0.839	1.02	< 0.001

[1] ADG = average daily gain, DMI = dry matter intake, DMI - Xm = DMI as a multiple of maintenance, FCR = feed conversion ratio, RFI = residual feed intake.

Table 2.4. Digestibility estimates of various nutrients measured in Study 1 (g/kg DM) using AIA as an internal marker in growing Santa Gertrudis steers

Item	Low RFI n = 16	High RFI n = 16	SE	*P* - value
DM	703.4	665.0	16.3	0.099
CP	597.3	555.0	23.2	0.207
NDF	548.2	495.8	24.0	0.131

numerically higher apparent CP digestibility (597.3 vs. 555.0 ± 23.2 g/kg DM). Phenotypic correlations are presented in Table 2.5. As expected, RFI was not correlated with final BW and ADG, but was positively correlated ($P < 0.001$) with DMI and FCR. Residual feed intake was negatively correlated with DMD and there was a tendency ($P = 0.12$) for RFI to be negatively (r = -0.26) correlated with apparent CP digestibility.

Descriptive statistics of the traits measured in study 2 are presented in Table 2.2. All animals (n = 468) in the study averaged 1.01 kg/d (range 0.59 to 1.53 kg/d) for ADG, 9.51 kg/d (range 6.94 to 12.68 kg/d) for DMI, and 9.55 kg DM/kg of gain (range 6.71 to 15.72 kg DM/kg gain) for FCR. Mean phenotypic RFI was 0.00 kg/d and ranged from -2.01 (most efficient) to 2.20 kg/d (least efficient). The least-squares means for performance and feed efficiency traits of heifers with divergent RFI used in study 2 are presented in Table 2.6. Heifers with low RFI consumed 18% less ($P < 0.001$) DMI and had 16% lower FCR than high RFI heifers. Animal performance (initial BW, final BW, and ADG) were similar amongst low and high RFI heifers.

Table 2.5. Phenotypic correlations among performance, feed efficiency traits, and digestibility in Santa Gertrudis steers

Trait[1]	ADG	DMI	FCR	DMD	NDFD	CPD	RFI
FBW	0.54*	0.64*	-0.02	0.07	0.09	0.13	0.16
ADG		0.64*	-0.60*	-0.08	-0.02	0.00	0.22
DMI			0.22	-0.23	-0.20	-0.14	0.83*
FCR				-0.11	-0.15	-0.13	0.57*
DMD					0.99*	0.95*	-0.34*
NDFD						0.94*	-0.32
CPD							-0.26

[1] FBW = final BW; ADG = average daily gain; DMI = dry matter intake; FCR = feed conversion ratio; DMD = apparent dry matter digestibility; NDFD = NDF digestibility; CPD = apparent CP digestibility.

* Correlations are different from zero at $P < 0.05$.

Table 2.6. Descriptive statistics for selected low and high RFI digestibility heifers in Study 2 (Brangus heifers)

Item[1]	Low RFI n = 78	High RFI n = 77	SD	*P* - value
Initial BW, kg	274.4	270.9	29.5	0.395
Final BW, kg	344.3	343.3	31.4	0.832
ADG, kg/d	1.01	1.04	0.18	0.187
DMI, kg/d	8.68	10.54	1.07	< 0.001
DMI - Xm	1.73	2.11	0.18	< 0.001
FCR	8.46	10.43	1.31	< 0.001
RFI, kg/d	-0.905	0.991	0.38	< 0.001

[1] ADG = average daily gain, DMI = dry matter intake, DMI - Xm = DMI as a multiple of maintenance, FCR = feed conversion ratio, RFI = residual feed intake.

Diet and nutrient digestibility estimates from study 2 based on 2 separate internal markers are presented in Table 2.7. Using AIA as an internal marker, heifers with low RFI had 4% higher ($P = 0.003$) DMD, 4% higher ($P = 0.01$) NDF digestibility, 5% higher ($P = 0.01$) ADF digestibility, and 5% higher ($P = 0.002$) apparent CP digestibility compared to high RFI heifers. When ADIA was used as an internal marker, heifers with low RFI had 4% higher ($P = 0.004$) DMD, 5% higher ($P = 0.006$) NDF digestibility, 6% higher ($P = 0.004$) ADF digestibility, and 6% higher ($P < 0.001$) apparent CP digestibility. Mineral digestibilities are presented in Table 2.7. Acid insoluble ash digestibility coefficients for heifers with low RFI were higher ($P = 0.05$) for P, and tended ($P = 0.07$) to be higher for Ca and Cu compared to high RFI heifers. Differences in Zn digestibility between heifers with low and high RFI were not detected, which was likely due to the high standard error. When using ADIA as an internal marker, low RFI heifers had higher ($P = 0.04$) P digestibility, Ca digestibility, and Cu digestibility vs. high RFI heifers. Acid insoluble ash and ADIA yielded similar results indicating that either marker would be acceptable to observe the relative differences in digestibility in growing heifers consuming a similar diet.

Ruminal and fecal pH, in vitro MPA, and calculated methane losses are presented in Table 2.8. There were no differences detected between low and high RFI heifers in ruminal and fecal pH and in vitro MPA. However, there was a difference detected in estimated methane energy losses expressed as a proportion of gross energy intake (Blaxter and Clapperton, 1965) between heifers with divergent RFI. Compared to heifers with high RFI, methane energy losses were 4.6% lower in heifers with low RFI.

Table 2.7. Digestibility estimates of various nutrients measured in Study 2 (g/kg DM) using two internal markers in growing Brangus heifers

	AIA				ADIA			
Item	Low RFI n = 78	High RFI n = 77	SE	*P* - value	Low RFI n = 78	High RFI n = 77	SE	*P* - value
DM	762.2	734.7	32.6	0.003	776.3	744.7	55.4	0.004
CP	726.3	693.1	37.4	0.002	742.3	702.3	44.7	0.001
NDF	706.6	678.2	41.9	0.012	717.3	681.0	65.2	0.006
ADF	674.6	644.7	39.7	0.013	690.0	649.7	62.1	0.004
Phosphorus	619.0	583.9	78.9	0.045	643.7	606.9	97.1	0.037
Calcium	563.1	531.8	41.6	0.069	598.5	560.9	65.2	0.041
Zinc	533.6	518.7	62.2	0.608	561.7	534.5	103.9	0.286
Copper	618.0	587.7	55.6	0.059	647.3	612.3	13.8	0.040

Table 2.8. Ruminal and fecal pH and in vitro methane producing activity in Study 2 for years 2, 3, and 4

Item	Low RFI[1]	High RFI[1]	SE	*P* - value
Rumen pH[1,4]	7.05	7.07	0.11	0.778
Rumen methane[2]	10.99	11.88	2.41	0.433
Fecal pH[1,4]	7.29	7.28	0.09	0.915
Fecal methane[2]	0.758	0.695	0.208	0.674
Methane[3], % GE	5.84	6.12	0.045	< 0.001

[1] Rumen pH: low RFI n = 40, high RFI n = 39; fecal pH: low RFI n= 40, high RFI n = 38; rumen and fecal methane: low RFI n = 56, high RFI n = 55.

[2] In vitro methane producing activity (μmol CH_4/mL fermentation fluid).

[3] Calculated using Blaxter and Clapperton (1965) as modified by Wilkerson et al. (1995); DMI was a significant covariate ($P < 0.001$).

[4] Rumen and fecal pH only measured on yr 3 and 4.

Volatile fatty acid concentrations are presented in Table 2.9. There were no differences in ruminal acetate or butyrate concentrations, however, ruminal propionate concentrations tended ($P = 0.08$) to be lower in low RFI heifers resulting in a increase ($P = 0.03$) in the acetate:propionate ratio for low RFI heifers (6.18 vs. 5.91 ± 0.10). There were no differences observed in fecal VFA concentrations amongst the RFI groups.

The variation in DMI explained by MBW and ADG using two different models for study 1 is presented in table 2.10. The base model included MBW and ADG, and accounted for 52.7% of the variation in DMI. The inclusion of DMD in the base model increased the R^2 to 58.1%, which suggests that variation in DMD accounted for an additional 11.5% of the variation in DMI not accounted for by MBW and ADG. Using ΔAIC, there is approximately a 65% chance that the reduced model, that only includes MBW and ADG, is more correct than the full model that includes MBW, ADG, and DMD, however, the proportion of additional variation that DMD accounts for in explaining DMI makes it a good candidate model to use.

The variation in DMI explained by MBW and ADG using models to combine data from multiple trials from study 2 (yr 1 – 4) is presented in Table 2.11. The base model accounted for 31.8% of the variation in DMI (model 1). The R^2 of model 2, which included trial as a fixed effect accounted for 37.4% of the variation in DMI while model 3, which accounted for the random effect of trial as well as the random effects of trial X MBW and trial X ADG, explained 38.5% of the variation in DMI. Using ΔAIC, there is almost a 100% chance that model 2 is more correct than model 1 indicating that

Table 2.9. Effect of phenotypic RFI group on ruminal and fecal VFA concentrations

Item	Low RFI n = 55	High RFI n = 54	SE	*P* -value
Rumen Concentration, mM				
Acetate	56.60	59.20	6.85	0.343
Propionate	9.27	10.19	1.09	0.083
Butyrate	9.32	9.38	1.40	0.924
Total VFA	75.18	78.77	9.26	0.347
A:P ratio[1]	6.18	5.91	0.10	0.032
Fecal concentration, mM				
Acetate	44.55	42.51	2.18	0.321
Propionate	9.19	8.87	0.33	0.508
Butyrate	4.72	4.70	0.27	0.961
Total VFA	58.43	56.06	2.46	0.388
A:P ratio[1]	4.92	4.88	0.20	0.735

[1] Acetate:propionate ratio.

Table 2.10. Percentage of variation explained (R^2) by different models to derive expected DMI for growing Santa Gertrudis steers

Regression[1]	Model number[2]
	1: $F_1 + e_1$
Base model (BM; ADG and mid-test $BW^{0.75}$	0.527
BM + DMD	0.581

[1] DMD = acid insoluble ash dry matter digestibility.
[2] F_1 = fixed effects of indicated variables; e_1 = random uncontrolled error.

Table 2.11. Percentage of variation explained (R^2) by different models to derive expected DMI for growing Brangus heifers for years 1, 2, 3, and 4

Regression[1]	Model number[2]		
	1: $F_1 + e_1$	2: $F_2 + e_2$	3: $F_1 + R + e_3$
Base model[3] (BM; ADG and mid-test $BW^{0.75}$	0.318	0.374	0.385
BM + DMD[3]	0.345	0.412	0.416

[1] DMD = acid insoluble ash dry matter digestibility; total VFA = ruminal total VFA; A:P = ruminal acetate : propionate ratio.

[2] F_1 = fixed effects of indicated variables; F_2 = fixed effects of indicated variables + fixed effect of trial; R = random effects of trial and trial X independent variable interactions; e_1 = random trial and uncontrolled error; e_2 = random uncontrolled error and error associated with fixed interactions of trial X independent variables; e_3 = random uncontrolled error.

[3] Includes analysis for yr 1, 2, 3, and 4.

accounting for variation due to trial is important in explaining variation in DMI beyond ADG and MBW. However, using the likelihood ratio test (LRT), there is no difference ($P > 0.05$) between models 2 and 3. Using model 2, the inclusion of DMD in the base model increased R^2 to 37.4% to 41.2%, which suggests that animal variation in DMD accounted for an additional 6.1% of the variation not explained by MBW and ADG [(0.412 – 0.374) / (1 – 0.374)]. The inclusion of DMD to model 3 explained minimal additional variation in DMI (41.6%).

To further explore the variation in RFI that was attributable to fermentation, ruminal VFA and ruminal acetate:propionate ratio was included in models 1 – 3, in addition to DMD, as stepwise regression identified these two variables as significant parameters. The variation in DMI explained by MBW and ADG using models to combine data from multiple trials of study 2 (yr 2 – 4) is presented in Table 2.12. The base model for yr 2 – 4 explained 27.1% of the variation in DMI, which is lower than was observed when using all 4 years in Table 2.10. When study was included as a fixed effect (model 2), 34.3% of the variation was explained, while model 3 explained 36.1% of the variation in DMI. Using ΔAIC, there is almost a 100% chance that model 2 is more correct than model 1 to explain variation in DMI, indicating that accounting for variation due to trial is important in explaining variation in DMI. Using LRT, there is no difference ($P > 0.05$) in the amount of variation in DMI that is explained between model 2 and 3, indicating that the random effect of trial and its interaction with MBW and ADG does not explain significantly more variation in DMI. Using model 2, the

Table 2.12. Percentage of variation explained (R^2) by different models to derive expected DMI for growing Brangus heifers for years 2, 3, and 4

Regression[1]	Model number[2]		
	1: $F_1 + e_1$	2: $F_2 + e_2$	3: $F_1 + R + e_3$
Base model[3] (BM; ADG and mid-test $BW^{0.75}$)	0.271	0.343	0.361
BM + DMD[3]	0.349	0.437	0.503
BM + DMD + total VFA + A:P[3]	0.408	0.458	0.532

[1] DMD = acid insoluble ash dry matter digestibility; total VFA = ruminal total VFA; A:P = ruminal acetate : propionate ratio.

[2] F_1 = fixed effects of indicated variables; F_2 = fixed effects of indicated variables + fixed effect of trial; R = random effects of trial and trial X independent variable interactions; e_1 = random trial and uncontrolled error; e_2 = random uncontrolled error and error associated with fixed interactions of trial X independent variables; e_3 = random uncontrolled error.

[3] Includes analysis for yr 2, 3, and 4 as rumen fluid was not collected in yr 1.

inclusion of DMD to the base model increased R^2 from 34.3% to 43.7%, which suggests that animal variation in DMD accounted for an additional 14.3% of the variation in DMI beyond ADG and MBW. The inclusion of ruminal total VFA and ruminal acetate: propionate ratio, in addition to DMD, increased the R^2 from 43.7% to 45.8%, indicating that an additional 3.7% of the variation in DMI is accounted for by ruminal total VFA and ruminal acetate:propionate ratio beyond ADG, MBW, and DMD.

Including DMD in model 2 accounts for an additional 14.3% of the variation in observed DMI not accounted for by MBW and ADG. The inclusion of ruminal total VFA and ruminal acetate:propionate ratio, in addition to DMD, to model 2 accounts for an additional 3.7% of the variation not explained by MBW and ADG. This indicates that more variation in DMI may be explained by DMD than by ruminal total VFA and ruminal acetate:propionate ratio.

Phenotypic correlations amongst animal performance, feed efficiency traits, AIA digestibility and fermentation parameters are presented in Table 2.13. Residual feed intake was not correlated to BW or ADG, which would be expected as the use of linear regression to compute RFI forces it to be phenotypically independent of the component traits. Residual feed intake was positively correlated ($P < 0.001$) with DMI ($r = 0.70$), FCR ($r = 0.68$), DMD ($r = -0.41$), NDF digestibility ($r = -0.38$), ADF digestibility ($r = -0.36$), and apparent CP digestibility (-0.42).

Table 2.13. Phenotypic correlations among animal performance, feed efficiency traits, AIA digestibility, and fermentation parameters in growing Brangus heifers

Trait[1]	ADG	DMI	FCR	DMD	NDFD	ADFD	CPD	PD	CaD	ZnD	CuD	rAc	rPr	rBy	rCH_4	fAc	fPr	fBy	fCH_4	CH_4, %GE	rA:P	fA:P	RFI
FBW	0.53*	0.44*	-0.14	0.07	0.07	0.05	0.08	0.02	-0.03	0.02	0.03	-0.15	-0.12	-0.18	-0.20*	0.01	-0.02	0.16	0.12	0.02	0.19*	0.07	-0.02
ADG		0.47*	-0.65*	0.06	0.01	0.01	0.06	0.16	0.03	0.10	0.08	-0.04	0.00	-0.10	-0.10	0.08	0.01	0.16	0.05	0.39*	-0.07	0.07	-0.02
DMI			0.29*	-0.36*	-0.30*	-0.33*	-0.34*	-0.27*	-0.26*	-0.28*	-0.25*	-0.11	-0.02	-0.18	-0.27*	-0.09	-0.06	0.13	0.00	0.89*	-0.12	-0.06	0.70*
FCR				-0.37*	-0.26*	-0.29*	-0.37*	-0.44*	-0.28*	-0.36*	-0.31*	-0.01	0.02	0.00	-0.12	-0.20*	-0.05	-0.14	0.04	0.25*	-0.05	-0.19*	0.68*
DMD					0.95*	0.93*	0.98*	0.82*	0.79*	0.81*	0.84*	0.05	-0.04	0.11	0.20*	0.03	-0.03	-0.04	-0.13	-0.36*	0.15	0.29*	-0.41*
NDFD						0.97*	0.94*	0.73*	0.71*	0.67*	0.77*	-0.10	-0.16	-0.05	0.10	-0.04	-0.04	-0.04	-0.04	-0.32*	0.16	0.08	-0.38*
ADFD							0.95*	0.79*	0.77*	0.77*	0.82*	-0.02	-0.09	0.04	0.11	0.00	-0.02	-0.03	-0.11	-0.33*	0.13	0.13	-0.36*
CPD								0.83*	0.80*	0.80*	0.85*	0.04	-0.05	0.10	0.18	0.04	-0.02	-0.04	-0.12	-0.34*	0.16	0.17	-0.42*
PD									0.86*	0.82*	0.91*	0.17	0.10	0.19*	0.20*	0.18	0.06	0.06	-0.22*	-0.20*	0.09	0.24*	-0.33*
CaD										0.91*	0.92*	0.11	0.04	0.17	0.15	0.08	0.00	-0.08	-0.18	-0.19*	0.12	0.22*	-0.27*
ZnD											0.94*	0.23*	0.14	0.29*	0.21*	0.13	0.00	-0.04	-0.24*	-0.21*	0.12	0.27*	-0.28*
CuD												0.09	0.00	0.14	0.15	0.05	0.00	-0.06	-0.15	-0.20*	0.15	0.16	-0.27*
rAc													0.93*	0.89*	0.53*	0.07	-0.05	-0.09	-0.15	0.02	-0.22*	0.20*	0.07
rPr														0.78*	0.48*	0.11	-0.04	-0.05	-0.11	0.13	-0.54*	0.23*	0.15
rBy															0.43*	0.02	-0.06	-0.15	-0.17	-0.05	-0.08	0.13	0.02
rCH_4																0.05	-0.12	-0.09	0.05	-0.16	-0.13	0.28*	-0.16
fAc																	0.81*	0.72*	-0.01	-0.06	-0.15	0.16	-0.07
fPr																		0.65*	0.05	-0.05	-0.03	-0.42*	0.02
fBy																			-0.04	0.08	-0.06	-0.03	0.00
fCH_4																				-0.09	-0.05	-0.09	0.00
CH4, %GE																					-025*	-0.03	0.76*
rA:P																						-0.18	-0.22*
fA:P																							-0.13

[1] FBW = final BW; ADG = average daily gain; DMI = dry matter intake; FCR = feed conversion ratio; DMD = apparent dry matter digestibility; NDFD = NDF digestibility; CPD = apparent CP digestibility; PD, CaD, ZnD, CuD = P, Ca, Zn, and Cu digestibility, respectively; rAc = rumen acetate; rPr = rumen propionate; rBy = rumen butyrate; rCH_4 = rumen CH_4 producing activity; fAc = fecal acetate; fPr = fecal propionate; fBy = fecal butyrate; fCH_4 = fecal CH_4 producing activity.

* Correlations are different from zero at $P < 0.05$.

Residual feed intake was correlated ($P < 0.01$) with P, Ca, Zn, and Cu digestibility (r = -0.33, -0.27, -0.28, -0.27, respectively). Dry matter intake was negatively correlated ($P < 0.05$) with all digestibility measures.

There was a tendency for RFI to be weakly correlated ($P = 0.11$) with ruminal in vitro MPA (r = -0.15), but not with fecal MPA. Residual feed intake was strongly correlated ($P < 0.05$) with calculated methane loss as a proportion of GE (r = 0.76). Residual feed intake was not correlated with ruminal or fecal VFA concentrations; however, RFI was negatively correlated ($P = 0.02$) with ruminal acetate:propionate ratio (r = -0.22), and tended ($P = 0.11$) to be correlated with ruminal propionate concentration (r = 0.15). Calculated methane was correlated ($P < 0.05$) with ADG, DMI, and FCR (r = 0.39, 0.89, 0.25). Diet and nutrient digestibilities were negatively correlated ($P < 0.05$) with methane loss as a proportion of GE further indicating that diet and nutrient digestibility as well as DMI serve to influence methane loss in growing Brangus heifers. Calculated methane was not correlated with VFA measures. Additionally, a Wolin fermenatation balance was calculated to estimate methane production from VFA [data not shown; (Wolin, 1960)]. As expected, Wolin ruminal methane was correlated ($P < 0.05$) with ruminal total VFA concentration (r = 068), as well as ruminal MPA (r = 0.35). Wolin fecal methane was only correlated ($P < 0.05$) with fecal VFA concentration (r = 0.50). This may indicate that the Wolin fermentation balance may be a proxy for in vitro ruminal methane producing activity, however, the usefulness of the fermentation balance to identify differences in RFI is limited as neither MPA nor the Wolin fermentation balance methane estimate were correlated with RFI.

Phenotypic correlations amongst animal performance, feed efficiency traits, ADIA digestibility, and fermentation parameters are presented in Table 2.14. When ADIA was used as an internal marker to estimate digestibility, RFI was correlated with DM digestibility (r = -0.38), NDF digestibility (r = -0.41), ADF digestibility (r = -0.39), and apparent CP digestibility (r = -0.43). Residual feed intake was correlated ($P < 0.05$) with P, Ca, Zn, and Cu digestibility (r = -0.30, -0.27, -0.26, -0.27, respectively). The congruency of correlations based on both markers further illustrates that AIA and ADIA were both adequate internal markers in this experiment.

Channon et al. (2004) found differences in starch digestibility in divergent RFI steers fed a high energy feedlot diet. Angus and Angus-cross steers used were progeny of lines selected for RFI. Fecal pH and fecal DM was used as a proxy for lower gut starch fermentation because when starch is fermented in the hindgut, fecal pH (Degregorio et al., 1982) and fecal DM are likely to be decreased leading to diarrhea (Huber, 1976), giving a visual appraisal of ruminal starch fermentation. Steers with low RFI (from efficient parents) had higher fecal pH and DM content compared to high RFI steers (from inefficient parents) suggesting that progeny from low RFI parents fermented more starch in the rumen. This provides evidence of genetic differences in starch digestion. The authors note that a measure of fecal starch would have been useful to definitively relate the fecal parameters to actual starch digestion; however, fecal starch is closely associated with total tract starch digestibility ($R^2 = 0.95$; Zinn, 1994).

Table 2.14. Phenotypic correlations among animal performance, feed efficiency traits, ADIA digestibility, and fermentation parameters in growing Brangus heifers

Trait[1]	ADG	DMI	FCR	DMD	NDFD	ADFD	CPD	PD	CaD	ZnD	CuD	rAc	rPr	rBy	rCH_4	fAc	fPr	fBy	fCH_4	CH_4, %GE	rA:P	fA:P	RFI
FBW	0.53*	0.44*	-0.14	-0.05	-0.06	-0.07	-0.05	-0.08	-0.12	-0.07	-0.08	-0.15	-0.12	-0.18	-0.20*	0.01	-0.02	0.16	0.12	0.02	0.19*	0.07	-0.02
ADG		0.47*	-0.65*	0.04	-0.04	-0.03	0.00	0.10	0.01	0.07	0.05	-0.04	0.00	-0.10	-0.10	0.08	0.01	0.16	0.05	0.39*	-0.07	0.07	-0.02
DMI			0.29*	-0.45*	-0.45*	-0.47*	-0.46*	-0.36*	-0.36*	-0.35*	-0.36*	-0.11	-0.02	-0.18	-0.27*	-0.09	-0.06	0.13	0.00	0.89*	-0.12	-0.06	0.70*
FCR				-0.42*	-0.36*	-0.37*	-0.41*	-0.45*	-0.34*	-0.38*	-0.37*	-0.01	0.02	0.00	-0.12	-0.20*	-0.05	-0.14	0.04	0.25*	-0.05	-0.19*	0.68*
DMD					0.95*	0.96*	0.98*	0.88*	0.86*	0.86*	0.87*	0.27*	0.18	0.34*	0.24*	0.10	-0.06	-0.06	-0.18	-0.35*	0.09	0.29*	-0.38*
NDFD						0.98*	0.97*	0.77*	0.78*	0.76*	0.79*	0.12	0.04	0.21*	0.17	0.00	-0.09	-0.10	-0.11	-0.39*	0.14	0.20*	-0.41*
ADFD							0.97*	0.82*	0.83*	0.82*	0.83*	0.17	0.09	0.26*	0.17	0.04	-0.07	-0.08	-0.15	-0.39*	0.11	0.23*	-0.39*
CPD								0.85*	0.84*	0.84*	0.85*	0.21	0.12	0.30	0.22*	0.06	-0.07	-0.09	-0.16	-0.39*	0.13	0.26*	-0.43*
PD									0.94*	0.96*	0.96*	0.30*	0.22*	0.35*	0.22*	0.20*	0.02	0.01	-0.25*	-0.22*	0.08	0.30*	-0.30*
CaD										0.96*	0.96*	0.28*	0.20*	0.34*	0.19*	0.13	-0.04	-0.09	-0.21*	-0.22*	0.10	0.30*	-0.27*
ZnD											0.96*	0.34*	0.25*	0.40*	0.22*	0.16	-0.02	-0.05	-0.25*	-0.22*	0.09	0.32*	-0.26*
CuD												0.27*	0.18	0.33*	0.20*	1.15	0.00	-0.07	-0.20*	-0.23*	0.11	0.26*	-0.27*
rAc													0.93*	0.89*	0.53*	0.07	-0.05	-0.09	-0.15	0.02	-0.22*	0.20*	0.07
rPr														0.78*	0.48*	0.11	-0.04	-0.05	-0.11	0.13	-0.54*	0.23*	0.15
rBy															0.43*	0.02	-0.06	-0.15	-0.17	-0.05	-0.08	0.13	0.02
rCH_4																0.05	-0.12	-0.09	0.05	-0.16	-0.13	0.28*	-0.16
fAc																	0.81*	0.72*	-0.01	-0.06	-0.15	0.16	-0.07
fPr																		0.65*	0.05	-0.05	-0.03	-0.42*	0.02
fBy																			-0.04	0.08	-0.06	-0.03	0.00
fCH_4																				-0.09	-0.05	-0.09	0.00
CH4, %GE																					-025*	-0.03	0.76*
rA:P																						-0.18	-0.22*
fA:P																							-0.13

[1] FBW = final BW; ADG = average daily gain; DMI = dry matter intake; FCR = feed conversion ratio; DMD = apparent dry matter digestibility; NDFD = NDF digestibility; CPD = apparent CP digestibility; PD, CaD, ZnD, CuD = P, Ca, Zn, and Cu digestibility, respectively; rAc = rumen acetate; rPr = rumen propionate; rBy = rumen butyrate; rCH_4 = rumen CH_4 producing activity; fAc = fecal acetate; fPr = fecal propionate; fBy = fecal butyrate; fCH_4 = fecal CH_4 producing activity.

* Correlations are different from zero at $P < 0.05$.

Richardson et al. (1996) found a difference in DMD in cattle fed a high-roughage pelleted ration (70 alfalfa hay:30 wheat mixture). A total of 575 head of cattle that included Angus, Hereford, and Shorthorn heifers and Angus bulls were tested for RFI. Of the tested animals, a smaller subset (n = 58) of calves was selected to determine DMD in metabolism crates. Low RFI animals had 1% unit higher ($P < 0.1$) DMD compared to high RFI animals which is lower than the 4 to 5% difference in digestibility observed in the current study. This may have been due to lack of sample size in the former study where 58 head of 575 were selected for fecal collections, less divergence in RFI between low and high steers, or variable/incomplete recovery rates of internal markers for digestibility (n-alkanes in this case). The authors calculated that the 1% unit difference in DMD equates to a 2.3% reduction in DMI in 450 kg cattle gaining 1.3 kg/d. In the current study, an approximate 3% unit difference in DMD in medium frame heifers gaining 1 kg/d equates to a 3.5% reduction in DMI for low RFI heifers (NRC, 1996), similar to Richardson et al. (1996). Richardson et al. (1996) estimated that DMD may account for up to 14% of the observed difference in feed intake between divergent RFI groups. Nkrumah et al. (2006) reported a tendency ($P = 0.1$) for low RFI Continental x British crossbred steers fed a high energy feedlot ration to have 6% higher DMD compared to high RFI steers. The authors also reported a tendency ($P = 0.09$) for low RFI steers to have 7% higher apparent CP digestibility. There were no differences in NDF and ADF digestibility. This is in congruence with the current study with higher DMD and apparent CP digestibilities in low RFI heifers; however, the current study showed differences in NDF and ADF digestibilities, as well. This is likely due to the

fact that the high energy diet that was fed in Nkrumah et al. (2006) had very low concentrations of NDF (20%$_{DM}$) and ADF (8%$_{DM}$) and resulted in a very high standard error negating any significance; numerically, the low RFI steers had higher NDF and ADF digestibilities. They also reported a tendency ($P < 0.1$) for a negative correlation between RFI and digestibility of DM (r = -0.33) and CP (r = -0.34).

It is generally recognized in ruminants that as DMI increases, DMD decreases (NRC, 2001) primarily due to a reduction in the amount of time digesta spends in the rumen (Staples et al., 1984). However, this does not appear to be the case in the current study. Dry matter intake and DMI X RFI group were evaluated as covariates and were found to be non-significant. In fact, the estimates for the covariates DMI and DMI X RFI group were not different ($P > 0.20$) than zero implying that, in this case, level of intake does not affect DMD resulting in low RFI calves having higher DMD compared to high RFI calves. However, a negative partial correlation between DMI and DMD was observed. Heifers with low RFI were consuming the high-roughage diet at 1.7X estimated net energy requirement for maintenance (**M**) while heifers with high RFI were consuming it at 2.1X M. This is not a wide spread in intake so it is reasonable that DMI would not affect DMD. Nkrumah et al. (2006) reported that DMI was a significant covariate when evaluating DMD; however, it did not eliminate the relationship between RFI and DMD indicating that part of the variation in divergent RFI steers in DMD might be independent of level of intake. The authors offer than the increased DMI in steers of high RFI might be partly related to the decreased metabolizability of consumed feed

(decreased DMD), and the associated increased need to attain the levels of energy intake required for maintaining BW and growth.

Robertson and Van Soest (1975) reported a decrease of 5% units in DMD when feeding level of a mixed diet was increased from maintenance to 2X maintenance in sheep. Tyrell and Moe (1975) using dairy cattle fed a total mixed ration, found that for each multiple of maintenance increase there was a 4% unit decrease in diet digestibility. However, Colucci et al. (1982) found that there was a greater degree of depression in digestibility with increased DMI when there was less forage in the diet. This is due to the fact that cell solubles and N digestibility account for the majority of the depression in digestibility with increased DMI so high fiber diets would be less affected by the depression in DMD when feeding level increases, similar to the high forage diet in the current study. Pearson et al. (2006) found no differences in DM, OM, CP, NDF, and ADF digestibility when adult dairy steers were fed short chopped alfalfa ad libitum and at restricted intake (75% of ad libitum). Ad libitum DM digestibility coefficient was 0.68 ± 0.05 vs. 0.68 ± 0.05 for restricted intake. In sheep fed short chopped alfalfa hay at 90% ad libitum and at 60% ad libitum, there were no differences in OM, CP, NDF, and ADF digestibilities. There were also no differences in VFA concentrations or molar proportions at low and high levels of intake, in agreement with the current study. Firkins et al. (1986) reported no differences in OM and NDF digestibility when British cross steers were fed ammoniated prairie grass hay at 90% or 60% of ad libitum intake. They did, however, observe a decrease in molar proportion of acetate and an increased molar proportion of butyrate. The authors note that while significant, the difference was

marginal and might not be of biological importance. Bhatti et al. (2008) fed steers Orchardgrass hay (*Dactylis glomerata* L.), chopped 20 cm in length, at 1% of initial BW (4.5 kg/d_{AF}; restricted level) or at ad libitum intake. There were no differences in DM, NDF, ADF, and apparent CP digestibility. Galyean et al. (1979) fed a high corn feedlot diet at 1X M, 1.33X M, 1.67X M and 2X M to growing Brown Swiss x Herford crossbred steers. There was a decrease in DM, OM, and starch digestion when 1X and 1.33X M were compared to 1.67X and 2X M, but there was no difference in 1X and 1.33X M or 1.67X and 2X M in DM, OM, CP, and starch digestion. This is in agreement with the current study that there is no depression in digestibility when the difference in the level of intake is negligible. Heifers in the current study consumed DM at 1.7X and 2.1X M with an absolute difference of 0.38X M. This is similar to the difference in feeding level of 1.67X and 2X M mentioned above. The authors also report no difference in rumen or fecal pH, as well as ruminal VFA in any of the four treatments; however, numerically, the 1.67X M had lower molar proportions of acetate and higher molar proportions of propionate compared to 2X M steers.

This research is the first to demonstrate a difference in mineral digestibility in divergent RFI heifers. Depending upon the internal marker used, the magnitude of the difference varies in the current study; however, low RFI heifers were able to digest more of the measured minerals compared to high RFI heifers. This is especially profound when considering environmentally important minerals such as phosphorus. Not only are less minerals being excreted in the feces there are potentially less environmental effects

of the feces through things such as direct run-off and plant phytotoxicity if the manure is used as fertilizer.

The relationship between RFI and microbial and subsequent ruminal VFA concentrations has been reported by Guan et al. (2008). They observed that low RFI steers fed a high corn finishing ration had a tendency for higher total VFA ($P = 0.06$) and acetate ($P = 0.07$), higher butyrate ($P < 0.001$) and valerate ($P < 0.01$). The current study found no differences in ruminal and fecal VFA; however, ruminal propionate tended ($P = 0.08$) to be lower in low RFI heifers. This may be due to a diet effect; the current study used a high roughage diet while Guan et al. (2008) used a high corn finishing ration. It is also plausible that animal breed may play an important role. Guan et al. (2008) found that there was distinctive microbial clustering when comparing only Angus profiles indicating a high degree of similarity but that clustering was lost when comparing all three breeds represented (Angus, Charolais, and crossbred). This may indicate that VFA concentration is not only dictated by diet but also by the host genetic background.

Dittmar (2007) reported no differences in fecal VFA concentrations of growing Brahman heifers with divergent RFI when fed a high-roughage pelleted ration at 2.2% of BW. This is in agreement with the current study that fecal VFA profile is not affected by RFI. In this study, no difference was detected in DMD due to RFI phenotype. The lack of agreement may have been due to the fact that calves in this study were limit fed; additionally, the author cautions that the interpretation must be questioned as the internal marker used (ADIA) was not optimal for the specific application. Richardson et al.

(2004) also reported no differences in DMD in steer progeny from 1 generation of divergent selection for RFI when fed a high-energy diet. Total collections were used to estimate DMD in steers housed in metabolism crates. There were no difference in DMI or RFI between low and high steers (-0.11 vs. 0.16 ± 0.13 RFI kg/d; $P > 0.1$) during the 52 d metabolism trial, so the likelihood of measuring a difference in DMD would be low. There was a phenotypic correlation between RFI and DMD ($r = -0.44$; $P < 0.05$) during the metabolism trial but not with the 180 d whole experiment (feedlot phase + metabolism phase). The authors note that the short duration of the metabolism trial to calculate RFI might not have been adequate; accurate assessment of individual animal RFI usually requires a minimum of 70 d following a 21 d adaption period (Archer et al., 1997). Additionally, the divergence in RFI during the metabolism trial was not enough to allow individual animal variation in DMD to be expressed. The difference in average RFI in the current study between low and high heifers was 1.9 kg feed DM/d while Richardson et al. (2004) reported an absolute difference in RFI of only 0.27 kg feed DM/d. In monogastrics, differences in DMD are minimal and not important sources of variation in RFI [chickens (Katle, 1991; Luiting et al., 1994) and pigs (De Haer et al., 1993)]. This is in contrast to the current study in growing heifers. This may be due to the fact that ruminants have more specialized digestive system that can adequately utilize fibrous feedstuffs via fermentation.

According to Blaxter and Clapperton (1965), DMI is a strong driver of methane emissions but diet digestibility also affects methane production, with higher diet digestibility resulting in less methane per unit GE intake. At high intakes of highly

digestible diets, low fractional methane losses occur compared to highly digestible diets at low intakes (Johnson and Johnson, 1995). Blaxter and Clapperton (1965) showed that when cattle were fed at maintenance, methane production increased with increased DMD. However, when cattle were fed at three times maintenance, methane emission decreased as a proportion of GE intake. In the current study, heifers with low RFI consumed feed at 1.7X M while feed intake of heifers with high RFI was 2.1X M. There was less methane loss in low RFI heifers not only due to decreased DMI but also to increased DMD. Increased fiber digestibility also can decrease methane production (Iqbal et al., 2008) as may be the case in the current study. Heifers with low RFI had 4 to 5% higher NDF digestibility also serving to reduce methane production.

Nkrumah et al. (2006) reported that low and high RFI steers fed a feedlot diet differed ($P = 0.04$) in methane loss as a percent of GE intake (3.19 vs. 4.28 ± 0.3 % of GE intake). This is in agreement with the current study that found low RFI heifers lost less ($P < 0.001$) methane as a percent of GE intake compared to high RFI heifers (5.84 vs. 6.12 ± 0.05 % of GE intake). The current study estimate is slightly higher as a high forage diet was used compared to a feedlot diet used in Nkrumah et al. (2006). It is well established that less energy is lost as methane as the concentrate proportion increases in the diet leading to lower methane losses in feedlot fed animals (Van Nevel and Demeyer, 1996); methane estimates in the current study are in line with the commonly predicted 6% of diet GE lost as methane (Johnson and Johnson, 1995). Hegarty et al. (2007) also reported that low RFI steers fed a feedlot diet had lower ($P = 0.01$) methane loss compared to high RFI steers (142.3 vs. 190.2 ± 16.5 g/d). However, RFI was calculated

based on the 15 d methane measurement period in that study, due to the fact that measuring methane modified DMI and ingestive behavior. They found no relationship between RFI and methane production when the 70 d RFI was used. This points out the need for less invasive ways to measure methane such that the high daily variation in methane production (Vlaming et al., 2008) can be accounted for with little risk in modifying DMI and ingestive behavior.

Methane producing activity is an indirect measure of the number of methanogens and theoretical methane yield (Gutierrez-Bañuelos et al., 2007). Methane producing activity, as measured in the current study, showed no difference between heifers with divergent RFI. This may be due to the single point in time measure that in vitro MPA was analyzed or that samples were collected before the morning feeding; however, it may be more likely due to the fact that the standard error was high and negated any differences.

The minimal alterations in ruminal VFA profiles due to RFI partially explain the lack of difference in the in vitro MPA. Fermentations that result in a predominance of propionate generally produce lower methane via decreased H^+ for methanogens (Iqbal et al., 2008) as propionate and butyrate are alternative H^+ sinks (Mathison et al., 1998). Ørskov et al. (1968) demonstrated that cows fed a diet of 60:40 forage:concentrate at 2.14 and 1.88 times maintenance had similar methane losses (Mcal/d). The forage:concentrate ratio and feeding levels were similar to the current study, however, the aforementioned cows had not been evaluated on efficiency. From this, it stands to reason that the current heifers consuming 1.7 and 2.1 times maintenance would have

similar methane loss per day; however, low RFI heifers produced less methane attributable to increased diet and nutrient digestibility.

It was expected that low RFI heifers produced less methane based on current literature (Hegarty et al., 2007; Nkrumah et al., 2006) with an expected increase in propionate or butyrate concentrations. With decreased methane, partial pressure of hydrogen in the rumen may be increased leading to increased formation of more reduced fatty acids (i.e. propionate and butyrate) to dispense excess reducing equivalents (Van Nevel and Demeyer, 1996). At present, it is unclear as to why low RFI heifers lost less energy as methane but also had a tendency for slightly lower propionate concentrations. Dry matter intake is a primary driver of methane production (Blaxter and Clapperton, 1965; Pelchen and Peters, 1998) such that as daily feed intake, regardless of digestibility, of any given animal increases, the percentage of dietary GE lost as methane decreases by an average of 1.6% per level of intake (Johnson and Johnson, 1995). However, as a greater amount of any carbohydrate fraction is fermented per day, methane production is decreased (Johnson and Johnson, 1995). This may partially explain why there was less methane produced by low RFI heifers with no increase in more reduced VFA such as propionate and butyrate.

The increased diet digestibility in low RFI heifers was such, that more carbohydrate was available for microbial growth, equally, amongst microbes with different end products of fermentation; perhaps higher DMD in low RFI heifers lead to increased ruminal passage rate to the small intestine with little effect on methane production (Johnson and Johnson, 1995).

Methane production can also be decreased by additional alternative hydrogen sinks. These include oxygen, unsaturated fatty acids, nitrates, and sulfites. Obviously, unsaturated fatty acids, nitrates, and sulfites would be a negligible source in the current experiment as all animals were fed the same ration and oxygen in the rumen is limited; however, differences in microbial growth between low and high RFI animals may play a role in methane production and VFA concentrations. There is known to be variation in supply of amino acids due to variation in efficiency of microbial protein production (microbial growth) (Kahn et al., 2000). They showed a 9% difference in microbial protein production, measured by urinary allantoin excretion, in sheep divergently selected for fleece weight. To date, no published reports are available relating microbial growth with RFI but it is plausible for low RFI heifers to have more microbial growth due to increased DM and nutrient digestibility compared to high RFI heifers.

Implications

Across species, about 30 to 50% of the phenotypic variation in intake is explained by the component traits BW and ADG (Luiting et al., 1994). In cattle, as much as 60% (Veerkamp et al., 1994) to 76% (Nkrumah et al., 2007) of the phenotypic variation in intake can be explained by the component traits BW and ADG. Residual feed intake is independent of MBW and ADG making it a prime selection criterion to increase animal efficiency without affecting mature body size and subsequent maintenance requirements. It has been shown that selection for RFI can decrease feed inputs, decrease methane emissions, and increase DMD, with little effect on animal performance; however, inconsistent results have been published concerning DMD and methane production. Even though the variation in RFI that is explained by DMD is small (≈ 11%), it still has a huge impact on feed inputs. Progeny of a single selection for low RFI parents required 5% less feed with no detrimental effects on growth translating into increased efficiency and economic profit to the cattle producer (Johnson et al., 2006). More research is needed to further elucidate the relationship between RFI and diet and nutrient digestibility and fermentation parameters in growing beef animals.

CHAPTER III

ASSOCIATIONS BETWEEN FEED EFFICIENCY AND GUT MICROBIAL ECOLOGY AND FERMENTATION PARAMETERS IN FEEDLOT STEERS

Introduction

Ruminants depend on the symbiotic relationship between them and the microbial population that inhabits their gastrointestinal tract. The rumen is a very diverse, inter-connected microbial ecosystem consisting of bacteria (10^{10} to 10^{11} cells/mL, representing more than 50 genera), ciliate protozoa (10^4 to 10^6/mL, from 25 genera), anaerobic fungi (10^3 to 10^5 zoospores/mL, representing five genera), methanogens (10^7 to 10^9 cells/g rumen content, representing five genera) and bacteriophages (10^8 to 10^9/mL) (Kamra, 2005). Nelson et al.(2003) suggests that 60 to 80% of the observable bacteria have not been cultivated due to the inadequacies of traditional techniques that are hampered by the fastidious growth requirements of many rumen bacteria (Whitford et al., 1998). This results in gaps in the literature concerning the interaction of the host and resulting microbial population, especially when animal efficiency and microbial community are considered together.

There is a scarcity of information published about the gut microbial ecology present in cattle. Methods to scan vast genomes and identify microbial populations, specifically to the species level, below 0.1% of the population using bacterial tag-encoded FLX 16s rDNA amplicon pyrosequencing (**bTEFAP**) has been developed

(Dowd et al., 2008), making it possible to examine the host-microbe interactions efficiently and economically.

Recently, work has been published relating host physiology, namely obesity, and gut microbial ecology in the mouse (Ley et al., 2005) and in humans (Turnbaugh et al., 2009; Turnbaugh et al., 2006). These studies have shed new light on the importance of understanding how animal performance may depend upon the specific microbial profile of an animal. Additionally, Guan et al. (2008), using PCR-denaturing gradient gel electrophoresis (**DGGE**), demonstrated a host-microbe interaction in finishing beef cattle. Their study showed a gross shift in the microbial populations present in low vs. high residual feed intake (**RFI**) cattle as well as a shift in the VFA pattern further illustrating the importance of understanding how the microbiome can affect animal response.

The objectives of this study were to examine the relationship between RFI and microbial ecology and the resulting VFA profile in finishing beef cattle. The hypotheses tested were: 1) that there was a difference in gut microbial diversity based on animal efficiency, 2) that there was a difference in VFA profiles in high vs. low RFI cattle, and 3) that there was a difference in methane production, as measured by in vitro methane producing activity (**MPA**) and ammonia producing activity (**APA**), in divergent RFI steers.

Materials and Methods

Animals and Management

All experimental procedures were approved by the Animal Care and Use Committee for Livestock at Texas A&M University and cattle were fed under feedlot conditions at the Texas A&M University Agriculture Research Center at McGregor, McGregor, TX. One-hundred seventy crossbred steers were fed a high corn finishing ration (ME = 3.0 Mcal/kg DM) for 70 d using the GrowSafe feeding system (GrowSafe Systems Ltd, Airdrie, AB, Canada). Diet ingredient composition and chemical composition is presented in Table 3.1. Individual feed intake was automatically recorded daily and BW recorded every two weeks. Residual feed intake was calculated as the residual of linear regression of DMI on ADG and mid-test $BW^{0.75}$. All calves were ranked based on RFI into high (> 0.5 SD) , medium and low RFI (< 0.5 SD) phenotypic groups. The six highest and six lowest RFI steers were selected to represent the phenotype extremes for rumen fluid and fecal sampling. Selected calves were restrained in a squeeze chute to allow for sampling 60 d after the completion of the RFI trial.

Rumen Fluid and Feces Collection for bTEFAP Analysis

Approximately 20 mL of rumen fluid was collected via stomach tube before the morning feeding. The fluid was collected in 25 mL serum vials that were capped immediately using rubber stoppers. Feces were collected into Whirl-Pak bags (NASCO, Fort Atkinson, WI) and the air removed and sealed; both rumen fluid and feces were stored at ambient temperatures until shipped. Samples were shipped

Table 3.1. Ingredient and chemical composition of the experimental diet

Item	Value, %
Feed Ingredients[1]	
Dry rolled corn	72.7
Sorghum-Sudan hay	5.5
Cottonseed meal	8.0
Cottonseed hulls	5.5
Molasses	5.0
Urea	0.8
Supplement[3]	2.5
Chemical composition[2]	
DM, %	87.65
ME, $Mcal/kg_{DM}$	2.77
CP, $\%_{DM}$	12.34
NDF, $\%_{DM}$	20.44
ADF, $\%_{DM}$	12.05

[1] Ingredient composition expressed on an as-fed basis.
[2] Chemical composition expressed on a dry matter basis.
[3] 1.32 g/kg monensin, 0.44 g/kg tylosin, 0.65% Cu, 0.14% Mn, 0.34% Zn, 0.001% Se, 0.003 % I, 0.003% Co, 220,460 IU/kg vitamin A, 3,527 IU/kg vitamin E.

overnight, on wet ice, to the laboratory for microbial bTEFAP analysis. Bacterial tag-encoded FLX 16s rDNA amplicon pyrosequencing analysis was done according to Dowd et al. (2008). Briefly, genomic DNA was extracted from rumen and fecal samples using a QIAmp stool DNA mini kit using manufacture's recommended procedures (Qiagen, Valencia, CA), DNA concentration equalized, and the products amplified. The 16s universal Eubacterial primers 530F (5'-GTG CCA GCM GCN GCG G) and 1100R (5'-GGG TTN CGN TCG TTG) were used for amplifying the 60 bp region of 16s RNA genes. After initial amplification, a secondary PCR was performed for FLX amplicon sequencing using appropriately designed special fusion primers with different tag sequences as: LinkerA-Tags-530F and LinkerB-1100R. The secondary PCR prevents amplification of any potential bias that might be caused by inclusion of tag and linkers during initial template amplification reactions. All amplification products were purified and underwent bTEFAP FLX massively parallel pyrosequencing using a Genome Sequencer FLX System following the manufacturer's instructions (Roche, Nutley, New Jersey). All post sequencing processing was done with custom written software in C#; quality trimmed sequences obtained from the FLX sequencing run were processed using a custom scripted bioinformatics pipeline. Tentative consensus FASTA files for each sample were evaluated using BLASTn against a custom database derived from the RDP-II database and Gen-Bank (http://ncbi.nlm.nih.gov). The identities of all hits were greater than 98%.

Rumen Fluid and Feces Collection for Methane, VFA, and Ruminal APA Analysis

Approximately 50 mL of rumen fluid and feces were collected via stomach tube before the morning feeding and stored at ambient temperature until laboratory analysis later that day. In vitro rumen fluid and fecal MPA was determined by combining, in a 18 x 150 mm crimp top tube, 5 mL rumen fluid or 2 g feces with 5 or 8 mL, respectively, anaerobic dilution solution (Bryant and Burkey, 1953) containing 60 mM sodium formate and 0.2 g finely ground alfalfa (to pass a 4 mm screen). Tubes were capped, vortexed to suspend the contents, and incubated 3 and 24 h at 39 °C under a H_2:CO_2 (50:50 mix) atmosphere. All incubations were performed singly. At the end of incubation, concentration of methane present in the headspace of the incubations was determined via gas chromatography (Allison et al., 1992). The GC used was a GOW-Mac with the following specifications: column: Haysep Q 80-100 mesh (2.44 m x 0.005 mm O.D.), injector temperature: 65 °C, detector temperature: 64 °C, column temperature: 40 °C, detector current: 75 mV, and flow rate: 25 mL/min. Argon was used as an inert carrier gas regulated at 52 psi. A single, 1 mL injection was made manually by the same person for all incubations using a gas-tight syringe.

For VFA analysis, a 1:10 dilution was made for both rumen fluid and feces, pH was recorded and the samples centrifuged and supernatant frozen (-20 °C) for subsequent VFA analysis. Volatile fatty acids were analyzed via gas chromatography (Agilent 6890N, Santa Clara, CA, USA) with the following parameters: column: 007 series bonded phase fused silica capillary column (25m x 0.25mm x 0.25µm) with a flame ionizing detector. One µl injections were performed with an automatic injector;

injector temperature: 240 ˚C, oven temperature: 80 ˚C (1 m hold), ramp to 120 ˚C (5 m hold), ramp to 165 ˚C (2 m hold); detector temperature: 260 ˚C. Ruminal APA was determined according to Yang and Russell (1993). Ammonia concentration was determined colormetrically using the method of Chaney and Marbach (1962).

Statistics

Least squares procedures using PROC MIXED of SAS (SAS Inst., Cary, NC) were used to examine the effects of RFI group on microbial ecology, VFA concentrations, pH, MPA and APA. Methane producing activity and APA included the repeated statement to account for repeated measures of these two variables (3 and 24 h samplings). Differences in RFI groups were determined by F-tests using Type III sums of squares. Significance was declared at $P < 0.05$.

Results and Discussion

Descriptive statistics of the traits studied are presented in Table 3.2. All animals (n = 170) averaged 1.50 kg/d (range 0.66 to 2.01 kg/d) for ADG, 11.60 kg/d (range 6.58 to 14.01 kg/d) for DMI, and 7.88 kg DM/kg gain (**FCR**; range 5.8 to 12.28 kg DM/kg gain). Mean phenotypic RFI was 0.00 kg/d and ranged from -2.01 (most efficient) to 2.17 (least efficient). Selected high and low RFI steers (n = 12; Table 3.3) averaged 1.47 kg/d (range 1.20 to 1.85 kg/d) for ADG, 10.47 kg/d (range 7.87 to 12.61 kg/d) for DMI, and 7.20 kg DM/kg gain (FCR; range 5.22 to 8.92 kg DM/kg gain). Mean phenotypic RFI was 0.01 kg/d and ranged from -1.65 (most efficient) to 1.95 (least efficient). This represents an absolute difference of 3.6 kg DM of feed per day. Low RFI steers (n = 6) consumed 23% less ($P < 0.001$) DMI and had 26% lower FCR compared to high RFI

Table 3.2. Descriptive statistics for the traits measured on all animals (n = 170)

Item[1]	Mean ± SD
Initial BW, kg	378.9 ± 29.7
Final BW, kg	486.7 ± 35.8
ADG, kg/d	1.50 ± 0.23
DMI, kg/d	11.60 ± 1.11
FCR, kg feed/kg gain	7.88 ± 1.11
RFI, kg/d	0.00 ± 0.82

[1] ADG = average daily gain, DMI = dry matter intake, FCR = feed conversion ratio, RFI = residual feed intake.

Table 3.3. Descriptive statistics for the traits measured on selected low (n = 6) and high RFI steers (n = 6)

Item[1]	Mean ± SD n = 12	Low RFI n = 6	High RFI n = 6	SE	*P* - value
Initial BW, kg	381.0 ± 26.6	380.9	381.2	7.68	0.981
Final BW, kg	483.8 ± 35.3	486.0	481.7	10.16	0.764
ADG, kg/d	1.47 ± 0.19	1.50	1.44	0.05	0.402
DMI, kg/d	10.47 ± 1.64	9.08	11.87	0.22	< 0.001
FCR; kg feed/kg gain	7.20 ± 1.24	6.10	8.30	0.14	< 0.001
RFI, kg/d	0.01 ± 1.49	-1.40	1.51	0.06	< 0.001

[1] ADG = average daily gain, DMI = dry matter intake, FCR = feed conversion ratio, RFI = residual feed intake.

steers (n=6); this represents the top and bottom 7% of the population sampled. Animal performance (initial BW, final BW, and ADG) were similar amongst both phenotype groups.

Ruminal microbial genus percentages present in each steer are presented in Table 3.4. Fecal microbial genus percentages are presented in Table 3.5. Fifty different genera were represented in rumen fluid while only 42 different genera were represented in the feces of the steers. Microbial data was further condensed into phyla (Table 3.6); rumen fluid samples contained 8 phyla (Bacteriodetes, Firmicutes, Proteobacteria, Spriochaetes, Cyanobacteria, Lentisphaerae, Verrucomicrobia, Tenericutes) while feces contained only 6 phyla (Bacteriodetes, Firmicutes, Proteobacteria, Spriochaetes, Cyanobacteria and Verrucomicrobia). Microbial ecology of low and high RFI steers is presented in Table 3.7. There were no differences ($P > 0.05$) between low and high RFI rumen fluid samples for ruminal Firmicutes:Bacteriodetes ratio (**F:B**), Bacteriodetes, Firmicutes, Proteobacteria, Spirochaetes, Lentisphaerae, and Verrucomicrobia; however, low RFI steers had 73% lower ($P = 0.01$) ruminal Cyanobacteria than high RFI steers. There was a tendency ($P = 0.1$) for low RFI steers to have higher Tenericutes as compared to high RFI steers (0.30 vs. 0.19 ± 0.08 %). In the feces, there were no differences ($P > 0.05$) between divergent RFI samples for fecal F:B, Bacteriodetes, Firmicutes, Proteobacteria, and Cyanobacteria. However, there was a tendency ($P = 0.06$) for fecal Verrucomicrobia to be higher in high RFI steers vs. low RFI steers (0.831 vs. 0.00 ± 0.279 %). Verrucomicrobia was present in the feces of 50% of the high RFI

Table 3.4. Microbial genus (percent) quantified in the rumen fluid of low and high RFI steers fed a finishing ration

Calf ID**	4	8	11	1	10	3	2	9	6	7	5
RFI group	low	low	low	low	low	low	high	high	high	high	high
RFI, kg/d	-1.654	-1.580	-1.445	-1.328	-1.216	-1.206	1.332	1.346	1.505	1.562	1.953
Genus											
Prevotella	40.04	31.09	45.16	30.52	37.89	53.63	56.93	59.15	25.87	31.44	36.69
Succinivibrio	34.53	27.22	2.86	6.70	1.59	1.90	0.59	17.43	38.12	1.40	9.49
Arsenophonus	0.56	0.97	3.44	23.77	18.11	3.59	2.73	0.87	3.62	20.15	1.14
Tannerella	3.22	4.69	3.54	4.41	1.10	3.69	1.20	0.82	1.83	0.77	17.77
Xenorhabdus	0.24	0.87	2.16	7.88	8.58	3.25	0.66	0.79	2.41	9.63	0.25
Hallella	3.22	0.92	1.46	1.19	0.52	12.09	5.04	1.72	1.57	0.95	1.02
Rikenella	0.80	1.99	3.67	1.64	0.74	3.49	6.49	0.71	3.11	4.29	1.46
Bacteroides	2.26	1.53	1.35	2.14	0.74	3.31	8.01	2.21	1.88	0.79	1.62
Ruminococcus	0.54	1.89	7.24	1.58	0.90	0.45	0.96	0.25	1.74	1.02	0.98
Selenomonas	0.73	2.19	1.85	0.58	2.99	1.59	0.39	1.12	0.42	0.38	4.25
Mitsuokella	0.14	0.31	0.68	0.31	7.32	1.24	0.64	0.05	0.13	2.43	2.22
Paludibacter	1.46	1.43	0.57	1.94	0.44	0.83	0.39	0.76	0.68	3.07	0.16
Butyrivibrio	0.89	1.63	0.60	0.36	0.93	0.21	0.27	0.79	0.99	0.54	4.44
Ruminobacter	0.89	4.79	0.18	0.28	0.00	0.00	0.00	1.66	3.27	0.03	0.00
Sporobacter	0.42	1.12	4.51	0.46	0.88	0.52	0.88	0.55	0.22	0.56	0.89
Oribacterium	0.56	0.92	2.03	0.66	1.32	0.69	0.15	0.49	0.60	0.84	2.48
Pasteurella	0.05	1.33	0.47	2.06	2.41	0.41	0.29	0.22	0.42	2.48	0.10
Papillibacter	0.47	0.66	0.91	1.23	0.60	0.73	0.49	1.36	2.01	0.72	0.79
Treponema	1.41	0.76	1.59	1.28	0.05	0.28	0.59	0.68	1.28	1.35	0.35
Succiniclasticum	0.26	1.58	1.04	0.22	1.34	0.52	0.22	1.34	1.13	0.72	1.17
Clostridium	0.07	2.65	1.72	0.67	0.99	0.31	0.54	0.22	0.38	0.66	0.44
Alkalilimnicola	0.35	0.20	3.88	0.19	0.14	0.55	2.29	0.52	0.00	0.05	0.48
Faecalibacterium	0.12	1.17	0.36	0.61	1.67	0.21	0.29	0.19	1.10	1.02	0.38
Xylanibacter	1.65	0.61	0.36	1.16	0.00	0.55	1.40	0.27	0.38	0.08	0.48
Roseburia	0.00	0.36	0.13	1.04	1.59	0.31	0.20	0.05	0.22	1.79	0.38
Synechococcus	0.24	0.20	0.13	0.28	0.00	0.17	0.12	0.55	0.57	0.82	0.76
Eubacterium	0.14	0.15	0.16	0.44	0.60	0.14	0.49	0.33	0.18	0.66	0.44
Anaerovibrio	0.40	0.20	0.21	0.38	0.22	0.35	0.10	0.41	0.44	0.03	0.95
Aquaspirillum	0.00	0.00	0.00	0.00	0.00	0.03	1.72	0.00	0.00	1.61	0.00
Brucella	0.16	0.00	0.91	0.18	0.00	0.45	0.61	0.03	0.02	0.13	0.51
Schwartzia	0.02	0.05	0.49	0.37	0.38	0.35	0.15	0.27	0.02	0.23	0.57
Parabacteroides	0.09	0.10	1.46	0.13	0.05	0.17	0.05	0.08	0.00	0.00	0.51
Dorea	0.21	0.46	0.23	0.20	0.08	0.07	0.05	0.16	0.18	0.13	0.83
Kopriimonas	0.16	0.05	0.10	0.36	0.03	0.24	0.59	0.30	0.13	0.38	0.10
Hespellia	0.00	0.00	0.18	0.15	1.78	0.00	0.05	0.05	0.11	0.03	0.06
Coprococcus	0.09	0.15	0.16	0.48	0.08	0.52	0.02	0.16	0.13	0.23	0.35
Moryella	0.02	0.10	0.29	0.05	0.14	0.07	0.00	0.25	0.73	0.18	0.48
Anaerovorax	0.09	0.31	0.44	0.35	0.22	0.24	0.07	0.08	0.11	0.05	0.13
Centipeda	0.09	0.10	0.00	0.09	0.38	0.52	0.42	0.03	0.15	0.00	0.22
Pseudobutyrivibrio	0.09	0.20	0.05	0.20	0.16	0.07	0.00	0.16	0.13	0.69	0.22
Victivallis	0.14	0.00	0.44	0.01	0.05	0.00	0.15	0.55	0.04	0.03	0.54
Spirochaeta	0.21	0.00	0.05	0.07	0.00	0.00	0.07	0.05	0.04	1.38	0.00
Opitutus	0.49	0.76	0.00	0.01	0.03	0.00	0.10	0.14	0.00	0.28	0.00
Odyssella	1.04	0.05	0.00	0.00	0.00	0.07	0.00	0.44	0.00	0.00	0.03
Holdemania	0.16	0.15	0.21	0.06	0.05	0.14	0.10	0.16	0.13	0.03	0.41
Shuttleworthia	0.00	0.05	0.00	0.11	0.08	0.10	0.20	0.00	0.00	0.26	0.79
Syntrophococcus	0.02	0.20	0.10	0.12	0.11	0.00	0.02	0.05	0.07	0.18	0.32
Rivularia	0.05	0.05	0.08	0.00	0.03	0.00	0.02	0.03	0.53	0.31	0.10
Asteroleplasma	0.02	0.10	0.10	0.37	0.27	0.00	0.17	0.08	0.04	0.00	0.00
Mycoplasma	0.00	0.51	0.39	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00
Sum %	98.87	96.84	97.97	97.30	97.59	98.03	96.93	98.58	97.28	94.76	97.75

** No ruminal data for calf ID 12 (vial broken during shipping).

Table 3.5. Microbial genus (percent) quantified in the feces of low and high RFI steers fed a finishing ration

Calf ID	4	8	11	1	10	3	2	9	12	6	7	5
RFI group	low	low	low	low	low	low	high	high	high	high	high	high
RFI kg/d	-1.654	-1.580	-1.445	-1.328	-1.216	-1.206	1.332	1.346	1.364	1.505	1.562	1.953
Genus												
Prevotella	48.01	42.48	57.93	65.62	12.54	25.19	8.33	25.40	23.89	51.08	11.14	12.80
Clostridium	6.33	7.73	3.76	3.15	18.10	9.09	20.81	9.88	10.60	5.73	15.10	10.59
Papillibacter	4.38	5.86	1.40	1.34	10.97	6.67	13.43	6.30	13.56	2.93	10.09	8.94
Tannerella	11.51	5.69	0.52	1.72	2.41	15.99	6.05	8.83	4.70	5.73	5.07	11.25
Roseburia	2.88	6.37	7.08	5.06	7.07	1.55	4.35	4.02	0.67	5.99	4.87	1.88
Bacteroides	0.93	1.44	0.74	0.48	9.48	6.56	4.64	5.29	0.54	1.91	12.15	5.41
Sporobacter	1.06	0.76	0.07	0.10	6.34	2.59	5.20	2.01	10.34	0.51	4.39	4.30
Ruminococcus	1.86	1.61	7.16	4.87	5.51	1.04	1.42	4.77	0.67	1.53	2.39	3.25
Faecalibacterium	0.62	5.44	3.03	4.78	1.41	1.44	3.03	2.89	1.07	3.57	1.56	2.04
Succinivibrio	2.04	7.56	1.55	1.15	3.42	1.27	0.47	2.97	0.13	4.20	2.48	2.87
Parabacteroides	3.59	2.29	0.07	0.10	1.22	5.75	0.28	4.02	1.21	0.38	1.28	2.54
Treponema	1.33	0.00	0.22	0.00	0.00	0.98	3.41	5.60	2.15	0.51	0.84	5.96
Alistipes	0.62	0.85	0.00	0.00	0.32	3.05	4.82	1.22	1.48	0.38	2.26	3.14
Eubacterium	0.44	1.10	4.50	1.91	2.01	0.35	0.38	1.84	0.67	2.42	1.20	0.55
Acidaminococcus	1.90	1.87	1.33	0.57	1.09	1.38	0.85	1.84	0.54	3.18	0.88	1.21
Coprococcus	1.82	1.27	2.36	0.38	0.95	0.35	0.19	1.62	3.49	2.04	0.67	0.50
Rikenella	1.06	1.10	0.22	0.29	0.54	2.53	2.93	1.18	0.54	0.51	3.88	0.77
Acetivibrio	1.06	0.08	0.00	0.00	0.22	2.30	1.61	0.66	1.34	0.00	2.54	3.42
Turicibacter	0.35	0.42	0.81	0.96	0.58	0.00	0.38	0.92	5.77	0.64	0.12	0.28
Anaerobacter	0.04	0.25	0.00	0.00	0.90	0.63	1.61	1.31	3.36	0.00	1.71	1.38
Dorea	0.66	0.59	0.96	0.38	2.69	0.75	0.66	1.44	0.27	0.89	0.80	0.88
Anaerovibrio	0.89	0.42	0.00	0.57	0.22	1.55	0.57	0.79	0.27	0.38	0.39	3.64
Coprobacillus	0.53	1.36	0.44	0.00	0.75	0.40	0.00	0.17	2.01	0.25	0.26	0.55
Peptococcus	0.49	0.00	0.07	0.00	0.51	0.86	0.38	0.22	2.15	0.51	0.67	0.83
Anaerovorax	0.18	0.00	0.00	0.00	2.21	0.23	0.47	0.22	1.21	0.13	1.24	0.39
Sarcina	0.09	0.17	0.66	2.39	0.20	0.23	0.57	0.26	0.40	0.38	0.60	0.22
Xylanibacter	0.49	0.17	0.00	0.00	0.59	1.04	0.09	0.39	0.54	0.51	0.99	0.66
Anaerostipes	0.22	0.00	0.00	1.62	0.84	0.06	0.76	0.26	0.81	0.13	0.40	0.06
Akkermansia	0.00	0.00	0.00	0.00	0.00	0.00	2.08	0.00	0.00	0.00	1.09	1.82
Anaerotruncus	0.13	0.08	0.00	0.00	0.63	0.46	1.70	0.13	0.13	0.00	0.99	0.55
Bulleidia	0.53	0.59	0.00	0.00	1.73	0.63	0.00	0.00	0.00	0.00	1.00	0.17
Butyrivibrio	0.27	0.25	0.15	0.10	0.71	0.23	0.47	0.22	0.40	0.25	1.22	0.11
Acetanaerobacterium	0.00	0.00	0.00	0.00	1.11	0.23	0.19	0.04	0.13	0.00	0.67	1.93
Propionispira	1.55	0.00	0.15	0.19	0.08	0.23	0.09	0.00	0.54	0.51	0.00	0.44
Ethanoligenens	0.00	0.00	0.00	0.00	0.33	0.17	0.57	0.31	0.13	0.00	0.96	0.50
Kopriimonas	0.09	0.00	0.07	0.00	0.01	0.12	1.04	0.17	0.67	0.00	0.06	0.39
Selenomonas	0.44	0.17	0.07	0.29	0.05	0.12	0.00	0.22	0.40	0.00	0.09	0.61
Pectinatus	0.00	0.00	0.00	0.00	0.00	0.06	0.85	0.00	0.27	0.00	0.43	0.72
Hallella	0.00	0.08	0.07	0.00	0.19	0.35	1.14	0.00	0.00	0.00	0.27	0.06
Synechococcus	0.13	0.59	0.07	0.00	0.00	0.06	0.00	0.13	0.40	0.25	0.41	0.06
Metabacterium	0.00	0.17	0.22	0.00	0.02	0.06	0.19	0.39	0.13	0.00	0.13	0.44
Sutterella	0.13	0.34	0.00	0.10	0.56	0.06	0.00	0.22	0.00	0.00	0.16	0.11
Sum %	98.67	99.24	95.72	98.09	98.51	96.55	96.03	98.16	97.58	97.45	97.43	98.18

Table 3.6. Rumen fluid and fecal bacteria grouped by phylum

Calf ID**	4	8	11	1	10	3	2	9	12	6	7	5
RFI group	low	low	low	low	low	low	high	high	high	high	high	high
RFI kg/d	-1.654	-1.580	-1.445	-1.328	-1.216	-1.206	1.332	1.346	1.364	1.505	1.562	1.953
Rumen fluid, %												
Bacteriodetes	52.74	42.35	57.58	43.15	41.48	77.76	79.51	65.72	---	35.32	41.38	59.70
Firmicutes	5.58	16.62	23.59	10.71	24.82	9.32	6.71	8.54	---	11.32	13.36	24.21
Proteobacteria	37.99	35.47	14.01	41.42	30.85	10.50	9.48	22.25	---	47.99	35.86	12.09
Spirochaetes	1.62	0.76	1.64	1.35	0.05	0.28	0.66	0.74	---	1.32	2.73	0.35
Cyanobacteria	0.28	0.25	0.21	0.28	0.03	0.17	0.15	0.57	---	1.10	1.12	0.86
Lentisphaerae	0.14	0.00	0.44	0.01	0.05	0.00	0.15	0.55	---	0.04	0.03	0.54
Verrucomicrobia	0.49	0.76	0.00	0.01	0.03	0.00	0.10	0.14	---	0.00	0.28	0.00
Tenericutes	0.02	0.61	0.49	0.37	0.27	0.00	0.17	0.08	---	0.18	0.00	0.00
sum %	98.87	96.84	97.97	97.30	97.59	98.03	96.93	98.58	---	97.28	94.76	97.75
Feces, %												
Bacteriodetes	66.21	54.12	59.56	68.19	27.29	60.44	28.29	46.35	32.89	60.51	37.03	36.62
Firmicutes	28.74	36.62	34.24	28.65	67.23	33.64	60.74	42.72	61.34	31.97	55.36	50.36
Proteobacteria	2.26	7.90	1.62	1.24	3.99	1.44	1.51	3.37	0.81	4.20	2.70	3.36
Spirochaetes	1.33	0.00	0.22	0.00	0.00	0.98	3.41	5.60	2.15	0.51	0.84	5.96
Cyanobacteria	0.13	0.59	0.07	0.00	0.00	0.06	0.00	0.13	0.40	0.25	0.41	0.06
Verrucomicrobia	0.00	0.00	0.00	0.00	0.00	0.00	2.08	0.00	0.00	0.00	1.09	1.82
sum%	98.67	99.24	95.72	98.09	98.51	96.55	96.03	98.16	97.58	97.45	97.43	98.18

** No ruminal data for calf ID 12 (vial broken during shipping).

Table 3.7. Effect of RFI on microbial phyla in low and high RFI steers fed a finishing ration

Item		Low RFI	High RFI	SE	*P* - value
Rumen fluid[1]					
	Bacteriodetes, %	52.210	56.235	6.804	0.701
	Firmicutes, %	15.107	12.828	3.189	0.526
	Proteobacteria, %	28.373	25.536	6.223	0.755
	Spirochaetes, %	0.951	1.161	0.348	0.680
	Cyanobacteria, %	0.205	0.761	0.121	0.010
	Lentisphaerae, %	0.109	0.260	0.093	0.277
	Verrucomicrobia, %	0.216	0.103	0.111	0.491
	Tenericutes, %	0.296	0.086	0.083	0.107
	Rumen F:B[2]	0.312	0.253	0.072	0.574
Feces[1]					
	Bacteriodetes, %	48.069	47.180	6.481	0.991
	Firmicutes, %	38.189	50.416	5.342	0.137
	Proteobacteria, %	3.075	2.658	0.827	0.729
	Spirochaetes, %	0.421	3.076	0.694	0.022
	Cyanobacteria, %	0.143	0.201	0.083	0.583
	Verrucomicrobia, %	0.000	0.831	0.279	0.062
	Fecal F:B[2]	1.068	1.175	0.309	0.811

[1] Rumen fluid: low RFI n = 6, high RFI n = 5; feces: low RFI n = 6, high RFI n = 6.
[2] Firmicutes:Bacteriodetes ratio.

steers (3 of 6) but was not detected in the feces of any of the low RFI steers (0 of 6). Low RFI steers had 86% lower ($P = 0.02$) fecal Spriochaetes compared to high RFI steers (0.421 vs. 3.08 ± 0.69 %). *Prevotella* spp. percent was also evaluated as they are a primary source of H^+ production during fermentation. There was no difference in ruminal *Prevotella* spp. (39.7 vs. 42.02 ± 4.8 %) in low vs. high RFI steers; however, low RFI steers tended to have higher ($P = 0.09$) fecal *Prevotella* spp. compared to high RFI steers (42.0 vs. 22.1 ± 7.4 %).

Methane and Ammonia Producing Activity, VFA, and pH

Methane producing activity and APA in vitro data are presented in Table 3.8. There was no difference ($P > 0.05$) in ruminal MPA in steers of divergent RFI, but numerically, low RFI steers had 5% lower in vitro MPA (44.9 vs. 47.3 ± 5.5 μmol CH_4/mL fermentation fluid). There was an interaction ($P = 0.02$) of RFI group x sampling time for fecal in vitro MPA (Figure 3.1). This is due to the fact that at the 3 h sampling time, both low and high RFI steers had similar MPA (0.12 vs. 0.16 ± 1.2 μmol CH_4/mL fermentation fluid), however, at the 24 h sampling point, low RFI steers had 172% higher ($P < 0.05$) in vitro MPA compared to high RFI steers (10.4 vs. 3.8 ± 1.2 μmol CH_4/mL fermentation fluid). No differences ($P > 0.05$) were detected for APA; however, low RFI steers had numerically lower APA as compared to high RFI steers (330.2 vs. 385.3 ± 25.6 mg/L). Ruminal and fecal VFA and pH data are presented in Table 3.9. Divergent RFI steers had similar ruminal and fecal pH. Ruminal VFA concentrations, total ruminal VFA and ruminal acetate:propionate ratio (**A:P**) was

Table 3.8. Effect of RFI on in vitro methane producing activity (MPA) and ammonia producing activity (APA)

Item	Low RFI n = 6	High RFI n = 6	SE	*P* -value RFI group	*P* – value time	*P* – value RFI group *Time
Rumen MPA[1]	44.89	47.25	5.48	0.727	0.001	0.501
Fecal MPA[1,2]	5.274	1.994	0.864	0.003	0.001	0.022
Rumen APA[1]	330.22	385.25	25.61	0.160	< 0.001	0.102

[1] MPA (μmol CH_4/mL fermentation fluid), APA (mg/L).
[2] See figure 3.1 to illustrate RFI group * sampling time interaction.

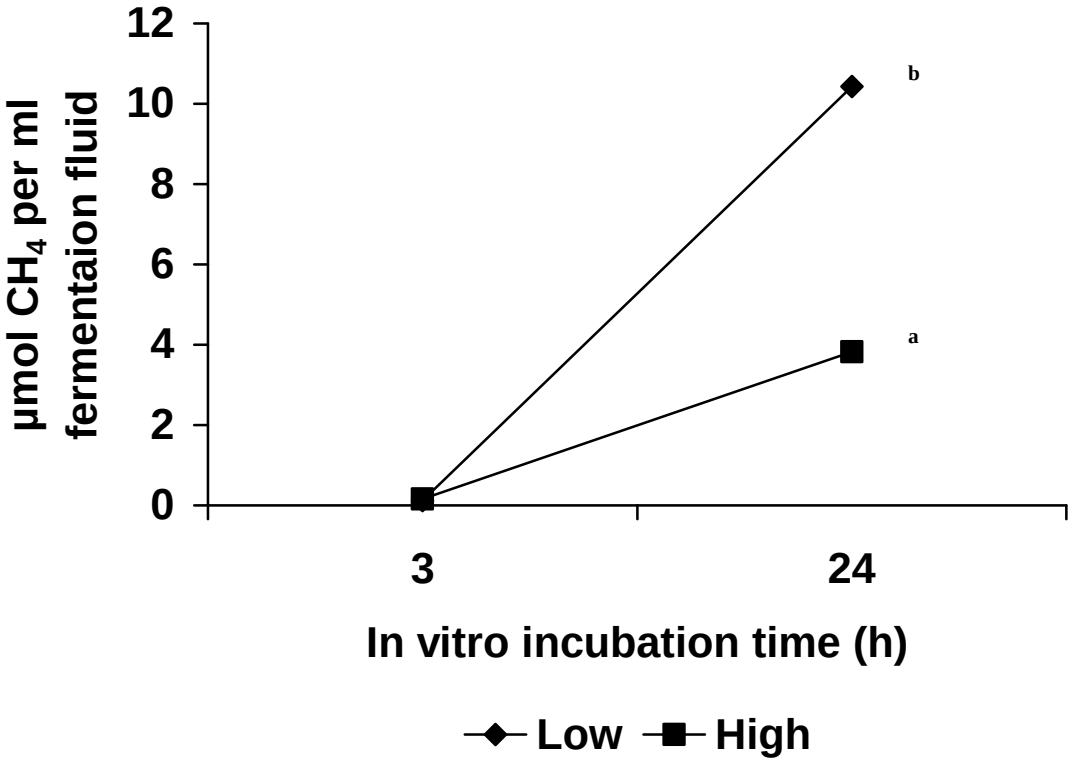

Figure 3.1. The interaction of RFI group and sampling time for fecal MPA.

[ab] Means in the same column with unlike superscripts are different at $P < 0.05$.

Table 3.9. Effect of RFI group on rumen and fecal pH and VFA concentrations

Item	Low RFI n = 6	High RFI n = 6	SE	*P* - value
Rumen pH	6.53	6.40	0.18	0.620
Feces pH	7.55	7.36	0.22	0.551
Rumen VFA, mM				
Acetate	47.31	46.49	3.85	0.883
Propionate	19.21	20.58	2.62	0.721
Iso-Butyrate	0.773	0.644	0.07	0.217
Butyrate	13.73	12.51	1.63	0.606
Iso-Valerate	2.53	2.48	0.31	0.911
Valerate	0.975	0.761	0.12	0.220
Total VFA	84.53	83.46	6.05	0.903
A:P[1]	2.68	2.42	0.34	0.592
Feces VFA, mM				
Acetate	40.78	41.50	2.54	0.847
Propionate	9.87	12.67	1.07	0.092
Iso-Butyrate	0.068	0.075	0.07	0.946
Butyrate	6.57	7.46	0.73	0.406
Iso-Valerate	0.22	0.13	0.08	0.447
Valerate	0.33	0.09	0.07	0.023
Total VFA	57.84	61.92	3.58	0.439
A:P[1]	4.20	3.41	0.30	0.900

[1] Acetate:propionate ratio.

not different amongst RFI groups. Fecal valerate was higher ($P = 0.02$) in low RFI steers and there was a tendency ($P = 0.09$) for fecal propionate to be lower in low RFI steers as compared to high RFI steers. There was no difference in fecal acetate, iso-butyrate, butyrate, iso-valerate concentrations, total fecal VFA, and fecal A:P amongst RFI groups.

Guan et al. (2008) reported that Angus, crossbred, and Charolais calves, fed a high corn finishing ration, had distinctive ruminal fluid microbial populations based on RFI. Not only were differences noted between RFI groups but low RFI steers were more similar to each other based on Dice similarity index (D_{sc} = 91% similarity) than high RFI steers (D_{sc} = 71% similarity). When additional data were collected on ruminal digesta from Charolais and Hereford-Angus crossbred steers that were raised at a different location but under similar conditions, denaturing gradient gel electrophoresis (**DGGE**) bacterial clustering based on RFI group was observed in the rumen digesta samples; clustering based upon RFI group was also observed in the rumen fluid samples. Additionally, when the digesta DGGE profiles were compared, two clusters formed, based on specific breed (Charolais or Herford-Angus crossbred). To examine the effect of breed type in the initial rumen fluid samples, breed information was included; when correlated to the DGGE profiles, no direct correlation was observed. When the DGGE profiles of the only the Angus calves were compared, clear separation patterns were observed for low and high RFI steers. This gives evidence of a host-microbe interaction such that the microbes present depend upon the host genetic make-up as all of the calves in the above study were fed the same diet and raised in a similar environment under the

same management conditions in both experiments (i.e. rumen fluid sampled steers and rumen digesta sampled steers).

Gastrointestinal microbial communities in cattle (Tajima et al., 1999; Whitford et al., 1998), pigs (Leser et al., 2002) and humans (Suau et al., 1999) appear to be dominated by the Firmicutes. This is in contrast to the current study that demonstrated that steers fed a high-corn diet have ruminal microbial communities dominated by the phylum Bacteriodetes (54%), followed by Proteobacteria (27%), Firmicutes (14%) accounting for 95% of the identified population. Fecal microbial communities are dominated by the Bacteriodetes (48%), followed by Firmicutes (44%), and Proteobacteria (3%) accounting for 95% of the identified population. Nelson et al. (2003) reported that about 48% of the identified bacteria in three types of free grazing wild African ruminants and zebu cattle were Firmicutes. Kocherginskaya et al. (2001) used 16s rDNA to determine the bacterial diversity in fistulated steers fed either a corn or hay based diet. In the corn fed animals, Bacteriodetes represented 58%, 27% Proteobacteria, and 15% Firmicutes. The hay fed animals had 67% Bacteriodetes, 3% Proteobacteria, and 25% Firmicutes. This is in close agreement to the current study with Bacteriodetes being the dominate phylum. There were also 23 and 19 phyla represented in corn and hay fed animals, respectively, based on sequence analysis of DGGE bands generated in their study. Based on sequencing in the current study, a much smaller number of phyla are represented in rumen fluid of corn fed animals (8 different phyla in rumen fluid and 6 in feces). The researchers also noted that Spirochaetes was found only in the hay fed animals, where as they were detected in grain fed animals in the

current study. One reason for the slight discrepancies is the fact that only rumen fluid was sampled in the current study and no particulate matter was sampled. This could serve to bias the microbial profile as many Firmicutes are associated with the feed particles (Tajima et al., 1999).

In contrast, Tajima et al. (2000) quantified the rumen microbial ecology in cannulated dry Holstein cows on a hay diet and then a high grain diet. Microbial profile of the hay only rumen fluid consisted of 90% of the sequences identified as Firmicute, 4% Bacteriodetes, 4% Proteobacteria, and 2% Actinobacteria. Twenty-eight d after the grain based diet was fed, the population remained relatively similar except for the absence of the Proteobacteria and Actinobacteria (95% Firmicutes and 5% Bacteriodetes). The slight shift in microbial population when animals were fed a high grain diet was also accompanied by a shift in VFA profile. Total VFA concentration tended to be higher in grain fed animals, the proportion of acetate was consistently lower in the high grain animals versus the forage fed animals, and the proportion of propionate was generally higher in the grain fed animals indicating a relationship between microbial populations and resulting VFA profile in the rumen. Whitford et al. (1998) analyzed rumen fluid, collected before the morning feeding, from mid-lactation dairy cattle fed haylage/corn silage/concentrate (65% roughage:35% concentrate) ration twice daily. Two experiments were done to evaluate microbial profiles present in these cows. In experiment 1, 55% of the sequences were identified as Firmicutes, 30% as Bacteriodetes, and the balance unnamed, while in experiment 2, 96% of the sequences were identified as Bacteriodetes and 4% Firmicutes. The authors speculate that the differences between

phyla indentified may be due to entirely different sampling procedures or to changes in the bacterial population over time. Additionally, they also attribute some of the greater microbial diversity in experiment 1 to the fact that perhaps more small particulate was collected in the rumen fluid increasing the proportion of Firmicutes in the sample.

It is obvious that research is merely scratching the surface in regards to accurately representing flora and fauna present in the ruminant animal. This has been recently demonstrated by Tajima et al. (2001). 16s rRNA analysis revealed the existence of a novel group of Archaea not associated with known methanogens. To further illustrate this, Whitford et al. (1998) identified up to 20 novel Gram-positive bacteria and six previously uncharacterized groups of Gram-negative bacteria in cannulated dairy cow rumen fluid. Dowd et al. (2008) reported the fecal microbial diversity in dairy cows fed a chopped alfalfa hay diet (20% of diet DM) and the balance a mixture of cracked corn, soybean meal, cottonseed meal, and trace mineral salts. The dominant genera found in the feces of all 20 cows was *Clostridium* spp. (accounting for about 20% of the total population), followed by *Bacteroides* spp. (9%), *Porphyromonas* spp. (7%), *Ruminococcus* spp. (4%), and *Alistipes* spp. (7%). This is in contrast to the current fecal data in steers on a high corn diet. *Prevotella* spp. represented 32% of the microbial population identified followed by *Clostridium* spp. (10%), *Papillibacter* spp. (7%), *Tannerella* spp. (7%), and *Roseburia* spp. (4%). Ouwerkerk and Klieve (2001) reported 97% of the sequences analyzed in feedlot manure were classified as Firmicutes and only 3% were Proteobacteria. To demonstrate the lack of congruency in published literature, Kocherginskaya et al. (2001) concluded that corn-fed animals display more diverse and

rich bacterial populations while Larue et al. (2005) concluded that animals consuming hay posses a more diverse microbiome than animals consuming a mixture of hay and grain. This indicates not only wide variation in the microbiome present, but that exogenous traits, too, must influence the microbial population in the rumen. The wide array of published results may be due to: 1) a host interaction such that different breeds of animals have different microbial populations (Guan et al., 2008), 2) environmental conditions (temperature and relative humidity) may affect the population present (Tajima et al., 2007), 3) sampling technique may serve to bias the microbial population [fluid vs. particulate; (Larue et al., 2005)], and 4) DNA extraction and amplification biases may manifest (Nelson et al., 2003).

Using cultivation techniques, *Streptococcus bovis* is commonly found to be a dominant starch utilizer often resulting in lactic acid production implicating it in ruminal acidosis (Tajima et al., 2000). However, *Streptococcus bovis* was not identified in any of the rumen samples in this study. There was no difference in rumen pH and *Megasphera elsdenii* was not identified. As *Megasphera elsdenii,* a lactate utilizer, is associated with *Streptococcus bovis*, it is plausible for *Streptococcus bovis* was not present in the current study. This was also the case in Tajima et al. (2000) in dairy cows fed a high grain diet for 28 d. Additionally, no food-borne pathogens were identified in the rumen fluid or feces in the current study.

Primers for kingdom Archaea were not used in this study so no methanogen microbial diversity data is available for the current study. There was no effect of RFI group on ruminal MPA and APA; however, there was a RFI group by sampling time

interaction for fecal methane producing activity such that at the 3 h sampling time both RFI groups had similar MPA (0.117 vs. 0.161 μmol CH_4/mL fermentation fluid) but at the 24 h sampling time low RFI steers had higher ($P = 0.01$) MPA compared to the high RFI steers (10.43 vs. 3.83 μmol CH4/mL fermentation fluid; Figure 3.1). The lack of difference at the 3 h sampling time may be due to similar lag times in the feces of low and high RFI steers that is overcome at the 24 h sampling time. Low RFI steers tended ($P = 0.09$) to have higher fecal *Prevotella* spp. compared to high RFI steers (42.0 vs. 22.1 ± 7.4%). *Prevotella* spp. are a primary H_2 producer which may help to explain the higher fecal CH_4 production at the 24 h sampling time. Rumen and fecal pH and VFA were similar amongst groups except that low RFI steers tended to have lower fecal concentrations of propionate and had increased fecal concentrations of valerate (0.33 vs. 0.09 ± 0.07 mM). Guan et al. (2008) also observed a shift in VFA between low and high RFI steers. Steers were fed a high energy finishing ration with an absolute mean difference in RFI of 2.8 kg/d. Low RFI steers tended to have higher total VFA concentrations as well as higher butyrate and valerate concentrations. When expressed as molar proportions, low RFI steers had higher butyrate compared to high RFI steers (15 vs. 6 mol/100 mol) and higher valerate (1.8 vs. 1.2 mol/100 mol). Differences in microbial clustering between low and high RFI steers was also demonstrated suggesting that differences in microbial population due to RFI can cause a change in ruminal VFA concentrations. Microbial differences were noted in the rumen fluid of low vs. high RFI steers but no differences were seen in the VFA profiles; however, there were subtle

microbial shifts in the fecal microbiome that resulted in a decrease in propionate and increased valerate.

Implications

Understanding the relationship between animal efficiency and the microbiome may explain animal variation in RFI. Differences in gross microbial ecology due to RFI was not observed in this study, however, subtle differences in fecal hydrogen producers (*Prevotella* spp.) may explain differences in fecal MPA. Logic leads one to believe that for a measurable difference to manifest in calves of divergent RFI there almost certainly has to be an underlying difference in the microbial ecology of low and high RFI animals. Small differences were observed but it is still too soon to tell the importance of these microbial differences. More research is needed to better understand not only the simple dynamics in ruminal and fecal microbial ecology but also the relationships between animal efficiency gut microbial ecology, and resulting fermentation in growing and finishing beef calves.

CHAPTER IV

EFFECTS OF HYDROLYSABLE AND CONDENSED TANNINS ON ANIMAL PERFORMANCE, RUMINAL FERMENTATION, AND CARCASS AND NON-CARCASS TRAITS IN STEERS FED A HIGH-GRAIN DIET

Introduction

Tannins are a complex group of polyphenolic compounds that plants have evolved to deter animal consumption (Foley et al., 1999). Tannins are commonly referred to as plant secondary compounds (as are oxalates, terpenes, saponins, to name a few) and generally have negative effects on animal production. Originally, the term "tannin" was applied to any substance that was able to tan leather; however, currently, it is generally used to denote any naturally occurring substance of high molecular weight which contains a large number of phenolic hydroxylic groups to enable it to form effective cross-links with proteins (Swain, 1979).

Tannins are classified into two categories: hydrolysable and condensed (**HT** and **CT**, respectively). Hydrolysable tannins consist of a carbohydrate core with phenolic carboxylic acids bound by ester linkage; Condensed tannins consist of oligomers of flavon-3-ols and related flavanol residues (Mueller-Harvey and McAllan, 1992). Tannins are ubiquitous in nature, and are widely found in feedstuffs, forages, fodders, and agroindustrial wastes. The plant endogenous compounds may have both positive and negative effects when consumed. For example, plant breeders have been able to

capitalize on plant's natural defense and have developed bird-resistant sorghum; the increased concentration of short oligomers results in bird avoidance (Butler, 1982).

Dietary tannins decreased DMI, growth, and caused damage to the gastrointestinal tract in mammals (Hervas et al., 2003; Mcleod, 1974; Robbins et al., 1991). Numerous studies have been conducted to examine the toxicity effects of HT with sparse information on CT toxicity (Hervas et al., 2003). The antimicrobial/bacteriostatic activity of tannins may make it useful as a novel agent to control food borne pathogens, such as *E. coli* (Henis et al., 1964; Min et al., 2008). The data presented in this paper is part of an overall larger project to elucidate the effect of dietary CT and HT on the prevalence of food borne pathogens in finishing beef cattle.

There is evidence of positive tannin effect on ADG in steers grazing winter wheat (Min et al., 2006) and in vitro methane production (Min et al., 2006; Min et al., 2005b). Tannins have also been shown to decrease bloat in wheat grazed steers (Min et al., 2006), as well as to decrease ruminal degradability of CP, increasing the amount of CP that reaches the abomasum and small intestine (Teferedegne, 2000). However, there are few studies that have examined the effects of tannins on animal performance while fed high-grain diets. Therefore, the objectives of this study were to examine the effects of added dietary CT and HT on animal performance, ruminal fermentation parameters, and carcass and non-carcass traits in beef cattle fed a high-grain diet.

Materials and Methods

Animals

Approval for care and use of animals used in this study was obtained from the Institutional Care and Use Committee of Texas A&M University. Thirty-six crossbred steers (414 ± 40 kg) were stratified by initial BW and randomLy assigned to one of three treatments (n = 12): control (**CN**), mimosa tannin (**CT**), and chestnut tannin (**HT**). Commercially available tannin extracts were supplemented in a total mixed ration at 15 g/kg DM (chestnut tannin: *Castanea sative* Mill; approximately 80 % hydrolysable tannins; mimosa tannin: *Acacia mearnsii* black wattle; approximately 70% condensed tannins; Chemtan ® Chestnut Powder KPN and Chemtan ® Mimosa Powder, Chemtan CO, NH, USA). Within treatment, steers were assigned to one of two pens (6 steers/pen) and each pen-group randomLy assigned to a pen location in the Calan gate facility resulting in two blocks. Each block consisted of one pen from each treatment; the second block was initiated one week following block I to allow for time sensitive sample processing and lab analysis. Steers were individually a high corn ration (Table 4.1) fed for 42 d using Calan gate feeders. The ration was formulated to meet nutritional requirements to support gains of 1.5 kg/d (NRC, 1996).

Animal Performance

Steers were fed their respective diet twice a day, following a 30 d diet adaption period, and were provided ad libitum access to water. During the 42-d feeding period, feed offered was recorded daily, and feed refusals and BW measured 7-d intervals, The treatment rations were mixed and placed into separate feed carts, and feed

TABLE 4.1. Ingredient composition of total mixed ration (g/kg DM)

	Treatment diets*		
Ingredient	Control	Chestnut	Mimosa
Corn	641.1	627.6	627.6
Hay-sorghum	71.1	71.1	71.1
CSH[†]	18.1	18.1	18.1
CSM[††]	100	100	100
Molasses	30.2	30.2	30.2
Limestone	14.9	14.9	14.9
TM Supplement[£]	19.8	19.8	19.8
Tannin mix	0.0	14.9	14.9

*Diets were formulated to meet nutritional requirements to support growth rates at 1.5 kg/d (NRC, 1996).

[†]Cottonseed hulls.

[††]Cottonseed meal.

[£]Trace minerals supplement: Guaranteed analysis: 500 mg/kg Co, 2,300 mg/kg I, 4,000 mg/kg Fe, 1,000 mg/kg Se, 4.5 g/kg Cu, 7 g/kg Mn, 19 g/kg Zn (Animal Science Products, Nacogdoches, TX).

handling equipment washed in between to prevent cross contamination of tannins. Initial and final BW and ADG were derived from linear regression of BW on days on test for each steer. Gain to feed (**G:F**) was computed as the ratio of gain:feed. Residual feed intake (**RFI**) was calculated as the residual of the linear regression of DMI on ADG and $BW^{0.75}$.

Ruminal Fermentation Parameters

On d 0, 21, and 42 rumen fluid and feces were collected for VFA, methane, ammonia, and pH analysis. Rumen fluid was collected via stomach tube, before the morning feeding, into 50 mL serum vials that were filled to capacity, capped immediately and stored at ambient temperature until analysis later that day. In vitro methane producing activity (**MPA**) of ruminal and fecal samples was determined by in vitro incubation of 5 mL rumen fluid or 2 g feces, mixed with 5 or 8 mL, respectively, anaerobic dilution solution (Bryant and Burkey, 1953) containing 60 mM sodium formate and 0.2 g finely ground alfalfa (to pass a 4 mm screen). The tubes were capped and incubated at 39 ˚C under a hydrogen:carbon dioxide (50:50) atmosphere. At the end of the 3 h incubation period, methane concentration was determined by gas chromatography according to Allison et al. (1992). For VFA analysis, 1 mL or 1 g of rumen fluid or feces were diluted 1:10 with water (pH = 7.0) and pH was recorded, samples were centrifuged and the supertnant frozen (-20 °C) for subsequent VFA and ammonia analysis. Ammonia concentrations were analyzed colorimetrically according to Chaney and Marbach (1962). Volatile fatty acids were analyzed via gas chromatography (Agilent 6890N, Santa Clara, CA, USA) with a 007 series bonded phase

fused silica capillary column (25m x 0.25mm x 0.25 μm) and a flame ionizing detector with the following parameters: 1 μl injection, injector temperature = 240˚C, oven temperature = 80˚C for 1 min, ramp to 120˚C hold for 5 min, ramp to 165˚C hold for 2 min, detector temperature = 260˚C.

Carcass and Non-Carcass Traits

Upon completion of the study, cattle were randomLy allocated to one of four groups and harvested at the Rosenthal Meat Science and Technology Center (Texas A&M University, College Station, TX). At harvest, visceral organs (heart, liver, and kidney) and total KPH fat were removed, dissected, and weighed. Esophagus, kidneys, and liver were externally and internally examined for lesions. At 24-h postharvest, carcass data was collected and the gastrointestinal tract emptied of contents and internal fat depots dissected. Weights of empty stomach complex, small and large intestines, and total internal fat depots were recorded.

Statistical Analysis

Individual animal was the experimental unit as individual intakes were recorded. Data were analyzed as a completely randomized block design using PROC MIXED of SAS (SAS v 9.1; SAS Inst. Inc., Cary, NC). Additionally, fermentation data utilized the repeated statement. If the interaction term was found not significant it was then omitted from the model. PROC FREQ with the FISHER option was used to examine the offal necropsy data (scale of 0 to 3; 0 = normal, 3 = significant abnormality). Significant differences were accepted if $P \leq 0.05$.

The model used to analyze fermentation measures was:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\gamma)_{ik} + e_{ijk}$$

where: Y_{ijkl} = observation, μ = population mean, α_i = treatment effect, β_j = block effect, γ_k = day effect, $(\alpha\gamma)_{ik}$, and e_{ijk} = residual error.

The model used to analyze all other data was:

$$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$$

where: Y_{ij} = observation,μ = population mean, α_i = treatment effect, β_j = block effect, and e_{ij} = residual error; except carcass data which also included a random effect of harvest group.

Results and Discussion

Animal Performance

In the current study, the diet offered was 15 g/kg DM of supplemental tannin which resulted in approximately 168 g tannin consumed per day and 0.38 g tannin/kg LW. Initially, tannin supplementation of 20 g/kg diet DM was used; however, there was a decrease in DMI in tannin treated calves in the initial days of tannin inclusion. After a 2-d period in which all calves were fed the control diet, the inclusion rate was lowered to 15 g/kg diet DM.

There was no effect of treatment ($P > 0.05$) on any of the animal performance or feed efficiency traits measured in this study (Table 4.2). Initial BW, final BW, ADG, absolute gain, DMI, gain:feed (**G:F**), and residual feed intake were similar across all treatments. Dietary tannins generally tend to decrease DMI; however, there are

Table 4.2. Effects of source of tannin on animal performance and feed efficiency traits in finishing steers

	Treatment				
Trait[1]	Control n = 12	Chestnut n = 12	Mimosa n = 12	SEM	*P* - value
IBW, kg	412.3	414.4	409.1	12.6	0.95
FBW, kg	481.3	478.5	479.2	14.2	0.99
ADG, kg	1.89	1.77	1.92	0.20	0.64
Gain, kg	79.27	74.43	80.44	8.45	0.64
DMI, kg/d	12.17	11.40	11.26	0.96	0.50
DMI, %BW	2.77	2.61	2.58	0.18	0.44
Gain:feed	0.17	0.164	0.177	0.02	0.65
RFI, kg	0.319	-0.452	0.132	0.58	0.62

[1] IBW = initial BW, FBW = final BW, ADG = average daily gain, DMI = dry matter intake, RFI = residual feed intake.

exceptions (Beauchemin et al., 2007; Puchala et al., 2005; Woodward et al., 2001). In cattle fed 70% forage ration supplemented with Quebracho CT, Beauchemin (2007) reported no adverse effect on DMI, BW, or ADG. Puchala (2005) reported increased DMI and decreased methane emissions in Angora does fed *Lespedeza cuneata* (CT containing forage) vs. a mixture of *Digitaria ischaemum* and *Festuca arundinacea*. Additionally, late lactation dairy cows consuming *Lotus corniculatus* (CT containing forage) had higher DMI and lower methane per unit milksolids yield compared to cows fed ryegrass silage. Frutos et al. (2004) reported no effect of chestnut HT on DMI and FCR in finishing lambs consuming a high-energy ration (3.4 Mcal GE/kg DM). Finishing lambs consumed approximately 0.84 g tannin/kg LW which is more than double the consumption rate in the current study of approximately 0.38 g tannin/kg LW.

In the rat, Mitjavila et al. (1977), demonstrated reduced intestinal permeability in response to tannin supplementation; this could lead to decreased nutrient absorption and lowered DMI as well. Decreased DMI in ruminants has also been reported (Donnelly, 1954; Mantz et al., 2009). Hervas et al. (2003) reported ewes fed alfalfa hay and intra-ruminally dosed with Quebracho CT at 3 g/kg LW had a 95% reduction in DMI after 3 d of dosing.

Reduced DMI due to tannins is thought to be caused by the astringent taste and decreased palatability possibly resulting in food avoidance (Kumar and Singh, 1984). Many mammals, especially browsers, are able to produce proline-rich salivary proteins (**PRP**) that are able to bind to dietary tannins to inactivate them (Austin et al., 1989). It is the binding of PRP and tannins that produce the astringent taste (Prinz and Lucas,

2000) and subsequent food avoidance. Cattle and sheep are devoid of PRP (Makkar, 2003) so the decrease in DMI due to astringent taste mechanism associated with tannins may not occur in sheep and cattle. However, other proteins are present in the saliva of cattle fed tannin-rich diets which have a high affinity for tannins but are not rich in proline; these salivary proteins tend to form soluble tannin-protein complexes (Makkar, 2003). The acceptability of the diet at 15 g/kg DM might have met a threshold of salivary protein production allowing tannin treated calves to consume as much as their control contemporaries.

The overall lack of effect on animal performance may be due to conservative dosing of tannins; however, it was not in the aim of this study to demonstrate toxicity but to examine the potential effects of added tannin on native food borne pathogens in finishing beef calves; it must also be understood that for a feed-through treatment to have a real-life practical effect, it must be in a palatable form so as not to disrupt cattle gains ultimately reducing feedlot profits.

Ruminal Fermentation Parameters

There was no effect ($P > 0.05$) of tannin supplementation on rumen pH and ammonia concentrations (Table 4.3). Added tannins had no effect on the molar proportions of acetate and propionate, as well as the acetate:propionate ratio or the total concentration of VFA. There was, however, a treatment * day interaction for butyrate (Fig. 4.1). For the mimosa treated calves, there was a linear increase in the molar proportion of butyrate while the chestnut calves remained relatively stable and the

Table 4.3. Effects of source of tannin on rumen fermentation parameters (n = 12)

	Day					
Item/treatment	0	21	42	SEM	*P* - value treatment	*P* - value day
pH				0.108	0.271	0.280
Control	6.2119	6.3876	6.375			
Chestnut	6.2805	6.2445	6.1143			
Mimosa	6.2504	6.3806	6.2181			
Methane[$]				1.372	0.508	0.008
Control	18.3144	21.4527	19.4414			
Chestnut	15.3225	22.1628	23.1442			
Mimosa	13.7268	20.0366	20.4013			
Ammonia[&]				0.515	0.550	< 0.001
Control	1.0954	2.6754	3.1704			
Chestnut	1.4954	2.1038	2.6471			
Mimosa	1.7688	2.9338	2.5421			
Acetate[^^]				2.827	0.116	< 0.001
Control	49.4768	51.5721	55.7572			
Chestnut	49.4263	51.2693	57.7118			
Mimosa	45.4648	49.8853	53.7012			
Propionate				5.083	0.311	< 0.001
Control	38.4713	34.1118	31.7971			
Chestnut	36.4499	34.845	26.5966			
Mimosa	43.0378	35.8556	28.4467			
Butyrate[#]				2.108	0.094	0.006
Control	9.6871	11.9512	10.0807			
Chestnut	11.7589	11.5207	13.3268			
Mimosa	9.1326	11.8942	15.4872			
A:P[1]				0.351	0.367	< 0.001
Control	1.306	1.3409	1.866			
Chestnut	1.2298	1.2926	2.4047			
Mimosa	0.8853	1.3135	1.8933			
Total VFA				6.779	0.788	0.394
Control	88.2823	85.7922	82.5616			
Chestnut	83.8532	87.509	93.3539			
Mimosa	82.2335	82.8931	93.661			

[$] µmol$CH_4$/mL fermentation fluid.
[&] mg NH_3/L.
[^^] Molar proportions.
[#] trt*day $P = 0.02$; see Fig. 4.1.
[1] Acetate:propionate ratio.

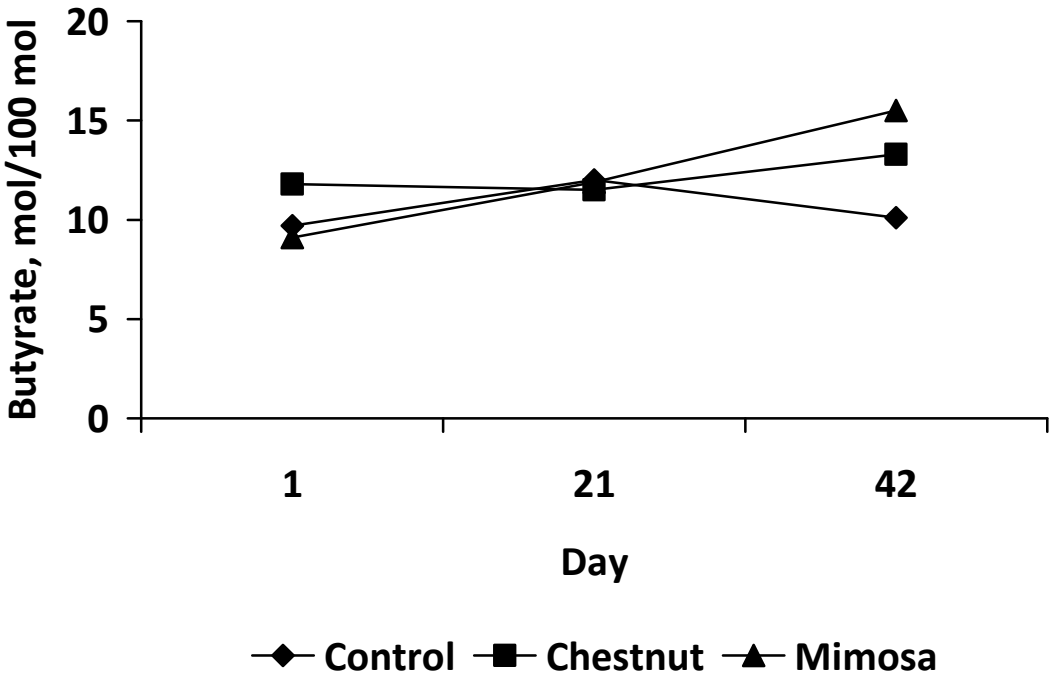

Figure 4.1. The interaction of treatment*day for ruminal butyrate molar proportions. Values are expressed as mol/100 mol. ♦ = control diet, no added tannin; ■ = chestnut diet, 15 g/kg DM added chestnut tannin; ▲ = mimosa diet, 15 g/kg DM added mimosa tannin.

control calves had numerically lower butyrate concentrations at d 42. Despite the significant interactions, treatment means at d 42 were not significantly different.

Previous studies that have examined the effects of tannin on fermentation parameters in various species have not been consistent. It is reported in the rat, that grape seed extract CT effectively decreased cecal pH and increased total cecal VFA concentration; specifically it increased acetate and decreased propionate and butyrate (Tebib et al., 1996). However, the physiologic differences in rats may limit inferences concerning cattle. Sheep fed a basal hay diet supplemented with *Elaeis guineense* at 0, 25, 50,% of the diet DM (0, 5, 9 g/kg DM CT; 0, 0.08, 0.14 g tannin/kg LW, respectively) had decreased rumen pH sustained 5 h post ingestion and increased ammonia concentrations; rumen pH began to return to pre-feeding levels after 5 h; total VFA concentration increased with 25% supplementation compared to the control diet and acetate concentration increased with *Elaeis guineense* treatment while butyrate concentration increased only in the 25% treatment (Osakwe et al., 2004). They also report decreased methane energy loss at 50% supplementation measured in a respiration chamber. Makkar et al. (1995) found that tannins decreased VFA production in vitro when added (0.8 mg/mL) to the medium and that CT decreased VFA production to a greater extent than HT.

In growing beef cattle fed a forage based diet, Beauchemin et al. (2007), found that supplementation with Quebracho tannin (1, or 2% diet DM), decreased the molar proportion of acetate, the acetate:propionate ratio, and ruminal ammonia compared to control animals. The concentration of tannins fed in the current study was

approximately 0.38 mg tannin/kg LW which is very similar to the concentrations used in the study of Beauchemin et al. (2007). In the current study, there was no effect of tannins on VFA, in contrast to Beauchemin et al. (2007) that utilized Quebracho as a source of CT, while in the current study, the source of CT was Mimosa. Waghorn and Shelton (1997) fed wethers a mixture of fresh cut *Lotus corniculatus* (37%) and ryegrss/clover for 32 d; the diet contained 10 g CT/kg DM. No effect of CT was observed on VFA concentrations or ammonia in these wethers.

There was no effect of tannin supplementation on in vitro methane producing ability (Table 4.3). Numerous studies have demonstrated that tannins decrease methane production in ruminants (Hess et al., 2006; Min et al., 2006; Puchala et al., 2005). Hess et al. (2006) reported a 13% reduction in methane emission measured in open circuit respiration chambers when wethers were fed a ryegrass based diet supplemented with 25 g CT/kg DM. Carulla et al. (2005) supplemented wethers at 25 g CT/kg DM fed a basal diet of ryegrass haylage; a 13% reduction in respiration chamber methane emission was reported. However, Beuchemin et al. (2007) reported no effect of CT tannin on enteric methane production in heifers fed a barley silage based diet. The diets fed in these studies were high in forage for which methane emission can be as high as 12% of GE (Johnson and Johnson, 1995). In high-grain diets, as little as 2 to 3% of GE may be emitted as methane, however, it is generally accepted that methane emission in high-grain diets is around 6% of GE (Johnson and Johnson, 1995). In the current study, a high-grain diet was fed resulting in decreased methane loss compared to much of the published literature, which may have negated any treatment effect or the sensitivity of

the methane procedure may not have been adequate to detect differences. The lack of methane response to tannin also reflects the lack of tannin effect on VFA.

The lack of response to tannin supplementation for ruminal VFA, ammonia, pH and methane producing activity, in the current study, could be due to the fact that samples were taken before the morning feeding or that they were obtained orally and salivary contamination may have served to dilute VFA concentration; this may have also served to mask any alteration in pH that may have presented itself. More simply, the lack of tannin effect in the present study may be due to the fact that cattle were fed a high-grain diet.

Tannins also have the ability to modify microbial populations which may serve to further alter subsequent diet and nutrient digestibility, VFA profile, ammonia, production, animal performance and ultimately animal efficiency. Henis et al. (1964) found an antimicrobial effect of carob pod tannin extract on *Cellvibrio fulvus* (a celluloytic bacterium) in vitro; tannin addition also resulted in morphological changes indicating tannin effect on this bacterium. Alteration in gut microbial population was demonstrated in rats, fed CT at 20 mg/kg diet, there was a shift in fecal microbial population favoring *Enterbacteriaceae* and the *Bacteroides* species (Smith and Mackie, 2004). Decreased celluloytic and proteolytic activity was also observed by Tagari et al. (1965) with carob pod extract in an artificial rumen indicating some effect on the microbial population. The lack of effect of tannin supplementation may indicate that ruminal microbial populations were not drastically altered as no difference in ruminal fermentation parameters were observed.

Carcass and Non-Carcass Traits

Condensed or HT tannin supplementation had no effect ($P > 0.05$) on HCW, REA, 12th rib fat thickness, KPH, yield grade, and marbling score (Table 4.4) or liver, heart, and kidney mass (Table 4.5). There was an effect ($P < 0.01$) of tannin supplementation on dressing percent. Calves fed CT had lower dressing percent than control calves with dressing percent intermediate for HT treated calves. This is likely due to numerically lower HCW in CT treated calves compared to HT and control calves. There was an effect ($P = 0.03$) of tannin supplementation on rumen mass. Calves fed HT had greater ($P < 0.05$) rumen mass than calves fed CT with rumen mass of control calves being intermediate. However, when rumen mass was expressed as a proportion of empty body weight, the tannin effect was no longer observed (Table 4.5).

Frutos et al. (2004) demonstrated no effect of chestnut tannin (HT) on lamb carcass traits when fed a high-grain diet (3.4 Mcal/kg DM) supplemented with approximately 0.84 g tannin/kg LW; there was no effect of HT supplementation on ADG, feed efficiency, and length of finishing period. They also reported that individual weight of offal (blood, skin, fat depots, and parts of the GIT) did not differ in weight between control and HT treated finished lambs. Additionally, chemical composition of the empty body weight was not different between control and HT treated lambs. Chickens fed high-tannin sorghum also showed no effect of tannins on carcass traits or yield of visceral organs (Kumar et al., 2005).

Table 4.4. Effects of source of tannin on selected carcass traits

	Treatment				
Trait[1]	Control n = 12	Chestnut n = 12	Mimosa n = 12	SEM	*P* - value
HCW, kg	286.4	279.8	271.4	8.76	0.46
12th rib FT, cm	0.86	0.94	0.91	0.01	0.82
REA, cm^2	80.06	76.83	77.85	2.18	0.52
KPH, %	2.04	1.77	2.20	0.35	0.08
Dressing, %	59.52[b]	58.50[ab]	56.72[a]	0.83	0.01
YG	2.70	2.75	2.72	0.07	0.88
Marbling score‡	5.09	4.88	4.76	0.21	0.52

‡ Slight[00] = 4.00, Small[00] = 5.00, Modest[00] = 6.00.

[1] HCW = hot carcass weight, FT = fat thickness, REA = ribeye area, KPH = kidney, pelvic, and heart, YG = yield grade.

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

Table 4.5. Effects of source of tannin on selected visceral organs

	Treatment				
Trait	Control n = 12	Chestnut n = 12	Mimosa n = 12	SEM	*P* - value
Mass of organs, kg					
Heart	1.69	1.75	1.67	0.11	0.55
Liver	6.49	6.26	6.17	0.24	0.44
Kidney	1.03	1.04	1.01	0.08	0.85
Rumen	12.36[ab]	13.59[b]	11.75[a]	0.47	0.03
Small intestine	4.53	4.35	4.24	0.17	0.51
Large intestine	2.40	2.31	2.36	0.12	0.85
GIT[2], % BW	4.34	4.54	4.13	0.14	0.06
Organs, %EBW[1]					
EBW, kg	405.27	409.20	384.83	11.03	0.24
Heart	0.44	0.41	0.44	0.02	0.55
Liver	1.55	1.60	1.62	0.06	0.71
Kidney	0.26	0.25	0.26	0.01	0.80
Rumen	3.37	3.04	3.08	0.14	0.20
Small intestine	1.08	1.12	1.11	0.05	0.83
Large intestine	0.58	0.59	0.62	0.04	0.68
GIT dissectible fat	4.15	4.52	4.39	0.34	0.73
GIT[2]	5.02	4.74	4.81	0.20	0.58

[1] Empty body weight.
[2] Gastrointestinal tract.

Maxson et al. (1973) demonstrated decreased DM, CP, and TDN digestibility in steers fed a high tannin sorghum finishing ration (2.15%$_{DM}$ tannin) compared to control steers consuming a high corn finishing ration (0.51 %$_{DM}$ tannin). The two diets were similar in energy and CP concentration and the sorghum based diet has decreased ME concentration; corn fed steers had higher diet and nutrient digestibility. The authors also reported decreased ($P < 0.05$) ADG in high-tannin sorghum fed steers, numerically higher F:G, and reduced dressing percent, hot carcass weight, and yield grade. This is in partial agreement with the current study as decreased HCW was observed in CT treated calves; however, in the study reported by Maxson et al. (1973), diet source was confounded with tannin addition.

McBrayer et al. (1983) fed feedlot heifers fed increasing levels (0, 10, 20%) of peanut skins [high in CT (Stansbury et al., 1950)] to provide 0.4, 2.2, and 3.9% tannin$_{DM}$. Heifers fed the peanut skin diets had lower ADG, DM and CP digestibility, and reduced DMI and FCR at 20% peanut skin diet. In a follow up experiment, steers supplemented (0, 4.8, 9.1% of diet) with peanut skins (0.6, 1.7, or 2.7% tannin$_{DM}$) showed no effect of peanut skin inclusion on ADG, FCR, and dressing percent. However, the marbling score, yield grade, quality grade, and back fat thickness were higher for the 4.5% peanut skin diet compared to the control steers with the 9.1% peanut skin diet intermediate . Decreased DMD in the peanut skin diets compared to the control diets was noted by the authors.

In rats fed graded levels of high-tannin sorghum (0, 20, 35, 50% of the diet), Larrain et al. (2007), observed no detrimental effects of tannin supplementation on BW,

ADG, G:F, and average daily feed intake when 13 wk old rats were fed for only two weeks. When 5 wk old rats were fed for ten weeks, no difference in G:F between control and tannin diets; however, the 35% high-tannin sorghum diet resulted in heavier ($P = 0.05$) rats at d 70, and higher ADG and intake during the first two weeks of the study; because G:F was not different at any time point, greater feed and energy intake seems to be driving higher ADG. In finishing pigs, Cousins et al. (1981) reported increased feed intake in pigs consuming the 75% high-tannin sorghum compared to control animals; feed conversion and ADG was similar fed the high-tannin sorghum diet compared to pigs fed the corn-based diet.

Tannins can cause toxicity in sheep (Hervas et al., 2003) and cattle (Garg et al., 1992). In the current study, there was no effect of tannin supplementation on offal and gastric lesion scores of the skin, tongue, esophagus, rumen, reticulum, omasum, abomasum, intestines, liver or kidney (data not shown) at the time of harvest. Offal lesions were not expected as the experimental diet contained well below the amount fed (0.75 mg/kg LW) to sheep that did not result in gastric lesions when fed for 60 d (Frutos et al., 2000).

Collectively, these studies indicate that the response to tannin supplementation is varied and depends on type of tannin, rate of supplementation, and species and in many cases suggest that tannin supplementation at low rates do not have a detrimental effect on economically important carcass and non-carcass traits.

The lack of tannin effect on HCW, REA, 12th rib fat thickness, KPH, yield grade, and marbling score and non-carcass traits is plausible as there was no effect of treatment

on animal performance or apparent rumen fermentation modification due to tannins; presumably, tannin treated calves were able to extract similar amounts of nutrients from their diets to allow similar growth and carcass component accretion. The current data indicate that CT treated calves have lower dressing percent compared to control and HT treated calves and is explained by numerically lower HCW. However, more research is needed to further substantiate this finding.

The difference in rumen mass is interesting, as calves were of similar size and would be expected to have similar gut masses. One possible explanation for this phenomenon is that HT supplies some growth factor that has a positive effect on rumen epithelium causing it to expand in size while CT has the opposite effect; HT can be metabolized in the rumen while CT-protein complexes pass through the gastrointestinal tract with little modification (Makkar et al., 1995). Hydrolysable tannins are largely hydrolyzed in the rumen to acetate and butyrate (Bhat et al., 1998) possibly resulting in slightly larger rumen mass in HT treated steers; however, cell proliferation or VFA uptake by the rumen wall was not measured. When rumen mass was expressed as a proportion of empty body weight, tannin effect was no longer evident indicating that the rumen mass increased in proportion to EBW.

Tannin supplementation offers other added benefits to the beef industry that may help offset possible decreased HCW. Tannins may serve to impede muscle oxidation during storage serving to increase shelf life of whole muscle products. It has been shown in rats consuming high-tannin sorghum, to have lowered markers of protein oxidation in rat muscle after 6 d of refrigerated storage (Larrain et al., 2007). Du et al. (2002) showed higher a* (redness) values in thigh patties after 7 d storage at 4˚C from chickens fed 10% high-tannin sorghum. This antioxidant effect of dietary tannins may serve to improve beef product acceptance by maintained redness and decreased oxidation. Evidence of this work was not found in the literature but may have research merit as tannin supplementation in finishing beef calves may help to meet product stability expectations of the consumer as well as being a "natural" product.

Implications

This study was part of a larger experiment looking at the antimicrobial effects of added dietary tannins on native food borne pathogens in finishing cattle. No detrimental effect was observed on animal performance, ruminal fermentation parameters, and non-carcass traits; however, decreased dressing percent was observed in CT treated calves. This may indicate that there is no obvious reason why tannins cannot be added at low levels in the diet to deter pathogens if bactericidal efficacy is established. Attention will need to be paid to inclusion ratios so as to avoid lowered DMI and subsequent animal performance. In this study, the original inclusion ratio of CT and HT was 20 mg/kg diet DM and was rejected by the steers; after decreasing the added dietary tannins to 15 mg/kg DM palatability was restored. Ultimately, for a feed-through additive to be effective it must be value additive; a supplement that is effective for its purpose but decreases animal performance or detrimentally alters carcass traits will not be accepted by industry. This research indicates that HT and CT supplementation at low levels has little ill effect on animal performance or other economically important traits, perhaps CT supplementation is detrimental to dressing percent, and would make a good candidate for further research on tannin effects of food borne pathogens and possibly meat product stability.

CHAPTER V

SUMMARY

The results of this dissertation indicate that residual feed intake is negatively related to diet and nutrient digestibility in growing Brangus and Santa Gertrudis beef calves such that more efficient animals have higher dry matter, NDF, ADF, CP, and various mineral apparent digestibilities. Low RFI calves also have lower methane emissions than high RFI calves, resulting from both decreased dry matter intake and increased diet digestibility. This confirms previous studies conducted at this location and around the world. Calves with divergent RFI also have slightly altered VFA profiles. Low RFI calves have lowered propionate concentrations and higher acetate:propionate ratio in the rumen compared to high RFI calves. Also, no differences were found in 3 h MPA in the first study. In study 2 (microbial ecology), no difference was detected in ruminal 3 h and 24 h methane producing activity (MPA) between low and high RFI calves. There was a RFI group * time interaction for fecal MPA such that at the 3 h sampling time, both phenotypes had very low MPA. At the 24 h sampling time, however, low RFI steers had higher MPA compared to high RFI steers. Microbial ecology is also different amongst low and high RFI calves. Low and high RFI calves share similar proportions of Firmicutes and Bacteriodetes but do exhibit subtle differences in microbial ecology. Low RFI calves have increased *Prevotella* spp. when compared to high RFI calves. *Prevotella* spp. are a major H^+ producer and increased H^+ production in the lower gastrointestinal tract may account for increased fecal 24 h MPA

in low RFI calves. Ultimately, many mechanisms interact together to result in a favorable RFI. These data indicate that diet and nutrient digestibility serve to explain a portion of the inter-animal biological variation that is observed phenotypically, as well as decreased methane emissions. Microbial ecology is responsive to RFI and understanding how the gut microbiome interacts with host efficiency and physiology is a pertinent research area for the future.

Tannin supplementation had no negative effect on animal performance, efficiency, ruminal fermentation parameters, and carcass and non-carcass traits in finishing steers at 1.5% of the diet dry matter. The lack of negative effect on economically important traits makes tannins a viable carcass intervention to decrease food borne pathogen associated illnesses. This research was able to demonstrate no detrimental effects of tannin supplementation, further bolstering the need for future research into the effectiveness of tannins as a feed-though product to control host gut pathogens. Additionally, no negative health effects were observed in vivo due to either hydrolysable or condensed tannins supplementation.

Further research is needed to fully understand the relationship between RFI and digestibility. Future research could include gut enzyme gene expression and protein quantification in low vs. high RFI calves, absorptive capacity in various parts of the gastrointestinal tract (i.e. villi length and density in the small intestine or VFA absorption in the rumen) in divergent RFI calves, and other physiological factors, such as gut volume, passage rate, and rumen dilution rate, that may affect RFI need to be examined. In addition to animal factors, rumen microbiome perturbations between high

and low RFI animals need to be examined. Static microbial ecology should not be the only focus of future research, but also variables such as microbial protein production, inter-species hydrogen transfer and microflora and microfauna interactions between high and low RFI calves need attention.

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