5-2013

Growth Performance and Nutrient Metabolism of Pasture-Finished Beef Steers and In Vitro Fermentation Characteristics of Pasture Forages in Continuous Cultures

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GROWTH PERFORMANCE AND NUTRIENT METABOLISM OF PASTURE-FINISHED BEEF STEERS AND IN VITRO FERMENTATION CHARACTERISTICS OF PASTURE FORAGES IN CONTINUOUS CULTURES

by

Cuk Tri Noviandi

A dissertation submitted in partial fulfillment
of requirements for the degree
of
DOCTOR OF PHILOSOPHY
in
Animal Science

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2013

ABSTRACT

Growth Performance and Nutrient Metabolism of Pasture-Finished Beef Steers and In Vitro Fermentation Characteristics of Pasture Forages in Continuous Cultures

by

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A 2-year grazing study was conducted to evaluate the growth performance, ruminal fermentation, carcass characteristics, and fatty acid compositions in subcutaneous adipose tissue of beef steers grazing tall fescue (*Festuca arundinacea* Shreb.; **TF**) pastures without or with N fertilization. Nitrogen fertilization increased crude protein concentration of TF pasture and average daily gain of beef steers. Increase in total volatile fatty acids (**VFA**) and ammonia-N (**NH_3-N**) concentrations were detected in steers grazing fertilized TF. In comparison with steers on feedlot, pasture-finished steers had greater proportions of *cis*-9, *trans*-11 CLA and C18:3 n-3, but lower n-6:n-3 ratio in adipose tissue.

In the first in vitro study using 2 energy supplements [corn or dried distillers grains with solubles (**DDGS**)] and 4 pasture mixture forages [TF without or with N fertilizer (**TF-NF** or **TF+NF**), TF-alfalfa mixture (**TF+AF**), and TF-birdsfoot trefoil mixture (**TF+BT**)], we found that corn supplementation increased total VFA and propionate

concentrations, while DDGS supplementation decreased total VFA concentrations. Lower NH_3-N concentration and methane (**CH_4**) production were observed due to energy supplementation, in particular when corn grain was supplemented. Similar NH_3-N:VFA ratios were detected in the cultures fed the TF+NF and the TF+BT. This result indicates that the TF+BT had similar fermentation efficiency on in vitro ruminal metabolism compared with the TF+NF.

The second in vitro study was performed to investigate the effects of grass-to-legume ratios of 3 different TF-legume mixed diets on in vitro fermentation characteristics in continuous cultures. Propionate concentration increased with the increasing of legume proportion in the mixed diets. The greatest propionate concentration was shown by cultures fed the TF+CM, while the TF+AF and the TF+BT maintained a similar propionate concentration. Increasing legume proportion in the forage diets also increased NH_3-N concentration, but decreased CH_4 production in the cultures. Further decrease of CH_4 production was recorded when the TF+BT was fed to the cultures.

Overall results from the grazing study demonstrate that N fertilizer can improve nutrient quality of TF as well as growth performance of grazing steers, while the in vitro studies showed positive effects of grass-legume mixture diets on in vitro microbial metabolism by improving ruminal fermentation and reducing CH_4 production.

(213 pages)

PUBLIC ABSTRACT

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ACKNOWLEDGMENTS

First, I have to address my great thanks to my advisor, Dr. Jong-Su Eun, who dedicated his life and time to educate me, so I can become a better scientist and a better person. I will never forget those good hours (as well as those bad hours) in his office, when he tried very hard to make me be able to "see beyond the mountain" and repeatedly asked me to always "think about it!" I appreciate his trust in my abilities, regardless of all failures and mistakes I made. I believe I could not go through this PhD program without his guidance and continuously help.

Second, I deeply appreciate all help and support from my committee members: Dr. Dale R. ZoBell, Dr. Blair L. Waldron, Dr. Robert E. Ward, and Dr. Allen J. Young. Their contributions are not limited to giving advice and checking my papers only, but they also challenged me to think beyond my knowledge and interests to broaden my view in my journey to become a professional scientist.

I would like to address my appreciation to the Directorate General of Higher Education, Ministry of Education and Culture of Indonesia, for offering me the scholarship during my PhD program. I also thank the Utah Agricultural Experimental Station Pasture Grants Program and Western Sustainable Agriculture Research and Education (WSARE) Grants Program for allowing me to join their excellent program as a part of my PhD research.

To all graduate students at the Ruminant Nutrition Lab., I am really glad to know them all. Their friendship, help, and support can only be paid back by God Almighty. Special thanks for Juan and Katie, for being such a great brother and sister during my stay in Logan. I am waiting your visit to Indonesia!

And last, but not least, I dedicate this dissertation to my mom, my wife, and my kids. Without their endless prays and support, I believe I would not be able to go through the most difficult part of my life these last 3.5 years. Special thanks for my beautiful wife for her endless love, support, and sacrifice during my absence. She is really the best woman I have ever known! For my beloved son and daughter, Athif and Bunga, I wish one day their achievements will be greater than what I have today!

Logan, June 11, 2013

Cuk Tri Noviandi

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LIST OF ABBREVIATIONS

ADF = acid detergent fiber

ADG = average daily gain

AF = alfalfa

BSA = bovine serum albumin

BT = birdsfoot trefoil

BW = body weight

CH_4 = methane

CLA = conjugated linoleic acids

CM = cicer milkvetch

CP = crude protein

CT = condensed tannins

DDGS = dried distillers grains with solubles

DM = dry matter

DMI = dry matter intake

EE = ether extract

FA = fatty acids

FLT = feedlot treatment

G:F = gain-to-feed

GEI = gross energy intake

GHG = green house gas

HT = hydrolysable tannin

iNDF = indigestible NDF

IVDMD = in vitro dry matter digestibility

LDL = low density lipoprotein

LSU = large sub-unit

NDF = neutral detergent fiber

NFC = non-fibrous carbohydrates

NH_3-N = ammonia nitrogen

OM = organic matter

PUFA = polyunsaturated fatty acid

RDP = rumen degradable protein

RPM = rising plate meter

RUP = rumen undegradable protein

SFA = saturated fatty acids

SSU = small sub-unit

TF = tall fescue

TMR = total mixed ration

TVA = *trans* vaccenic acid

UFA = unsaturated fatty acids

VFA = volatile fatty acids

CHAPTER 1

INTRODUCTION

Forage-based beef cattle producers in the Western United States currently face 2 large challenges; first, they must demonstrate that their production methods are compatible with the desires of the American public, promising ecologically and environmentally sound operation, and second, they must remain economically viable in times of increased competition. Consequently, one of the major objectives of the forage-based cattle system is to develop a sustainable farming system with environmentally-friendly production management. Efficient use of nutrients is one of the major assets of sustainable agricultural production systems, because inefficient nutrient use not only results in excessive and potentially harmful losses to the environment but also affects economic performance (Oenema and Pietrzak, 2002). For instance, in ruminants fed high quality fresh forage diets, most proteins are rapidly solubilized releasing between 56 and 65% of the N concentration in the rumen during mastication; consequently large losses of N occur (25 to 35%) as ammonia is absorbed from the rumen (Min et al., 2000). As forages are the foundation upon which good forage-based cattle programs are built, it is important to identify sources of forage that will accomplish this goal with maximal nutritional efficiency while minimizing environmental concerns and economical costs.

For many years, tall fescue (*Festuca arundinacea* Schreb.; **TF**) has been important forage for beef cattle producers in the Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada). Tall fescue is known for its ease of establishment, wide range of adaptation of periodic drought, fluctuating seasonal temperatures, low fertility and low pH soils (Ball et al., 2002). However, it has been

reported that N fertilizer increased biomass production and crude protein (**CP**) concentration of TF (Berg and Sims, 2000; Teuton et al., 2007) and positively affected beef cattle performance (Berg and Sims, 1995). Unfortunately, N fertilizer application on pasture may not be efficient due to the rising price of N fertilizers in recent years and its negative impacts on the environment.

Interseeding legumes into grass stands may be an attractive alternative to N fertilizer due to their ability to fix atmospheric N through a symbiotic relationship with bacteria that infect the roots forming nodules. As the nodules slough off and decay over time, some of the N that has been fixed is released for uptake by the associated grasses. Similar to the N fertilizer, the added N from the decaying nodules can increase the yield and CP concentration of the grasses. Interseeding legumes into grass stands can also improve forage quality, because legumes are typically higher in CP concentration and digestibility compared with grasses. In addition, total productivity often increases, because the root systems of many legumes (e.g., alfalfa and red clover) occupy a different part of the soil profile, exploiting water and nutrients that would otherwise not be utilized by the grasses (Brummer et al., 2011). The broad leaves of legumes also help to increase capture of incoming solar radiation, especially in a grazing situation where plants are kept much shorter (Brummer et al., 2011).

Monounsaturated fatty acids (**FA**) and saturated FA comprise the largest percentages of FA in beef fat. Beef fats are also the richest natural sources of conjugated linoleic acids (**CLA**; Chin et al., 1992) and *trans*-vaccenic acid, which has been shown to have health benefits (Bhattacharya et al., 2006; Huth, 2007). Monounsaturated and C18:3 n-3 FA acid in reducing the risk of heart disease, whereas some saturated FA increase serum

cholesterol concentrations (Groff and Gropper, 1999). Benefit of pasture-finishing beef is an increase in the CLA concentration of beef (French et al., 2000; Steen and Porter, 2003; Realini et al., 2004). Conjugated linoleic acids are believed to have several health benefits; they have been shown in many animal studies to contribute to cancer prevention, decreased atherosclerosis, improved immune response, and altered protein or energy metabolism (Belury, 2002; Pariza, 2004; Palmquist et al., 2005). An increase in CLA and C18:3 n-3 FA in food products may be beneficial to consumer health. Thus, finishing beef steers on pasture diets may be one method of increasing these beneficial FA in animal products.

One problem with pasture systems is that they do not provide the proper combination of protein and energy to allow growing steers to gain weight at an optimum rate. It is well documented that steers finished on all forage diets have lower average daily gains (**ADG**) and take longer time to reach market weight when compared to those finished on a high concentrate diet in a feedlot (Steen et al., 2003; Jenkins et al., 2009; Faulkner et al., 2010). Numerous studies reported that addition of corn grain to a pasture finishing system can improve ADG (Reis et al., 2001; Pavan and Duckett, 2008; Duckett et al., 2009). However, starch-based energy supplementation, such as corn grain, has been reported to reduce forage intake and fiber digestibility (Pordomingo et al., 1991). It was reported that supplements containing highly digestible fiber do not depress forage intake or digestibility (Bowman et al., 2004).

In recent years, dried distillers grains with solubles (**DDGS**) has been used as a protein and energy source for grazing cattle. Recent studies reported that DDGS supplementation in forage-based diets increased ADG (Buttrey et al., 2012; Griffin et al.,

2012; Williams et al., 2012) and N utilization efficiency (Greenquist et al., 2011) without negatively affecting ruminal fermentation (Zhang et al., 2010; Islas and Soto-Navarro, 2011).

As pasture forages mature, there is decreased digestibility of nutrients with increased fiber and lignin concentrations in the forages (Minson, 1990). Reduced forage digestibility is accompanied by decreased forage intake and shift in ruminal fermentation such as an increased acetate-to-propionate ratio, which favors increased methane (**CH_4**) production per unit of forage consumed (McAllister et al., 1996). Methane production from cattle constitutes 2 to 12% of gross energy loss and is also a major contributor to atmospheric greenhouse gas emissions (Johnson and Johnson, 1995). As a result, strategies to reduce greenhouse gases from every sector of the animal industry have intensified in recent years. Many investigations that focused on controlling ruminant CH_4 emission from grazing beef has been accomplished (DeRamus et al, 2003; Chaves et al., 2006; Pinares-Patiño et al., 2007). For instance, McCaughey et al. (1999) reported that CH_4 emission from cows grazed grass-alfalfa pasture was lower compare to those only grazed grass (374 vs. 411 L/d, respectively). The CH_4 reduction was attributed to the secondary compounds in alfalfa (saponin), which reduced total protozoal count in the rumen, and thus decreased CH_4 production (Bhatta et al., 2009; Tan et al., 2011). This result indicates that improving pasture quality through interseeded legumes to the grass pasture can reduce CH_4 emissions from ruminants.

Other studies reported that feeding forages containing condensed tannins (**CT**) to ruminants may reduce CH_4 emissions (Waghorn et al., 2002; Woodward et al., 2004; Puchala et al., 2005). For instance, Woodward et al. (2004) showed that cows fed

birdsfoot trefoil (*Lotus corniculatus* L.; **BT**) exhaled less CH_4 by 13% per kg dry matter intake compared to those fed good quality ryegrass. In addition, animal trials have shown that the CT in temperate legumes may protect dietary protein from ruminal degradation and can increase absorption of essential amino acids from the intestine, resulting in improvement of animal performance (Waghorn et al., 1998; Waghorn et al., 1999). Condensed tannins also have inhibitory effects on biohydrogenation of FA in the rumen, which may be beneficial by increasing *trans*-vaccenic acid proportion in the rumen which is a major precursor to synthesize CLA in the adipose tissue and the mammary grand (Al-Soqeer, 2008; Khiaosa-Ard et al., 2009; Vasta et al., 2009).

Several forages containing CT are well adapted to growing conditions in the western United States and show potential to be used for grazing. Birdsfoot trefoil and cicer milkvetch (*Astragalus cicer*; **CM**) are non-bloating forage legumes that are similar to alfalfa in feeding value. Alfalfa contains low concentration of CT, whereas BT has high concentration of CT. Although CM does not contain CT, it possesses a unique plant structure that alters microbial digestion in the rumen; the rate of microbial digestion is decreased by its thick epidermal layers and vein pattern of the leaf (Lees et al., 1982).

Despite some advantages of the grass-legume mixed pastures, they have still some limitations. Perhaps the single largest limitation is their poor stand persistence during the grazing season. For instance, BT seedlings are small, slow to establish, and cannot tolerate much competition, while TF is considered to be an aggressive and competitive forage grass in mixtures with BT (Beuselinck et al., 1992). Competition for light with the grass canopy is a major factor limiting successful BT establishment with grasses. Wen et al. (2002) found that there was a rapid decline in total yield of BT monoculture during

spring to fall grazing season, which led to decreased proportion of BT in TF and BT mixed pasture from 31.5 to 13.5%. In addition, the significant change in production and proportion of BT also negatively affected the total weight gain of the steers (Wen et al., 2002).

Overall hypothesis in a series of grazing and in vitro studies reported in this dissertation was that fertilizing TF with N would improve ruminal fermentation and performance of beef steers. In addition, supplementation of energy sources in TF grass monocultures or CT-containing grass-legume mixtures (TF and BT) would improve in vitro ruminal fermentation by increased N utilization and decreased CH_4 production in continuous cultures. The objectives of this research were to: 1) evaluate relationships between application of N fertilizer to pasture grasses and its impacts on the ruminal fermentation profiles and growth performance of beef steers; 2) assess beneficial effects of grazing steers by comparing the adipose tissue FA profiles between pasture- and feedlot-finished beef steers; 3) investigate effects of corn or DDGS supplementation in high-forage diets on ruminal fermentation characteristics in continuous culture system; and 4) evaluate ruminal fermentation characteristics in continuous culture system fed fresh sources of TF-legume mixed forages combined with different ratio of TF-legumes.

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CHAPTER 2

REVIEW OF LITERATURE

Considerations for Pasture-Based Beef Production

The ability to produce high-quality beef to fulfill increasing demand for naturally raised animal products while minimizing input costs is an ongoing challenge for beef producers. One of the challenges facing producers in applying grazing systems in dry areas, such as the Intermountain West, is maintaining the forage quantity, particularly during the grazing season. As forage matures with the progression of grazing season, crude protein (**CP**) concentration and digestibility decrease, whereas fiber and lignin concentration increase (Johnson et al., 1998). Nitrogen, especially rumen degradable protein, can be deficient in pasture forages consumed by cattle in the late grazing season (Cline et al., 2009). This suggests that intake and forage quality may decline with advancing grazing season.

Producers may rely on N fertilizer to improve forage dry matter (**DM**) production and persistence of grasses. However, interest in substituting legumes for N fertilizer in beef cattle grazing systems has recently increased with rising fertilizer prices. Including forage legumes in grass pasture mixtures has increased pasture quality and livestock average daily gain (**ADG**; Popp et al., 1999). Ruminants grazing legumes vs. grasses displayed faster growth and increased productivity per unit of land area (Popp et al., 1999; Wen et al., 2002; Mouriño et al. 2003). Campbell (1981) found similar increases in total DM yield in a study comparing grass and grass-alfalfa pastures. Although alfalfa only made up 15 to 25% of the sward, the mixed pasture resulted in increased production

compared with the grass pasture by nearly 35%. Legumes also provide N fixing capabilities to the pasture system, which reduces the amount of fertilizer producers are required to apply on the pasture and decreases production costs. Additionally, environmental and health concerns, food safety, and sustainability issues also have encouraged beef producers to look for alternative methods of production that may keep the industry viable.

Performance of Pasture-Finished Beef Steers

It has been known that pasture-finished steers usually have lower ADG than those finished on a high-energy grain diets. In early research accomplished by Bowling et al. (1978), there was evidence that steers fed grain in a feedlot reached slaughter weight (518 kg) in 100 to 230 d sooner than those finished on forage systems. However, in a later study, Elizalde et al. (1998) reported that lower ADG and final body weight (**BW**) of steers grazed tall fescue (*Schedonorus arundinaceus* Schreb.; **TF**) pasture compared to those with energy supplementation were detected only during the growing stage (0.64 vs. 0.74 kg/d and 302 vs. 309 kg, respectively), but no differences on ADG and final BW were observed on finishing steers (averaged 1.26 kg/d and 472 kg, respectively). Recently, Faulkner et al. (2010) reported that steers on pasture were 5 kg lighter and took an additional 10.5 d to reach a slaughter weight of 532 kg compared to those fed high-grain diets. However, the authors also noticed that pasture-finished steers had $35.50 lower feed cost and had a $37.50 lower total input cost per steer compared to those on high-grain diets (Faulkner et al., 2010). This finding suggests that although pasture-finished steers gained less ADG, they are more profitable than those raised on high-grain diets.

Ruminal Fermentation of Pasture-Finished Beef Steers

Low performance of ruminants fed a pasture-based diet has been reported to be a consequence of decreased feed intake and changes in ruminal fermentation (Bargo et al., 2002). Lardy et al. (2004) reported that beef steers fed medium-quality grass hay showed lower total intake (DM basis) compared to those fed with barley supplementation. The primary cause of physical limitation on intake on steers fed high forage diets is long retention time of the fibrous fraction of the diet. Although fiber is crucial to maintaining a healthy rumen environment, digestion of the fibrous feed fraction is slow and can increase ruminal retention time if particles cannot be broken down and passed from the rumen (Allen, 1997). In comparison between grasses and legumes, Smith et al. (1972) reported that grasses often have a greater proportion of potentially digestible neutral detergent fiber (**NDF**) to indigestible NDF. Grasses also have higher in vitro NDF digestibility than legume forages, but the rate of digestion of legume potentially digestible NDF is faster (Smith et al., 1972), resulting in an increased total amount of NDF digested per day in vivo. Thus, grass-legume mixture forages could increase DM intake (**DMI**) of grazing steers (McCaughey et al., 1999).

Effects of feeding high- vs. low-forage diets on ruminal fermentation have been extensively investigated (Hess et al., 1996; Fieser and Vanzant, 2004; Lee et al., 2006). High-forage diets decreased total volatile fatty acid (**VFA**) concentration (Hess et al., 1996; Fuentes et al., 2009), with greater acetate but lower propionate concentrations (Hess et al., 1996; Lee et al., 2006; Fuentes et al., 2009). Propionate is quantitatively the most important VFA precursor for glucose synthesis, and therefore has a major impact on hormonal release and tissue distribution of nutrients (Nagaraja et al., 1997), which is

particularly important to rapidly growing cattle. Consequently, lower propionate concentration in high-forage diets may reduce nutrient utilization for growth by grazing cattle.

Ruminal environment of steers grazing fresh grasses is often characterized by having a greater concentration of readily fermentable soluble CP and fiber (van Vuuren et al., 1991) compared with those consuming TMR. As a result, most dietary CP is rapidly solubilized, releasing between 56 and 65% of the N in the rumen. Consequently, large losses of N occur (25 to 35%) as ammonia-N (**NH_3-N**) in the rumen (Min et al., 2000). It has been reported that steers fed high forage diet resulted in greater ruminal NH_3-N concentration (Lardy et al., 2004; Lee et al., 2006) and lower efficiency of microbial protein synthesis (Lee et al., 2006). Because ruminal NH_3-N concentration is a result of a balance between production (proteolysis) and assimilation (de Visser et al., 1997), any efforts to maximize N utilization in the rumen should involve an optimal balance between those 2 metabolic processes. Thus, any efforts to decrease protein degradation in the rumen and increase N use by ruminal microbes may improve dietary N utilization (Hoover and Stokes, 1991).

Ruminants have the unique advantage of converting indigestible cellulose-rich plant material into meat, milk, wool, and leather, while they do not compete directly with humans for food. However, this process is inefficient, because methane (**CH_4**) is an unavoidable end-product formed during anaerobic microbial fermentation of carbohydrates in the rumen. Methane represents a dietary energy loss to the animals between 4 and 10% of gross energy with extremes of 2 and 15% (McCaughey et al.,

1997; Flachowsky and Lebzien, 2012), and thus reducing enteric CH_4 production may also improve feed efficiency in ruminants (Beauchemin et al., 2008).

In ruminants, production of CH_4 in the rumen is necessary to remove excess H_2 formed during the ruminal fermentation process. Methane production is greater when ruminants are fed high forage diets, as a result of increasing H_2 production during acetate and butyrate productions from structural carbohydrates (Blümmel et al., 1997). Hydrogen is then used in conjunction with CO_2 by rumen methanogens to form CH_4 (Blümmel et al., 1997). Methane produced by enteric fermentation in grazing cattle is of interest, because enteric CH_4 emission from ruminants accounts for 17 to 37% of anthropogenic CH_4 (Steinfeld and Wassenaar, 2007; Lassey, 2008) or 3 to 5% of global greenhouse gas emissions (Steinfeld et al., 2006). Thus, enteric CH_4 emissions from ruminants are considered as a strong contributor to various climate change scenarios associated with global warming.

Reducing CH_4 emissions from beef cattle by improving grazing management systems provides economic as well as environmental benefits. The best strategy for mitigation of CH_4 emissions by cattle is probably through enhancing the efficiency of feed energy utilization. Form and rate of dietary carbohydrate fermentation influence the relative proportions of total VFA produced during feed fermentation. Generally, diets rich in soluble sugars favor propionate production which results in lower CH_4 emissions (Moss et al., 1995; Hindrichsen et al., 2004). Therefore, starch or grain supplementation in grazing system could be a way of reducing ruminal CH_4 emissions. However, in pasture-based systems, CH_4 mitigation strategies that require daily supplementation in

basal diets are not feasible, and manipulation of pasture species composition seems to be a better alternative for the CH_4 mitigation strategies.

Feed quality of grass for grazing ruminants can be manipulated by increasing the leaf-to-stem proportion through grazing management. Growing grass together with legumes has been proven to increase the leaf proportion of the sward (Bélanger and McQueen, 1998) and improve forage quality and crop yield (Hoveland et al., 1997; Brummer et al., 2011). With increasing leaf-to-stem proportion, NDF digestibility increased with increased sugar availability from the fiber. In addition, legumes also contain secondary plant components (tannins and saponins) that can decrease CH_4 emissions (Animut et al., 2008; Guo et al., 2008). Recent studies (Waghorn et al., 2002; Woodward et al., 2004) have shown that the use of forages containing condensed tannins (**CT**), such as birdsfoot trefoil (*Lotus corniculatus* L.; **BT**), can reduce enteric CH_4 emissions. The reduction of CH_4 emissions from dairy cows fed BT ranged from 13 to 16% (Woodward et al., 2004).

Fatty Acid Profiles of Pasture-Finished Beef Steers

The argument that pasture-fed beef is more beneficial for human health than grain-fed beef has been raised among researches and consumers. Health professionals world-wide recommend a reduction in the overall consumption of saturated fatty acids (**SFA**), *trans*-fatty acids (**FA**), and cholesterol, while emphasizing the need to increase intake of n-3 polyunsaturated FA (Griel and Kris-Etherton, 2006; Kris-Etherton and Innis, 2007). This nutritional recommendation with regard to fat consumption is largely due to epidemiologic studies showing strong positive correlations between intake of SFA and the incidence of cardiovascular disease, which is believed as the result of increasing low-

density-lipoprotein cholesterol due to increasing SFA intake (Posner et al., 1991; Hu et al., 1997). However, more recent lipid researches (Kris-Etherton and Yu, 1997; Mensink et al., 2003) suggest that not all SFA have the same impacts on serum cholesterol. For instance, C12:0 and C14:0 can increase total cholesterol more than C16:0, whereas C18:0 has a neutral effect on total cholesterol concentration.

Recent research findings suggest that grass-only diets can alter the FA composition of beef. Scollan et al. (2001) and Realini et al. (2004) reported that beef produced from cattle grazing forages has greater concentrations of *trans*-vaccenic acid (**TVA**) and conjugate linoleic acids (**CLA**). Furthermore, other studies reported that grass-fed steers showed 2 to 3 times more CLA in their meat products than those fed in confinement on high grain diets (French et al., 2000; Rule et al., 2002). Health benefits, attributable to the actions of CLA, have been demonstrated by experimental animal models, including actions to reduce carcinogenesis, atherosclerosis, and diabetes (Ip et al., 1999; Kritchevskyet al., 2000; Steinhart et al., 2003). These findings suggest that grass-finished beef can produce a more favorable SFA than grain-fed beef.

Other SFA, C18:2 n-6 and C18:3 n-3, are also essential in human nutrition, thus their presence in food are critical to human health. Typical western diets tend to contain 15 to 17 times more n-6 FA than n-3, which may promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases (Simopoulos, 2008). Based on his paper review, Simopoulos (2002) concluded that n-6:n-3 ratio between 2:1 and 3:1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5:1 had a beneficial effect on patients with asthma, whereas a ratio of 10:1 had adverse consequences. These data indicate that the optimal ratio may vary with the

disease under consideration. Therefore, a lower ratio of n-6:n-3 FA is more desirable in reducing the risk of many of the chronic diseases of high prevalence in western societies.

Grass-finished beef consistently produces a higher concentration of C18:3 n-3 within the lipid fraction of the meat, while C18:2 n-6 levels were left unchanged, resulting in a more favorable n-6:n-3 ratio (Nuernberg et al., 2005; Leheska et al., 2008). In their study to determine the differences on FA composition between grass- and TMR-fed beef, Leheska et al. (2008) found that grass-fed beef had lower n-6:n-3 ratio than those on TMR diet (2.5:1 vs. 9.6:1, respectively). Furthermore, Noci et al. (2005) examined the FA composition of tissues from beef heifers grazed on grass for different lengths of time (0, 40, 99, or 158 day). The authors reported that increasing days of grazing linearly increased n-3 concentration of lipids in muscle tissue, resulting in a lower n-6:n-3 ratio (averaged at 1.7:1; Noci et al., 2005). These findings suggest that cattle fed high-forage diets can be beneficial for human health through lowering n-6:n-3 in their meat.

Pasture Forages in Intermountain West

The Intermountain West in the United States is a semi-arid (150 to 600 mm annual precipitation) and high-elevation (1,200 to 2,000 m) region lying roughly between 37 and 43°N latitude. Soils are calcareous and neutral to alkaline, and irrigation water is often moderately saline. Because most cool-season pasture grass cultivars were developed in a more humid region than a semiarid region (Waldron et al., 2002), a cautious selection of grass and legume cultivars that fit with the semiarid condition in the region should be considered.

Nitrogen Fertilization and Its Impacts on Forage Quality and Environment

For years, synthetic N fertilization has been widely adopted as a part of modern farming practices to increase forage production and animal productivity. It has been reported that N fertilization of cool-season grasses increased CP concentration and crop yield (Zemenchik and Albrecht, 2002), but it also reduced persistence of the grasses (Van Esbroeck et al., 1995). In addition, N fertilizer applied in excess of what the grass utilizes may lead to N leaching below the effective rooting zone (Stout and Jung, 1992), thus increasing N waste into the environment.

Although N fertilization has shown to affect both CP concentration and DM yield, research results on the effects of N fertilization on digestibility have been inconsistent. For example, Peyraud and Astigarraga (1998) reported that cell wall concentration was unaffected by N fertilization, because a decrease in CP concentration was compensated for by an increase in water-soluble carbohydrates. However, in their literature review, the authors also cited reports on both moderate rise and moderate fall in NDF concentration with decreasing N fertilization (Peyraud and Astigarraga, 1998). Bélanger and McQueen (1999) reported that N deficiency increased the NDF concentration of leaves, but had a lesser effect on the NDF concentration of stems. Nitrogen fertilization may affect the NDF concentration indirectly by delays or advances in maturity (Peyraud et al., 1997) and by altering the leaf-to-stem ratio (Bélanger and McQueen, 1998). The conflicting results from N fertilization on chemical composition and NDF degradation characteristics are probably caused by interactions between N fertilization and environment factors like soil, temperature, rainfall, and water (Peyraud and Astigarraga, 1998; Berg and Sims, 2000).

Synthetic N fertilizer may greatly increase crop yield, but at the same time applications can be inefficient. The amount of N applied as fertilizer to cool-season grasses is often in excess of plant uptake (Mosier et al., 2001), and the apparent plant N recovery rates range from 17 to 50% (NRC, 2000; Mosier et al., 2001). Some of the N taken up by plants remains in roots and other crop residues, and this fraction will not be included in the harvested N, and consequently the amount of N actually removed during harvesting can be as low as 35% (NRC, 2000). The inefficiency of synthetic N uptake contributes directly to environmental issues related to the over abundance of N compounds released into the environment, which can become air and water pollutants.

Tall Fescue

Rising beef based on grazing systems in semiarid areas, such as the Intermountain West, is challenging for producers due to limited water availability that required for pasture growth. Waldron et al. (2002) conducted a study to determine which species of cool-season grass would be best for intensively grazed pasture with limited water availability. They found that TF, orchardgrass (*Dactylis glomerata* L.), and meadow brome (*Bromus riparius* Rehm.) were the best for use under conditions where water may be limited, especially if DM yield would be the primary selection criteria (Waldron et al., 2002). In addition, the authors reported that TF had superior forage yield at all water levels compared with the other grasses (Waldron et al., 2002).

Tall fescue is native to Europe and was introduced to the United States in the 1800's (Hoveland, 2009). It soon became the most important pasture grass in the United States, covering approximately 15 million ha (Buckner et al., 1979). It is used as both turf- and forage-type grass in the United States, and researchers have developed improved TF

cultivars in recent years (Kelly et al., 2009). Today, TF is known as a high yielding perennial cool-season grass that is among the most stable in maintaining yield across varied irrigation levels (Waldron et al., 2002; Lauriault et al., 2005).

Drought is considered a major challenge for grass management in the Intermountain West. Cool-season grasses are commonly under drought stress during summer. However, due to its extensive root system, TF is more adaptive to drought, but it is also capable of surviving in poorly drained soils (Buckner and Cowan, 1973). Tall fescue has spring and fall growing seasons and can also be stockpiled for winter grazing. Additionally, TF has good adaptability to poor soil and heavy grazing, established easily, and pest-resistance (Panaccione et al., 2001). Tall fescue is also regarded as a high-quality cool-season forage due to its CP, digestible DM, and amino acid and mineral concentrations (Bush and Buckner, 1973). Therefore, TF can be considered as ideal forage for grazing system in the Intermountain West region.

Alfalfa

Alfalfa (*Medicago sativa* L.) is one of the oldest known domesticated forages, and has been used for more than 3,300 years (Bolton et al., 1972). Alfalfa is widely grown as a hay crop in the United States today and is known for its aggressive N fixing capabilities as well as providing high-protein, low-fiber forage for livestock (Barnes et al., 1988). Hoveland et al. (1997) reported that TF cultivars in mixture with alfalfa produced greater yields during warm seasons. Another study by Brummer et al. (2011) reported that grass-alfalfa mixture increased forage DM yield compared with grass fertilized with 89.7 kg N/ha by over 784.6 kg/ha. Due to its high yield, high quality of protein, and broad adaption, alfalfa is referred to as “Queen of the Forages” (Lacefield et al., 1987).

Alfalfa is one of the few types of forage with a potential to increase ADG of beef cattle by 1.0 to 1.5 kg/d (Popp et al., 1997; Schlegel et al., 2000). A grazing study using 3 different pasture compositions (TF only and TF combined with either alfalfa or red clover) was performed by Dierking et al. (2010). They found that although steers grazing mixtures with either alfalfa or red clover had lower final weight than TF only (456 and 480 kg vs. 476, respectively), greater ADG were noticed (0.30 and 0.40 vs. 0.24 kg/d, respectively). Hoveland et al. (1997) performed a grazing study to determine the relationship between pasture compositions and grazing pressure and their effects on beef steer performance. They observed that TF with alfalfa pasture improved ADG by 30% at low grazing pressure and 84% at high grazing pressure, although alfalfa only represented 6% of the available forage. The positive effects of TF and alfalfa pasture on ADG of steers as well as excellent stand persistence of the mixed pasture on grazing pressure indicate the potency of alfalfa as grazing pasture for beef steers.

Establishment of alfalfa pasture for grazing beef cattle has been limited due to its propensity to cause pasture bloat. Bloat on grazing ruminants is attributed to soluble protein in rumen fluid, which is produced by legume forages (Majak et al., 2003). The gas from the substrate mixes with digesta in the rumen and forms stable foam (Moate et al., 1997). This foam inhibits the opening of esophagus, thus trapping gases in the rumen, preventing eructation and hindering breathing. Elevated ruminal pressure eventually results in suffocation, if the pressure is not relieved through rumenectomy or administration of defrothing agents like poloxalene (Popp et al., 1999). Cattle are reported to be more susceptible to legume bloat than other ruminants, and the susceptibility of individuals varies widely (Colvin and Backus, 1988). According to

Lauriault et al. (2005), hungry animals that are turned into a pasture that contains a fresh stand of legumes are more likely to bloat. Bloat on ruminants grazing alfalfa can be prevented by a number of methods, including gradually adapting cattle to grazing alfalfa, adding grasses to alfalfa pastures, not turning hungry animals out on lush alfalfa, treating livestock with antifoaming agents, or providing mineral blocks containing surfactants (Howarth, 1988; Ball et al., 2002).

Although alfalfa is high in protein, the full benefit of alfalfa cannot be achieved due to its poor utilization by the animal. In the rumen, alfalfa protein is extensively degraded to NH_3-N, resulting in inefficient use of dietary N and thus increased N waste excretion into environment. Slowing the protein degradation rate of alfalfa in the rumen is difficult to address via the alfalfa plant. However, previous studies provided some evidences that some plant secondary compounds, such as CT, may improve the protein metabolism in the rumen by reducing protein degradation via formation of insoluble CT-protein complexes and decreasing the solubility of protein (Min et al., 2000).

Birdsfoot Trefoil

Birdsfoot trefoil is a tannin-containing legume that is well adapted to the Intermountain West of the United States (Williams, 2010). Birdsfoot trefoil is an excellent legume for increasing production of permanent pastures and can be used as an alternative to alfalfa due to its non-bloating characteristics (Marten et al., 1987; Wen et al., 2002). Birdsfoot trefoil is better adapted than alfalfa to soils that may be slightly acidic. It tolerates drought better than most clovers and is more tolerant to wet and infertile soils than alfalfa (Williams, 2010).

Birdsfoot trefoil has been reported to be similar to alfalfa in CP and fiber concentrations, with some tendency for fiber to be lower in BT. Based on a 3-year study, Cassida et al. (2000) reported that concentration of CP, NDF, and acid detergent fiber (**ADF**) in BT averaged 20.9, 35.9, and 29.5% DM, respectively, while alfalfa averaged 19.7, 39.0, and 28.4% DM, respectively. Williams et al. (2010) reported similar results, with 20.0 and 18.5% CP for alfalfa and BT, respectively, while NDF for alfalfa and BT were 40.8 and 35.8%, respectively.

Effects of feeding BT to ruminants on intake and animal performance have been extensively investigated. Owens et al. (2012) observed that feeding BT prior to TF to lambs enhanced intake of TF and resulted in greater nutrient intake. They suggested that CT in BT inhibited the effects of the alkaloids that limit intake of TF. In their 2-year grazing study, Wen et al. (2002) reported that although total forage production of BT was low, steers grazing pure stand of BT showed greater ADG and lower grazing days. Surprisingly, they also reported that ADG for steers grazing TF and BT mixtures did not differ to those grazing pure stands of TF, which was caused by the lower DM yield of TF and BT mixtures compared with that of pure stands of TF. The findings from these 2 studies suggest that the positive effects of BT on animal performance are dependent on feeding methods as well as its availability.

Stand persistence is the major limitation of BT. In early study, Beuselinck et al. (1984) observed substantial stand losses, reporting 68 to 90% reductions within the first 2 years of establishment, with the majority of stand loss occurring during the first year of grazing. However, a recent study showed 54% decrease in BT in pastures during first year of establishment (Brummer and Moore, 2000). Consequently, BT is sometimes

described as a short-term perennial. Beside problems with stand persistence, BT is not easily established and does not compete well with many forage plants and weeds. Reseeding at regular intervals by allowing the plants to blossom and set seed helps alleviate these problems (Wen et al., 2002).

Cicer Milkvetch

Cicer milkvetch (*Astragalus cicer* L; **CM**) is a perennial legume that is adapted to a wide range of growing conditions and does not contain CT (Acharya et al., 2006). It does not produce bloat in ruminant animals (Twidwell and Kephart, 2002) and has been grown mainly in the northern and central Rocky Mountains of the United States and western Canada (Stroh et al., 1972; Gabrielsen et al., 1985; Kephart et al., 1990). In some areas with long growing seasons, CM produces similar yields to alfalfa (Baldridge and Lohmiller, 1990). However, it was reported that CM produced 70 to 80% of the yield of alfalfa when grown in western Canada in dryland conditions (Johnston et al., 1975), but considerably out-produced alfalfa when grown under irrigation in Montana and New Mexico (Stroh et al., 1972; Melton, 1973). Townsend et al. (1978) reported satisfactory yields (16.2 tonne/ha), stand persistence, and forage quality when grown in Colorado. In addition, CM is also highly tolerant to high grazing pressure due to its vigorous root systems (Twidwell and Kephart, 2002; Tilley et al., 2008).

Wide variation in nutrient compositions among varieties of CM has been reported by previous researchers (Dahlberg et al., 1988; Acharya et al., 1996, 2006), ranging from 20.0 to 28.7% CP and from 31.0 to 44.8% NDF. For instance, CM cultivars ‘Oxley’ and ‘Monarch’ contained 28.0 and 21.7% CP and 41.4 and 36.1% NDF, respectively (Acharya et al., 1996). In comparison with alfalfa, Dahlberg et al. (1988) observed

similar CP levels in alfalfa and CM (26.0 and 28.7% of DM, respectively), while also observing decreased concentrations of NDF and ADF in CM (31.0 and 23.1% DM, respectively) when compared with alfalfa (41.8 and 27.5% DM, respectively). Cicer milkvetch has high CP concentration because of the high leaf-to-stem ratio, which is 40% greater than the leaf-to-stem ratio of alfalfa (Baldridge and Lohmiller, 1990).

Although CM does not contain any CT, it owns a unique plant structure that alters microbial digestion in the rumen. Lees et al. (1982) reported that CM leaves have bundle sheath cells surrounding every vein that connect secondary and tertiary veins to both the upper and lower epidermal layers of the leaf. These veins have a reticulate pattern that compartmentalizes the leaf, confining microbial digestion to discrete areas. Furthermore, the epidermal layers of the leaf are very thick, improving mechanical strength and reducing fragmentation. In contrast to CM, the epidermal layers of alfalfa are less resistant to microbial digestion, allowing the epidermal layers to lift away from leaflets in large sheets and exposing the mesophyll to rapid digestion (Lees et al., 1982). This difference in plant structures may explain the low ruminal degradation of CM.

Studies focused on the feeding value of CM on animal performance are very limited. In a 3-year study, Rumburg (1978) reported that although grass-CM pasture produced a lower yield of digestible energy, steers grazed on grass-CM pasture showed similar ADG with those on N fertilized grass. Marten et al. (1987) reported that although CM improved in vitro DM digestibility, the lower DMI of heifers grazing CM resulted in no increase in ADG when compared to heifers grazing alfalfa. This suggests that although CM may be an alternative for N fertilized grass pastures, its effects on animal performance are still less than that of alfalfa.

Grass-Legume Mixed Pasture

Benefits of legumes in livestock production systems are well documented (Wilkins and Jones, 2000; Frame and Laidlaw, 2005). These include N fixation, high nutritive value, increasing yield, and high voluntary feed intake. When grown together with grasses, legumes not only improve the soil quality, such as organic matter, porosity, structure, pH, and recycling nutrients, but also provide N for grasses (Sheaffer and Evers, 2007). In adjacent to that, the increasing fertilizer costs, changes in environmental protection legislation, and increasing demand of organic farming systems in recent years give rise to a greater interest of the use of legumes for ruminant feed (Rochon et al., 2004).

Interseeding legumes into grass-dominated stands can benefit pasture by increasing yield and quality of the forage as well as reducing the need of N fertilizer. Grass-legume mixtures have been reported to have more consistent forage yields across a wide range of environments compared with grass monocultures (Haynes, 1980). Legumes are also an option to replace fertilizer due to their ability to fix atmospheric N through a symbiotic relationship with bacteria that infect the roots forming nodules (Giller and Cadisch, 1995). As the nodules slough off and decay over time, some of the N that has been fixed is released for uptake by the associated grasses. Thus, increasing yield and CP concentration of the grasses due to the added N from the decaying nodules may be achieved (Brummer et al., 2011).

Legumes contain a high concentration of CP, which typically ranges from 15 to 23%, although in leaves CP concentrations can exceed 25%. These high concentrations of CP make legumes one of the most important sources of protein in animal diets. However, the

fibrous components of legumes are mostly concentrated in the stem of the plant, resulting in legume leaves being highly digestible. Fiber components found in legume stems are slightly less digestible than grass fiber due to a greater level of legume fiber lignification. However, legume fiber is greater in overall nutritive value despite its lignification due to increased solubility (Vasiljević et al., 2009).

One of the most important factors for using legumes in pasture systems is the legumes' impacts on animal performance. Various combinations between grasses and legumes have been reported to increase ADG of grazing steers (Hoveland et al., 1997; Clem, 2004). For instance, 6% alfalfa presence in TF stand improved ADG of grazing steers (Hoveland et al., 1997). In a 5-year grazing study, Clem (2004) reported that legume-grass mixed pastures showed an animal live weight gain of 120 kg/head/year in the first year of establishment, with improvement in the following 4 years, ranging from 160 to 200 kg/head/year. In addition, legume-based pastures also resulted in larger animals at a younger age due to high ADG, leading to a decreased period of grazing (Hill et al., 2009). The increase in animal performance on legume pastures when compared with grass pastures can be linked to the greater CP concentration and digestibility of the legumes (Bhatti et al., 2008; Hill et al., 2009). A pasture that contains legumes in conjunction with grass has the ability to provide more protein for a longer period of time. This prolonged protein supply helps legume-grass mixed pastures to outperform grass-only pastures in terms of animal production (Coates et al., 1997).

Another advantage of legume inclusion in grass pasture is associated with their ability to reduce enteric CH_4 emission. A cow-calf study comparing performance and enteric CH_4 emissions of grass-alfalfa and grass only pastures (McCaughey et al., 1999)

resulted with greater DMI and lower CH_4 production for cows grazing grass-alfalfa pastures than for grass-only pastures (11.4 vs. 9.7 kg/d and 0.53 vs. 0.58 g/kg BW/d, respectively). In addition, an 11% increase of calf growth rates on grass-legume pasture was reported. McCaughey et al. (1999) also reported a lower energy lost as enteric CH_4 emissions from calves on grass-legume pasture than those grazed on grass-only pastures (7.1% vs. 9.5% of gross energy intake, respectively). The lowered CH_4 loss observed with legumes is attributed to the lower proportion of structural carbohydrates and faster rate of passage of legumes, which can shift the fermentation pathway toward higher propionate production.

Energy Supplementation for Beef Cattle on Pasture

Desired levels of livestock performance are not always achieved when forage is consumed alone, because fresh forages commonly contain rapidly degradable protein but lack energy, resulting in low N utilization efficiency in the rumen. Therefore, diets based on forages often require energy supplementation to ensure optimal performance (Caton and Dhuyvetter, 1997). However, forage intake and digestibility may be negatively affected by the addition of cereal grains (Dixon and Stockdale, 1999). For instance, use of corn as an energy supplement often results in decreased forage intake and lower forage digestibility, especially with lower-quality forages (Pordomingo et al., 1991). Because the objective of feeding cattle with forages is to maximize utilization of available nutrients in the forages and decrease grain consumption, alternative forms of energy supplementation should be considered to achieve desirable production. It was reported that supplements containing highly digestible fiber did not depress forage intake or digestibility (Bowman

et al., 2004). Therefore, feedstuffs that are relatively high in fiber but also highly digestible, such as dried distillers grains with solubles (**DDGS**), may be a potential alternative source of energy supplementation for grazing cattle.

Corn Supplementation

Supplementing corn to grazing steers has been shown to be a practical method of shortening the finishing period of beef steers (Pavan and Duckett, 2008; Duckett et al., 2009). There is a body of evidence that corn grain supplementation positively affects performance of beef steers fed high forage diets. As an example, Goetsch et al. (1991) reported that yearling steers grazing annual spring forages with 0.5% ground corn supplementation had greater ADG than those without supplementation. On low-quality forage, corn supplementation increased efficiency of energy utilization, as forage quality declined (McMillin et al., 1990). These results suggest that animal performance on forage diets is usually limited by energy intake, and rate of gain is generally increased by energy supplementation.

As a negative effect, low forage intake due to corn supplementation has been widely reported, especially when corn supplementation is higher than 0.5% of BW (Pordomingo et al., 1991; Matejovsky and Sanson, 1995). As a nonstructural carbohydrate, corn grain starch is highly digestible (60 to 90%) in the rumen, and only a small portion of it passes on to the intestines for post-ruminal digestion (Allen, 1997; Owens and Zinn, 2005). Due to its high digestibility and fermentability, starch may create an acidic environment (pH of 5.7 to 6.2) in the rumen (van Vuuren et al., 2010). The low pH of the rumen reduces the ability of cellulolytic bacteria to degrade fiber in the forage and thereby lowers fiber digestion (Dixon and Stockdale, 1999), resulting in prolonged retention in the rumen and

hence lower forage intake. The extent to which fiber digestion begins to decline as a result of starch inclusion in a forage-based diet depends on various interrelated factors, including level and frequency of supplementation and processing method of grains. If supplemented at lower quantities, such as at 0.25% of BW, corn supplementation may not present the same negative associative effects on forage intake and digestibility compared when supplemented at level higher than 0.50% of BW (Sanson et al., 2004). Thus, when corn is considered as an energy supplement for grazing steers, it is important to apply the appropriate level of corn to avoid a decrease in forage intake and digestibility.

Corn grain has been proved to have positive impacts on performance of beef steers grazing on TF. Elizalde et al. (1998) reported that when supplemented with low levels of cracked corn, steers grazing TF resulted in a higher ADG compared to unsupplemented steers (0.74 vs. 0.64 kg/d, respectively). Similarly, a study on steers grazing TF pasture with corn supplement at a level of 0.4% BW caused a higher ADG than those grazing TF alone (1.01 vs. 0.82 kg/d, respectively; Judkins et al., 1997). Increased ADG, carcass weight, and subcutaneous fat thickness in carcasses have also been reported when steers grazing TF were supplemented with corn grain at 0.5% BW (Pavan and Duckett, 2008).

DDGS Supplementation

Dried distillers grains with solubles is a co-product of fuel ethanol production, which is known as an excellent source of both protein and energy. During ethanol production, starch of corn is mostly fermented to ethanol, and the other nutrients associated with the grain become more concentrated. For instance, CP increases from approximately 9% in the original corn grain to 27% in the whole stillage (Stock et al., 2000), whereas NDF concentration increases from 9 to 46%, and fat increases from 4.3 to 10.3% on a DM

basis (NRC, 2000). Because DDGS does not contain nonstructural carbohydrates, energy is supplied in the form of highly digestible fiber and fat, when DDGS is used as energy supplement (Stock et al., 2000). In addition, DDGS contains approximately 52% ruminally undegraded protein (**RUP**; NRC, 2000). Because DDGS contains high digestible fiber and RUP, it is considered as an ideal energy supplement as well as a protein supplement for growing steers (Patterson et al., 2003).

While supplementation with starch-based energy supplements, such as corn, can depress forage intake (Pordomingo et al., 1991; Doyle et al., 2005), supplementation with DDGS can lessen these negative associative effects. Leupp et al. (2009) performed a study using cannulated steers to assess the effects of DDGS supplementation on ruminal fermentation and digestion. They found that ruminal pH increased, as dietary DDGS increased, which is the result of decreased starch levels in DDGS compared with corn grain (Leupp et al., 2009). The other studies (MacDonald et al., 2007; Griffin et al., 2012) also reported an increase in ADG and carcass weight of grazing heifers and steers supplemented with DDGS.

Many studies have been performed to evaluate the optimal inclusion rate of DDGS in diets to improve animal performance. Buckner et al. (2008) observed an increase in gain-to-feed (**G:F**) ratio when cattle were fed levels of 0, 10, 20, 30, and 40% DDGS. A meta-analysis completed by Klopfenstein et al. (2008) showed a positive response in ADG and G:F as level of DDGS in the diet increased from 0 to 40%. They noted that the maximum ADG was between 20 and 30%, while maximum G:F ratio was observed between 10 and 20% DDGS (Klopfenstein et al., 2008).

The effects of DDGS supplementation in increasing ADG and carcass weight of the grazing steers have been reported (Greenquist et al., 2009; Griffin et al., 2012). Using 2 different levels of DDGS (0.6 vs. 1.2% BW), Griffin et al. (2012) found that ADG of steers grazing meadow grass increased at greater level of DDGS supplementation. Greenquist et al. (2009) performed a study to evaluate DDGS as a supplement for forage and as a substitution for N fertilizer in yearling steers grazing smooth bromegrass. They reported that steers offered 2.3 kg/d DDGS (DM basis) had greater ADG than those grazed meadow grass fertilized with 90 kg N/ha. Furthermore, when compared with non-fertilized pastures and non-supplemented steers, total BW gain per hectare was increased by 53% with N fertilization and by 105% with DDGS supplementation. These results suggest that DDGS positively affected animal performance and is also a potential source of substitution for N fertilizer on pasture systems.

In order to mimic the changes on forage quality during the grazing season, Morris et al. (2005) conducted a study using low- and high-quality forages supplemented with increasing levels of DDGS. They found that although forage intake decreased as DDGS increased, the ADG increased linearly with increasing DDGS supplementation. Since the 2 qualities of forage were selected to stimulate grazing-like conditions, the feed intake and ADG could be projected for cattle grazing pasture at different times of the year; for the spring/summer with the high-quality forage and fall/winter with the low-quality forage. The higher ADG and forage intake seen with the high-quality forage are similar to what would be observed on spring/summer range. In addition, they also observed that the rate of increase in gain was greater for low-quality diet with higher levels of DDGS supplementation, which indicate that higher levels of DDGS supplement is required

during fall/winter seasons in order to eliminate loss in ADG. Based on their economical analysis, Morris et al. (2005) also noticed that supplementation of DDGS at any level with either high- or low-quality forage appears to be more profitable than not supplementing at all. In addition, they noted that DDGS is valued higher with the low-quality than with the high-quality forage.

Effects of Condensed Tannins

Tannins are polyphenolic polymers of relatively high molecular weight with the capacity to form complexes mainly with proteins due to the presence of a large number of phenolic hydroxyl groups (Patra et al., 2012). They are usually classified into 2 major groups: hydrolysable tannins (**HT**) and CT. Hydrolysable tannins contain a carbohydrate as a central core and in most plants occur mainly in fruit pods and plant galls as protection against insect herbivory (Haslam, 1989). Condensed tannins are polymers of flavan-3-ol (catechin) or flavan-3, 4-diol (proanthocyandins) units linked by C4–C8 and C4–C6 interflavonoid linkages (Ferreira et al., 1999; Figure 2.1) and are not susceptible to anaerobic enzymatic degradation (McSweeny et al., 2001). Both HT and CT may be found in the same plant, but some plants may contain predominantly HT, whereas others contain CT (Haslam, 1989).

Condensed tannins are typically found in various plant species, including forage trees, shrubs, legumes, cereals, and grains. Generally, CT concentrations are greater in vulnerable parts of the plants such as new leaves and flowers (Frutos et al., 2004), and various factors such as temperature, light intensities, water and nutrient stress, soil

quality, and topography influence the concentrations of tannins in plants (McMahon et al., 2000; Frutos et al., 2004).

Condensed tannins form complexes with many compounds, including protein and carbohydrates (cellulose, hemicellulose, and pectin) to form stable complexes (Barry and McNabb, 1999). Condensed tannins bind protein by hydrogen bonding at near neutral pH (pH 6.0 to 7.0) in the rumen to form CT-protein complexes, and then dissociate and release bound protein at pH less than 3.5, as they enter the abomasum and small intestine (Barry et al., 2001; Mueller-Harvey, 2006). Therefore, CT-containing plants can protect dietary protein against degradation in the rumen and increase N utilization, resulting in an increase in dietary N flow into small intestine, decreased nitrogenous waste excretion, and improved nutritional status of the animal (Figure 2.2). Due to their ability to bind protein, feeding CT-containing forages in beef diets is a promising means to improve N utilization and reduce excretion of nitrogenous waste by beef steers.

In the past, CT was primarily considered as anti-nutritional biochemicals due to their adverse effects on feed intake and nutrient utilization (Kumar and Vaithiyanathan, 1990). However, in recent years, they have been recognized as useful phytochemicals for beneficially modulating rumen microbial fermentation, such as decrease in ruminal protein degradation (Min et al., 2000), inhibition in methanogenesis (Animut et al., 2008; Patra and Saxena, 2010), and modification in biohydrogenation of FA in the rumen (Khiaosa-Ard et al., 2009).

Nitrogen Metabolism

Two of the major factors determining the productivity of ruminant livestock are the quantity and the quality of protein flowing to the intestine. When ruminants are fed fresh

forages containing high concentration of N (2.5 to 3.5% DM), most of the proteins become rapidly soluble in the rumen and are degraded by rumen microbes, resulting in surplus levels of NH_3-N in the rumen (20 to 35%), which is absorbed from the rumen and excreted in urine (Ulyatt et al., 1975). The flow of protein from the rumen to abomasum depends on proteolysis by rumen microbes and the efficiency of microbial protein synthesis in the rumen. Therefore, reducing ruminal protein degradation may allow more protein to reach the abomasum and small intestine, thus avoiding possible losses of amino acids in the rumen.

Protein degradation in the rumen should provide only the necessary amount of N to meet microbial requirements, improving bacteria growth and consequently nutrient digestion. Several studies have reported that losses of N may be reduced by decreasing protein degradation in the rumen (Atkinson et al., 2007; Pina et al., 2009). In comparison between 2 BT cultivars containing 0.25 and 1.45% CT, John and Lancashire (1981) found that the cultivar containing greater CT decreased protein solubility and NH_3-N concentration in the rumen. Other studies (Waghorn and Shelton, 1997; Makkar, 2003) reported that low concentrations of CT could increase dietary RUP fraction flow into abomasum and intestine. This increase occurs due to the capacity of CT to bind protein through hydrogen bonds, forming a CT-protein complex which is stable in the rumen (pH 5.0 to 7.0) and resistant to microbial degradation. Consequently, CT-protein complexes reach the abomasum where they are dissociated due to the difference in pH (Mezzomo et al., 2011). Thus, the animal can use dietary protein more efficiently.

An increase in dietary protein flow to the abomasum may also be due to the inhibitory effects of CT on proteolytic bacteria and proteolytic enzyme activity. Jones et

al. (1994) studied the protease activity of 4 strains of ruminal bacteria as affected by CT from sainfoin (*Onobrychis viciifolia*). Total protease activity in cultures of *Butyrivibrio fibrisolvens* and *Streptococcus bovis* was reduced by 48 and 92%, respectively, at a concentration of 25 mg of CT/L. In contrast, the total protease activity in cultures of *Provotella ruminicola* was 36% higher in the presence of 100 mg of CT/L than in the control. Using CT extracted from BT and big trefoil (*Lotus pedunculatus*), Molan et al. (2001) reported that CT from BT reduced the growth of 5 major strains of ruminal proteolytic bacteria (*S. bovis*, *Eubacterium* sp., *Prevotella bryantii*, *B. fibrisolvens*, and *Clostridium proteoclasticum*) when used at a level of 600 µg/mL, although the CT from big trefoil proved to have greater inhibitory effects. Min et al. (2002) reported that when the diet was changed from perennial ryegrass/white clover pasture (which does not contain CT) to BT (3.2% CT) in sheep, populations of the proteolytic rumen bacteria *C. proteoclasticum*, *Eubacterium* sp., *S. bovis*, and *B. fibrisolvens* decreased. These studies suggest that CT not only reduce protein degradation in the rumen by binding with protein, but also altered the microbial population, particularly those strains most involved in ruminal proteolysis.

Research focused on the factors that can affect the reactivity between CT and proteins has been performed. McNabb et al. (1998) found that CT extracted from BT and big trefoil had a much greater affinity for Rubisco extracted from white clover than for bovine serum albumin protein at pH 7.0. It was suggested that proteolysis of the large sub-unit of Rubisco from alfalfa occurred relatively fast, but that the small sub-unit of Rubisco was more resistant to ruminal degradation (McNabb et al., 1998). Min et al. (2000) reported that the large sub-unit (MW 54 kDa) of Rubisco was consistently

solubilized and degraded faster than the small sub-unit (16 kDa), and CT extracted from big trefoil had more effect on reducing the degradability of the large sub-unit (Aerts et al., 1999). These results suggest that the reactivity between CT and protein depends on the source of CT as well as the type of substrates.

Ruminal Methanogenesis and Methane Emissions

One of the beneficial effects associated with CT is the reduction of ruminal CH_4 production. Methane is produced by methanogens in the rumen during anaerobic fermentation of soluble and structural carbohydrates contained in forage-based diets (Waghorn et al., 2002; Tavendale et al., 2005). Methane produced during anaerobic fermentation in the rumen represents 3 to 9% feed energy loss and contributes to greenhouse gas emissions in the environment (Woodward et al., 2001). Therefore, reducing CH_4 emissions has been of great interest for ensuring sustainability of ruminant production.

Tannin-containing forages and CT extracts have been demonstrated to decrease CH_4 production both in vivo and in vitro. For instance, diets containing CT from BT at levels of 2.6% DM resulted in decreased CH_4 emission in dairy cattle (Woodward et al., 2001), while similar in vitro reduction on CH_4 production due to the presence of CT originated from BT was reported (Williams et al., 2011). On grazing cows, a 66% reduction of CH_4 from cows grazed BT pasture has been reported (Woodward et al., 2004). The inhibitory effects of CT on rumen methanogenesis have been attributed to direct effects on methanogenic archaea, protozoal associated CH_4 production, and indirectly through a depression of fiber digestion in the rumen (Patra et al., 2012).

Several factors can affect the inhibitory action of CT on methanogenesis in the rumen. Tavendale et al. (2005) demonstrated the inhibitory effect of CT from big trefoil on the growth of a pure culture of the common rumen methanogens *Methanobrevibacter ruminantium* strains YLM-1 and DSM 1093. They found that low molecular weight of CT completely inhibited CH_4 production for the DSM 1093 and decreased CH_4 production by 65% for the YLM-1. Field et al. (1989) also found that due to low molecular weight CT could form a strong binding with microbial enzymes, thus making them a more effective inhibitor of methanogens compared with high molecular weight CT. It has been suggested that high molecular weight CT are not able to penetrate into bacterial proteins, causing lower toxicity of methanogens (Field et al., 1989). Results from these studies suggest that the direct inhibitory effect of CT on methanogens depends on the chemical structure of CT and methanogen species.

Direct inhibitory effect of CT on the growth of methanogens also depends on type and dose of CT. Using mixed rumen cultures, Bhatta et al. (2009) observed that inclusion of a quebracho (*Schinopsis lorentii*) tannin that contained 3.94% HT and 1.33% CT at 5.0% of mixed substrates did not reduce rumen methanogen populations, while at 10.0% of substrates caused an inhibitory effect on methanogens, but no further inhibitory effect was detected when the concentration increased up to 25.0% of substrates. Another quebracho tannin sample containing 7.62% HT and 3.67% CT inhibited methanogens at 5.0% of substrates and further inhibitory effects were noted at 25.0% of substrates (Bhatta et al., 2009). Using mixed rumen cultures collected from goats, Animut et al. (2008) found that replacing 33 to 100% of sorghum-sudan grass with *Lespedeza striata*

(15.1% CT) progressively produced less CH_4, indicating a decrease in methanogen populations by *L. striata*.

Although toxic effects of CT to rumen protozoa appear to be less pronounced and vary among studies, anti-protozoal properties of CT from different plants have been reported. Using Rusitec system, Makkar et al. (1995) reported that quebracho CT (0.1 to 0.4 mg/mL media) reduced the numbers of total protozoa (both entodiniomorphs and holotrichs) with the effect being higher on holotrichs. Out of the 15 tree fodders, Monforte-Briceno et al. (2005) found inhibitory effect of sweet acacia (*Acacia farnesiana*), *Calliandra calothyrsus*, and false tamarind (*Lysiloma latisiliquum*) on protozoa occurs when CT concentration was higher than 1% DM. Lower total protozoa due to CT extracted from myrobalan (*Terminalia chebula*) or kobe lespedeza have been reported (Patra et al., 2006; Animut et al., 2008). Because protozoal associated methanogenesis can account for about 37% of rumen methanogenesis (Finlay et al., 1994), the anti-protozoal effects of CT may be beneficial for improving protein utilization and inhibiting methanogenesis in the rumen. However, the mechanisms of inhibition of rumen protozoa by tannins are not clearly known, yet.

Besides directly inhibiting methanogenic archaea in the rumen, CT have also shown indirect effects by reducing fibrolytic bacterial populations in the rumen, thus reducing the fiber digestion, and decreasing fiber digestibility. A number of studies showed that tannins also selectively inhibited the growth of fiber degrading microbes in the rumen. For instance, addition of 2 to 3% CT from *Calliandra* in the diet (2 to 3% tannin) reduced the population of *F. succinogenes* and *Ruminococcus* spp. (McSweeney et al., 2001). Feeding of *Ficus infectoria* leaves containing 8 to 12% CT at 50% of the diet to goats did

not decrease the number of *F. succinogenes*, but reduced the number of *R. flavefaciens* (Singh et al., 2011), while CT extracted from BT inhibited the growth of *F. succinogenes* at a concentration of 400 mg/L, but had no noticeable effect on growth at concentrations below 400 mg/L (Bae et al., 1993). Condensed tannins of BT also reduced the populations of *C. proteoclasticum*, *B. fibrisolvens*, *Eubacterium* spp. and *S. bovis* in sheep (Min et al., 2002). These studies showed that the capability of CT in inhibiting the major fiber degrading bacteria in the rumen, such as *F. succinogenes*, *R. albus* and *R. flavefaciens*, and their response to CT depend on the dose and type of CT.

Biohydrogenation of Fatty Acids

Ruminal microbial community is responsible for the biohydrogenation of the dietary unsaturated FA (**UFA**) ingested by ruminants, which result in the production of SFA. During the biohydrogenation process, many intermediates are formed in the rumen; however, the 2 compounds that are important to final levels of CLA in ruminant products are 18:2 *cis*-9 *trans*-11 CLA isomer and C18:1 *trans*-11 TVA (Figure 2.3). While CLA present in the rumen is generated only during the biohydrogenation of C18:2 n-6, TVA is synthesized during the hydrogenation of both C18:2 n-6 and C18:3 n-3. Because up to the 90% of CLA found in meat and milk is originated from the desaturation of TVA in the adipose tissue or in the mammary gland by Δ^9-desaturase (Santora et al., 2000; Piperova et al., 2002), the CLA synthesized during ruminal biohydrogenation contributes little to the overall CLA concentration in meats and in dairy products. Therefore, the best strategy to increase CLA concentration in meat and milk is by enhancing TVA formation in the rumen and its uptake in the duodenum, rather than increasing CLA post-ruminal absorption.

Many ruminal bacteria species have been involved in ruminal biohydrogenation, including species of the genera *Butyrivibrio*, *Ruminococcus*, *Treponema-Borrelia*, *Micrococcus*, *Megasphaera*, *Eubacterium*, *Fusocillus*, and *Clostridium* (Harfoot and Hazlewood, 1997; Maia et al., 2007; Durmic et al., 2008). *Butyrivibrio* group are most active species among the group A bacteria, which form CLA from C18:2 n-6, while few species of bacteria such as *Fusocillus* spp. and *C. proteoclasticum* (group B) convert TVA to C18:0 (Maia et al., 2007; Paillard et al., 2007; Durmic et al., 2008; Figure 2.4). Therefore, selective suppression of group B bacteria without affecting group A bacteria would provide more UFA, including TVA and CLA, escaping the rumen to be absorbed and incorporated into animal tissues.

In recent years, the ability of plant extracts including CT to modify the FA composition of ruminant-derived food products (i.e., milk and meat) has received great attention. Some studies (Jones et al., 1994; Molan et al., 2001) have shown that CT from different legume forages inhibit cell growth and division of ruminal microorganisms, particularly *B. fibrisolvens*, that are responsible for ruminal biohydrogenation (Jenkins et al., 2008). In vitro incubation of CT extracted from *Acacia mearnsii* (Khiaosa-Ard et al., 2009) or quebracho (Vasta et al., 2009) in the ruminal fluid inhibited the last step of the biohydrogenation, thus leading to the accumulation of TVA at the cost of C18:0. Durmic et al. (2008) reported that when CT extracts from *Acacia iteaphylla* were incubated in ruminal fluid from sheep, the production of TVA increased, while C18:0 decreased. Similar trends of FA in ruminal fluid were also observed in in vivo studies with lambs supplemented with quebracho tannins (Vasta et al., 2009, 2010). These results suggest

that inclusion of legumes containing CT in diets may affect ruminal biohydrogenation by elevating TVA proportion in the ruminal fluid.

Several studies have been performed to evaluate the selective inhibitory effect of CT. It has been reported that CT from BT (Min et al., 2002) or from *Acacia* spp. (Durmic et al., 2008) reduced the proliferation of *C. proteoclasticum* B316 T and *C. proteoclasticum* P18, respectively. Using CT extracted from carob (*Ceratonia siliqua*), acacia leaves (*Acacia cyanophylla*), and quebracho, Vasta et al. (2009) found an increase in TVA and a reduction in C18:0, suggesting an alteration in the activity of microorganisms. Therefore, it is likely that different bacterial strains may have been differently sensitive to tannins. Furthermore, Durmic et al. (2008) tested the inhibitory power upon biohydrogenating bacteria of large number of plants containing secondary compounds, most of which also contained CT. They found that the minimum dose of plants needed to inhibit the proliferation of *B. fibrisolvens* was much greater than the dose needed to inhibit *Cl. proteoclasticum*, suggesting that *Cl. proteoclasticum* is more sensitive to tannins than *B. fibrisolvens* (Durmic et al., 2008).

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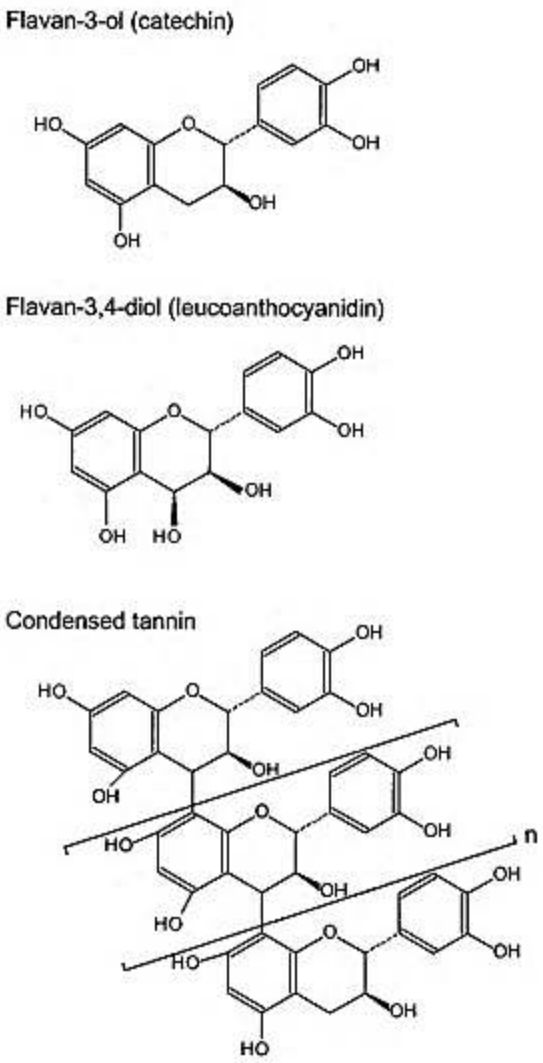

Figure 2.1. Chemical structure of condensed tannins (McMahon et al., 2000)

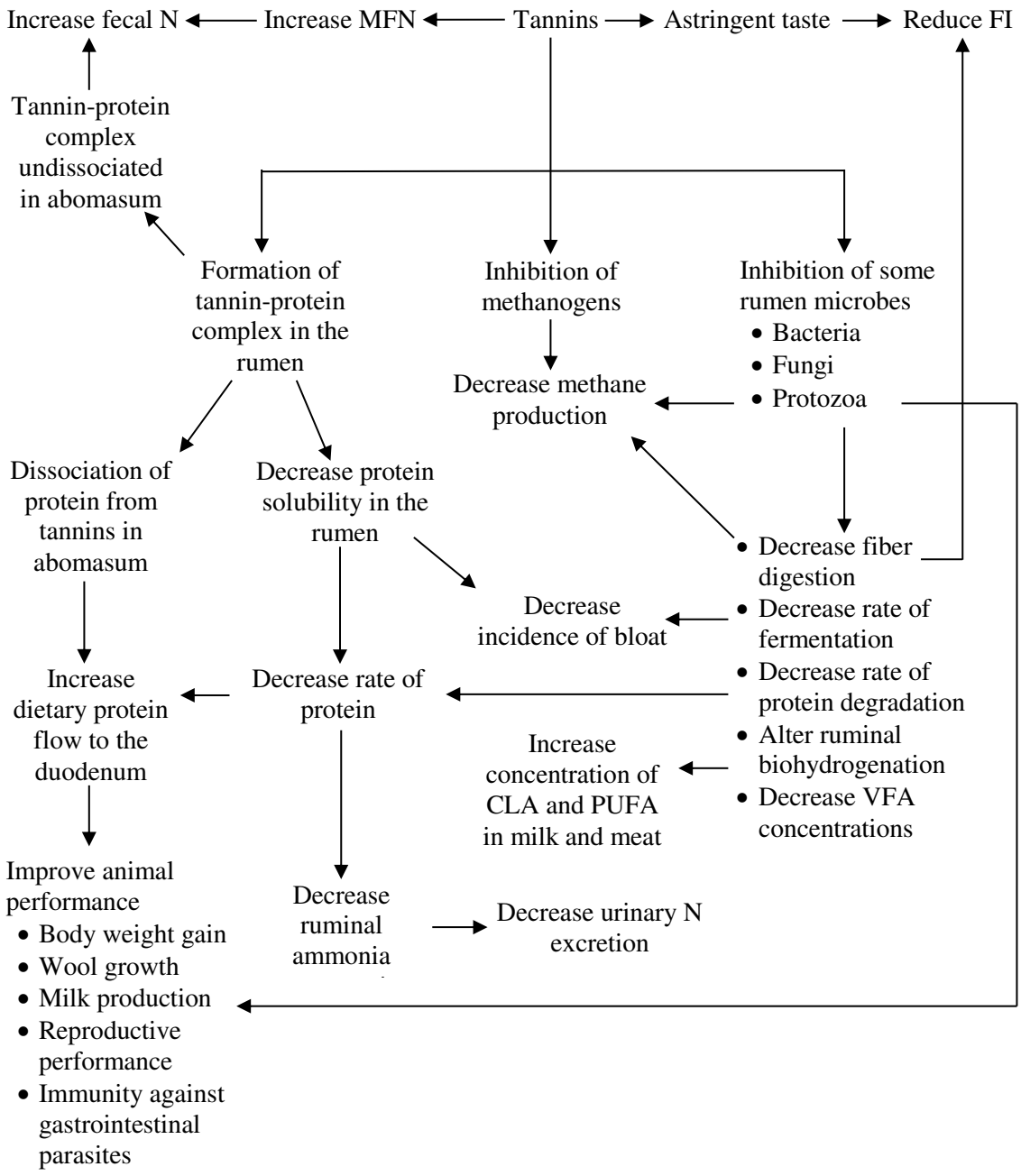

Figure 2.2. Schematic representation of the effects of tannins on ruminal metabolism and ruminant performance. MFN = metabolic fecal nitrogen; FI = feed intake; CLA = conjugated linoleic acids; PUFA = polyunsaturated fatty acids; and VFA = volatile fatty acids (Patra and Saxena, 2011)

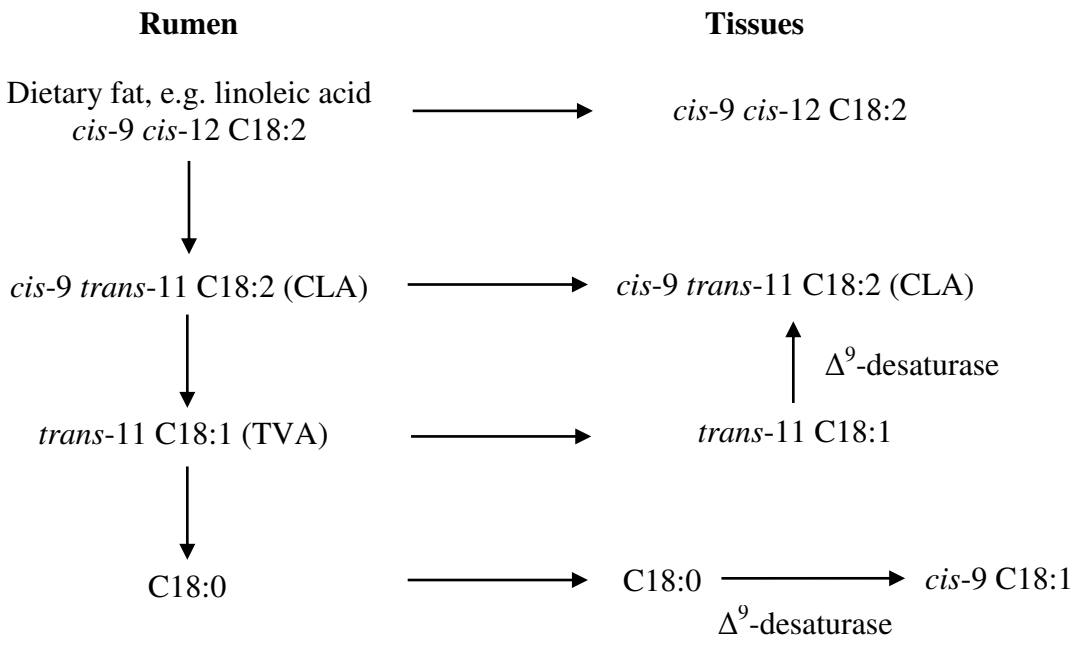

Figure 2.3. De novo synthesis of CLA from *trans*-11 C18:1 vaccenic acid (Bauman et al., 1999)

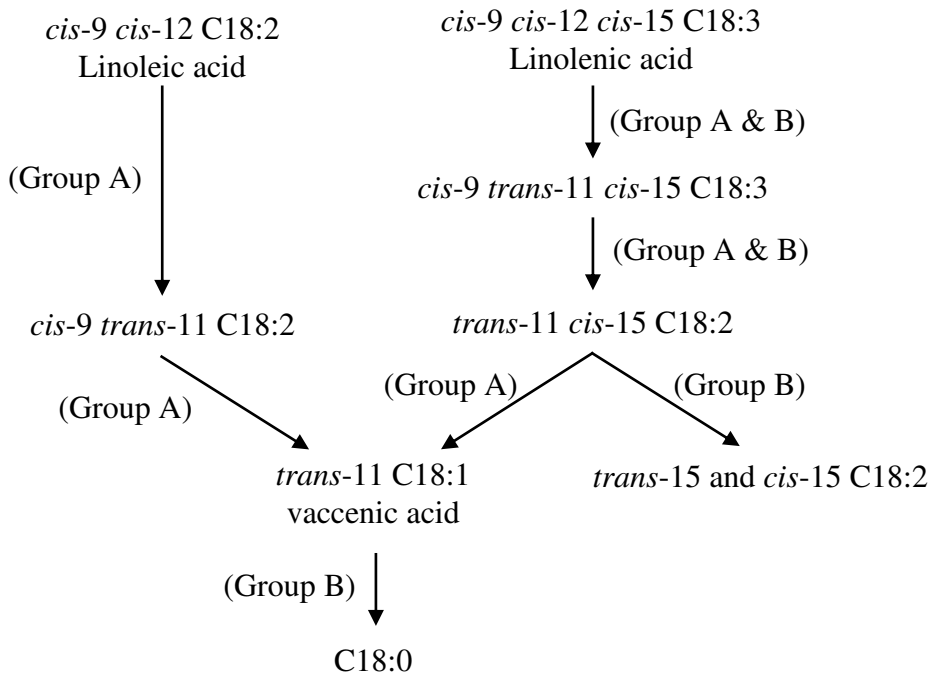

Figure 2.4. Biochemical pathways for the biohydrogenation of linoleic and linolenic acids in the rumen (Harfoot and Hazlewood, 1997)

CHAPTER 3

GROWTH PERFORMANCE, RUMINAL FERMENTATION PROFILES, AND CARCASS CHARACTERISTICS OF BEEF STEERS GRAZING TALL FESCUE WITHOUT OR WITH NITROGEN FERTILIZATION[1]

Introduction

Tall fescue (**TF**; *Festuca arundinacea* Schreb.) is a coarse-textured grass, and it is considered a moderately drought tolerant turfgrass because of its deep root system (Christians, 2004). This forage has a broad range of adaptation due to its tolerance to periodic drought, low soil fertility, and fluctuating seasonal temperatures, and thus it is a popular pasture grass in the Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada). Although TF tolerates low soil fertility, N fertilization increases biomass production and N concentration of TF (Berg and Sims, 2000; Teuton et al., 2007) and positively affects beef cattle performance (Berg and Sims, 1995). Wolf and von Boberfeld (2003) reported that application of N fertilizer in 2 split applications resulted in greater CP concentration of TF, and its effect was cumulative, causing a greater rate of CP increase at later N application.

When N fertilization improves biomass production and N concentration of TF, the process demands energy and leads to less concentrations of non-fibrous carbohydrates (**NFC**), leading to increased fiber concentration and decreased digestibility. For example, Peyraud et al. (1997) reported that N fertilizer treatment increased NDF and ADF

[1] Noviandi, C. T., B. L. Waldron, J.-S. Eun, D. R. ZoBell, R. D. Stott, and M. D. Peel. 2012. Growth performance, ruminal fermentation profiles, and carcass characteristics of beef steers grazing tall fescue without or with nitrogen fertilization. Prof. Anim. Sci. 28:519–527.

concentration from 50 to 53% and 25 to 28%, respectively, while Probasco and Bjugstad (1980) found that IVDMD decreased from 63 to 60% due to N fertilization. On the contrary, greater NDF degradations were reported when pasture grasses were fertilized with N fertilizer (Valk et al., 1996; Galdámez-Cabrera et al., 2003; Nordheim-Viken and Volden, 2009). Forages containing high CP concentrations are often considered to supply the CP required by rapidly growing ruminants, but its utilization efficiency depends on several factors such as carbohydrate availability. Berg and Sims (1995) indicated that steer BW gain/ha during the 4 summer grazing periods increased with N fertilization at rates up to 68 kg N/ha when animals grazed Old World Bluestem (*Bothriochloa ischaemum*) pasture. Increase in BW gain as a response to N fertilization on pasture may have resulted from increases in forage production and ADG. Berg and Sims (2000) also reported that BW gain of steers responded linearly to N fertilization up to 102 kg N/ha/yr with an increase of BW gain from 185 to 505 kg/ha. In the rumen, N fertilized grasses increased ruminal ammonia-N (**NH_3-N**) concentration as a result of increasing CP concentration (Peyraud et al., 1997; Valk et al., 2000). When applied on perennial ryegrass (*Lolium perenne*), N fertilizer increased total VFA concentration with greater proportion of acetate, less proportion of butyrate, and similar proportion of propionate (Peyraud et al., 1997). By increasing both NH_3-N and total VFA, an improvement of animal growth performance can be expected, as a result of greater protein-energy efficiency between NH_3-N and VFA.

Research on forage quality and animal performance often focuses on improving forage yield and N utilization efficiency in pastures. Much is known about fertilizer type, amount, and timing of application for maximizing crop yield. However, major gaps still

exist in our knowledge of the relationships between application of N fertilizer to pasture grasses and its impacts on the ruminal fermentation profiles and growth performance of beef steers. The objective of this grazing study was to test the overall hypothesis that fertilizing TF with N would improve the ruminal fermentation profiles, and thus positively influence growth performance and carcass quality of beef steers.

Materials and Methods

This study was conducted under the approval of the Institutional Animal Care and Use Committee at Utah State University, and the animals were cared for according to its guidelines.

Grazing was completed at Utah State University Pasture Research Farm (Lewiston, UT) from May through September in each year of study in 2010 and 2011. Prior to grazing, all steers were administered with brucellosis vaccination, parasite treatment (Dectomax®, Pfizer Animal Health, Exton, PA), 8-way Clostridial vaccine (Pfizer Animal Health) and an intranasal respiratory product (BoviShield™, Pfizer Animal Health). In addition, animals were implanted with Ralgro® (36 mg of Zeranol; Schering Plough, Madison, NJ).

Animals and Experimental Design

Eighteen Angus crossbred steers (402 ± 9.9 and 379 ± 7.9 kg of BW in 2010 and 2011, respectively) were allocated to 1 of 2 treatments in a completely randomized design: TF without N fertilizer (**TF–NF**) and TF with N fertilizer (**TF+NF**), with 3 replications per treatment and 3 steers per paddock. A total of 168 kg/ha N fertilizer was applied in 3 split applications at 56 kg/ha to the TF+NF in each study year. Each of 0.47-

ha treatment pasture was divided evenly into 4 paddocks (approximately 0.12 ha/paddock), with a single strand of polywire electrified by a battery-powered fence charger. Three steers were allotted to each paddock with a pasture allowance of 215 kg of DM per paddock. Pasture availability was continuously monitored on daily basis to ensure sufficient forages for 3 steers in each paddock. Grazing seasons lasted for 118 d (May 27 to September 21, 2010) and 120 d (May 25 to September 21, 2011). Pastures were managed under rotational stocking during the grazing seasons. Each paddock was grazed for 7 d, and then the same paddock was rested for 21 d until wk 12. However, starting on wk 13 in 2010 and wk 14 in 2011, each paddock was grazed for 3 to 5 d, and then it was rested for 9 to 15 d until the end of grazing study due to availability of forage. All animals were rotated to new paddocks between 0900 and 1000 h. All steers had ad libitum access to fresh water and mineral supplement (Right Now Emerald®, Cargill Animal Nutrition, Minneapolis, MN), and were weighed every 4 wk to determine BW (Figure 3.1).

Determination of DMI and Pasture Sampling and Analysis

Pasture yield was estimated using rising plate meter (**RPM**; 3 Research Park UMC, Columbia, MO) on a weekly basis. Seven measurements were obtained on each paddock at pre- and post-grazing to determine the total DMI per paddock. Pasture sampling for DM yield calibration was done every 4 wk on each paddock (Figure 3.1). Due to differences in pastures, weather, and environment, the total DM yield (DM_{yield}) per paddock was calculated by using the modified equation according to the methodology supplied by the manufacturer as follows: $DM_{yield} = RPM_{average} \times DM_{cal} \times Area_{paddock}$, where $RPM_{average}$ = average of RPM height (cm), DM_{cal} = DM yield calibration per

quadrat (g/cm), and $Area_{paddock}$ = area of paddock on each week (1,165 m^2). Pasture intake was measured according to the herbage disappearance method based on the difference between pre- and post-grazing herbage mass (Lantinga et al., 2004). Intake of DM (g/kg BW) was estimated by using the equation: DMI (g/kg BW) = {(pasture intake ÷ 3 cows) ÷ BW} × 1000.

Pasture forage samples were collected at 4-wk intervals throughout the study, beginning at approximately 0900 h. Forage samples were collected from the paddocks where the steers were grazing by clipping 6 quadrats of 0.102-m^2 each during the grazing period. Clippings were made at the same time of the day, approximately 0900 h on the first day of grazing. Pastures were clipped at 2–3 cm above ground level with the aid of a battery-powered portable mower (SSC 1000, Black & Decker, Inc., Towson, MD), and care was taken to avoid soil contamination. Samples were placed in paper bags and immediately transported to the laboratory. Samples were weighed, dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ), and stored for subsequent analyses. Analytical DM and OM concentration of samples was determined by oven drying at 105°C overnight and by ashing at 550°C, respectively, while N concentration was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) (AOAC, 2000). The NDF and ADF concentrations were sequentially determined using an ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination, and pre-treatment was performed with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO).

Analysis of Ruminal Fluid and Carcass Characteristics

Ruminal fluid samples were obtained using Geishauser probe at wk 4, 10, and 16 (Figure 3.1). The fluid was collected with a solid, tube-like probe with rows of small holes on the end (Geishauser, 1993). The pH of the ruminal fluid was measured within 5 min of collecting the samples using a portable pH meter (Oakton pH 6; Oakton Instruments, Vernon Hills, IL). Five milliliters of the ruminal fluid were mixed with 1 mL of 1% sulfuric acid and stored frozen (-40°C) for NH_3-N analysis. Concentration of NH_3-N in the ruminal contents was determined as described by Rhine et al. (1998), using a plate reader (MRX[e], Dynex Technologies, Chantilly, VA). Another 5 mL of the ruminal fluid were collected and added with 1 mL of 25% meta-phosphoric acid, and then stored at -40°C for VFA determination. Ruminal VFA were separated and quantified using a GLC (model 6890 series II; Hewlett Packard Co., Avandale, PA) with a capillary column (30 m × 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame-ionization detection. The oven temperature was held at 170°C for 4 min, increased to 185°C at a rate of 5°C/min, then increased by 3°C/min to 220°C and held at this temperature for 1 min. The injector and the detector temperatures were 225 and 250°C, respectively, and the carrier gas was helium (Eun and Beauchemin, 2007).

At the end of the grazing season, all steers were scanned using ultrasound (Aloka SSD-500V, Wallingford, CT) to determine the carcass characteristics (back fat thickness, ribeye area, and intramuscular fat concentration) using proprietary analysis software (Brethour 1991, 1992).

Statistical Analysis

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Cary, NC). All data were assessed using a one-way analysis of variance in a completely randomized design. Paddocks were considered as the experimental units. The model included the fixed effects of treatment, sampling week, and treatment × week interaction. The replication within treatment was considered as random effect. Data collected in 2-year grazing seasons were analyzed separately by each year, because the weather and environmental conditions were different between the years. In addition, due to variations in nutritive composition of pastures during the 16-wk study, data of animal growth performance and ruminal fermentation profiles from each year were also compared within week. All means reported are least squares means. Pair-wise comparisons and contrasts were used to compare effects of treatment, sampling week, and interaction effects. In all cases, significant effects were declared at $P < 0.05$, and trends were discussed at $P < 0.10$.

Results and Discussion

Nutrient Composition of TF Pastures

In both 2010 and 2011 grazing seasons, N fertilization did not show any effect on the DM of TF pasture, but increasing DM of TF pasture ($P < 0.01$) from wk 4 to wk 16 (averaged 18.6 and 25.4%, respectively) was noticed in both grazing years (Table 3.1). A year effect was detected ($P < 0.01$) on pasture DM, with less DM in 2011 compared with that in 2010 (20.0 vs. 24.5%) due to greater precipitation during the grazing season (May

to September) in 2011 (3.0 vs. 1.5 cm, data not presented). However, there was no interaction effect between treatment and week in 2010 and 2011.

Numerically, N fertilization always resulted in greater CP, but did not significantly affect the CP concentration of TF pasture in 2010. In contrast, greater CP concentration was detected on TF+NF compared with TF–NF in 2011 (averaged 11.9 vs. 10.6% DM, respectively; $P < 0.01$; Table 3.1). No significant effect on CP in response to N fertilization on TF pasture in 2010 is likely to be caused by residual N in the soil, which would be often the case for the seedling year. This condition may dilute the overall effects of applying N fertilizer on TF pasture (Noviandi et al., 2011). Peyraud and Astigarraga (1998) stated that a greater dilution of grass CP contents may occur on highly fertilized pastures, which was related to the enhancement of sward mass production. In contrast, an accumulative effect of N fertilization in 2011 increased the CP concentration of TF+NF. There is a body of evidence to indicate changes in forage composition due to N fertilization and its accumulative effect from multiple applications (Wolf and Boberfeld, 2003; Teuton et al., 2007; Pecetti et al., 2008). The accumulative effect of N fertilization in this study was apparent in the differences of CP concentration between TF–NF and TF+NF from wk 4 to 16 that gradually increased in 2010 as grazing progressed (0.5, 1.1, 1.3, and 2.5 percentage units at wk 4, 8, 12, and 16, respectively). However, the differences of CP concentration between the treatments were relatively stable during 2011 grazing season (1.2, 1.4, 1.2, and 1.5 percentage units at wk 4, 8, 12, and 16, respectively). The gradual increase in CP concentration differences between treatments in 2010 indicates that by applying 168 kg N/ha in 3 different times on TF contributed to increasing CP concentration of TF in a cumulative manner. Similarly,

Wolf and von Boberfeld (2003) observed the accumulative effect of N fertilization on CP concentration of TF when 50 kg N/ha were applied at 2 different times, but the authors reported no cumulative effects of CP concentration on TF when more than 100 kg N/ha were applied. In addition to the rate of N fertilization, its application frequency may affect the accumulative effect of N fertilization. For instance, Wolf and von Boberfeld (2003) applied 100 kg N/ha in two applications, while we applied 168 kg N/ha at three times.

Nitrogen fertilization resulted in greater NDF concentration in 2010, but it did not influence NDF concentration in 2011 (Table 3.1). Concentration of ADF between treatments was similar in both grazing seasons. However, greater NDF and ADF concentrations of TF pasture in 2010 were detected ($P < 0.01$) compared with those in 2011. The difference in NDF concentration caused by N fertilization in 2010 is likely due to differences in growth rates of TF pastures. Nitrogen fertilization increases the DM yield of TF through accelerating growth rate (Read and Hipp, 1998). Rapid growth of plants demands high energy input, which typically leads to less NFC retention in the plant tissue. In our study, fiber concentrations peaked on wk 12 ($P < 0.01$), whereas NFC concentrations were the lowest on wk 12 (6.4 and 4.6% to TF–NF and TF+NF, respectively; $P < 0.01$). This decrease in NFC concentration is attributable to an increase in the utilization of carbon chains for protein synthesis and for production of the energy required for the nitrate reduction step before protein synthesis (Peyraud and Astigarraga, 1998).

Growth Performance of Steers

Intake of DM (g/kg BW) of the grazing steers did not differ between fertilized and unfertilized TF pastures in either year except wk 8 of 2010 when steers grazing TF+NF showed increased DMI compared to the non-fertilized (Table 3.2). In addition, overall DMI in 2010 tended to increase ($P = 0.07$) on TF+NF compared with TF–NF (19.4 vs. 18.1 g/kg BW). The tendency of increasing overall DMI on TF+NF may be related to greater NDF degradation rate due to N fertilization. In in situ studies using pastures, N fertilizer application increased NDF degradation on bromegrass (*Bromus inermis*; Messman et al., 1991), perennial ryegrass (Valk et al., 1996), bermudagrass (*Cynodon dactylon*; Galdámez-Cabrera et al., 2003), and timothy grass (*Phleum pratense*; Nordheim-Viken and Volden, 2009). Greater DMI of TF+NF at wk 8 in 2010 compared with TF–NF may be associated with the time of N fertilization. Messman et al. (1991) reported that greater NDF degradation occurred when N fertilizer was applied in the early stage of growth, which would increase DMI of grazing steers. In contrast, N fertilization did not affect overall DMI in 2011. With progression of the grazing season, DMI gradually decreased from 21.7 to 14.7 g/kg BW and from 23.1 to 19.0 g/kg BW in 2010 and 2011, respectively ($P < 0.01$). The decreasing trend of DMI is likely induced by the decreasing NFC concentration of TF during the grazing seasons. Peyraud et al. (1997) reported that DMI decreased as soluble carbohydrates decreased. This result may be related to a shortage of soluble carbohydrate in the rumen, which may limit microbial activities, thus increasing rumen fill and consequently limiting pasture intake. High concentration of cell wall contents can suppress microbial activity by reducing the availability of rapidly fermentable carbohydrates (Wilson and Hatfield, 1997). Similarly,

lignification limits microbial access to structural polysaccharides in the cell wall, resulting in slower digestion and decrease in DMI (Waghorn and McNabb, 2003).

While ADG did not differ between TF−NF and TF+NF at wk 4, 8, 12, and 16 in 2010, it increased on the TF+NF pastures at wk 4 in 2011(Table 3.2). In both grazing years, overall ADG increased by steers grazed with TF+NF compared to those grazed with TF−NF ($P < 0.05$). The positive effect of N fertilizer on overall ADG may have resulted from increased ruminal fermentation evidenced on increased VFA concentration with N fertilization as discussed later. Regardless of N fertilization, maximum ADG of grazing steers was achieved at wk 4 and 8 in both grazing years (averaged 1.05 kg/d; $P < 0.01$), and then declined until the completion of this grazing study. The gradual decrease of ADG during later phases in the grazing season is likely due to increased energy demand of rapidly growing steers that could not met by available energy in TF pasture. As shown in Table 3.1, NFC concentration in our study decreased by 8.0 percentage units on average from wk 4 to 12 in both grazing seasons. Wolf and Boberfeld (2003) reported that the energy concentration of TF decreased throughout the grazing season.

Nitrogen fertilization did not affect G:F at any week in 2010 and 2011, whereas steers grazed with TF+NF tended to increase overall G:F ($P = 0.07$) compared to those grazed with TF−NF in 2010, but they had similar overall G:F in 2011. In 2010 and 2011, G:F gradually decreased ($P < 0.01$) with progression in grazing season. Increased overall ADG in response to N fertilization in 2010 supported increased overall G:F of steers grazed TF+NF. As discussed previously, lack of energy availability in TF pastures led to decreased G:F with the progression of grazing seasons.

Ruminal Fermentation Profiles

Ruminal pH ranged from 6.85 to 7.21, and it was not affected by N fertilization (Table 3.3). Steers that grazed TF+NF pastures had greater total VFA concentration at wk 10 and 16 in 2010 and at all weeks in 2011 ($P < 0.01$). Greater concentration of total VFA on TF+NF compared with TF–NF may have resulted from enhanced ruminal fermentation, particularly the NDF fraction. Messman et al. (1991) and Valk et al. (1996) indicated that rate of degradation of cell walls increased with N fertilization. Peyraud et al. (1997) reported that a high level of N fertilization (80 kg N/ha/cut) to perennial ryegrass increased NDF concentration in pasture and digestibility by lactating dairy cows, which resulted in increased total VFA concentration. The increase in total VFA concentration due to N fertilization on TF pasture was consistent in 2010 and 2011 in our study. The positive effect on ruminal fermentation may have contributed to the improved animal growth performance of steers grazed with TF+NF, providing more precursors toward BW gain and consequently resulting in increased overall ADG and G:F.

There were minor effects of N fertilization on molar proportions of individual VFA (acetate, propionate, and butyrate) and acetate:propionate in both grazing seasons (Table 3.3). However, increased propionate proportion by N fertilization ($P < 0.05$) was detected at wk 16 and 4 in 2010 and 2011, respectively. In addition, less acetate:propionate on TF+NF compared with TF–NF was noticed at wk 4 in 2011.

Steers that grazed TF+NF had greater ruminal NH_3-N concentration ($P < 0.01$) compared with those that grazed TF–NF at wk 4 and 16 in 2010 and at all weeks in 2011 (Table 3.3). Regardless of N fertilization, NH_3-N concentration gradually increased ($P < 0.01$) with the progression of grazing season in 2010 and 2011. Grass under grazing

conditions is usually in vegetative stage, which contains high amounts of rapidly rumen degradable N fraction (Valk et al., 2000). Applying N fertilizer on TF further influenced dietary N utilization in the rumen as indicated by increased NH_3-N concentrations throughout grazing in our study. Greater ruminal NH_3-N concentration of TF+NF compared with TF–NF during 2-yr grazing seasons implies that dietary N utilization in the rumen was less efficient. Concentration of dietary CP can influence microbial activity, as RDP supplies peptides, amino acids, and NH_3-N derived from microbial proteolysis for use in microbial protein synthesis (Wallace et al., 1997). A minimum of 7.0% CP in diets is required for efficient microbial digestion (Hariadi and Santoso, 2010), and 5.0 mg/100 mL is considered the minimum NH_3-N concentration for bacterial growth (Satter and Slyter, 1974). The minimum levels of CP concentrations and NH_3-N productions of all pastures in this study indicate that TF pastures regardless of N fertilization supplied the minimally required N source for microbial fermentation, but they lacked energy to optimize ruminal fermentation. Increased ruminal NH_3-N concentration on TF+NF may reflect reduced ruminal capture of the NH_3-N for microbial protein synthesis. Ruminal NH_3-N concentration is a result from a balance between production (proteolysis) and assimilation (De Visser et al., 1997), and thus any efforts to maximize N utilization in the rumen should involve an optimal balance between the two metabolic processes. Yet, in order to optimize dietary N utilization, particularly pasture forages, in ruminants, protein degradation in the rumen should be decreased, whereas N use by ruminal microbes must be increased (Hoover and Stokes, 1991). It is believed that energy is the most limiting factor in microbial growth (Bach et al., 2005), and consequently decreased NFC concentration may contribute to increased difference on

NH_3-N concentration between TF–NF and TF+NF. De Visser et al. (1997) reported that ryegrass (*Lolium perenne*) that was fertilized with the high amount of N (450 kg of N/ha) contained more N and less sugar than did ryegrass that was fertilized with less N (150 kg of N/ha). In ruminal fluid, the concentration of NH_3-N was less for ryegrass that was fertilized with the low amount of N (De Visser et al., 1997).

Applying N fertilizer on TF pasture did not affect NH_3-N:total VFA concentration in both years of study (Table 3.3). However, the ratio increased ($P < 0.01$) with the progression of grazing season (from 0.072 at wk 4 to 0.116 at wk 16 in 2010 and from 0.061 at wk 4 to 0.108 at wk 16 in 2011). In this study, greater total VFA concentration of TF+NF was noticed, as NH_3-N concentration increased. However, the sizable increase of NH_3-N concentration was not followed by the increase in total VFA concentration at a similar level, thus resulting in greater NH_3-N:total VFA concentration with the progression of grazing seasons.

Carcass Characteristics

Nitrogen fertilization on TF pasture did not influence carcass characteristics (back fat thickness, ribeye area, and intramuscular fat concentration) of grazing beef steers (Table 3.4). These results agree with those of Keane and Allen (1999) who reported that levels of N fertilizer (57, 204, and 227 kg N/ha) did not affect carcass composition and meat quality traits of grazing steers. Back fat deposition of ruminants is largely dictated by acetate production in the rumen (Berger and Pyatt, 2005). In our study, N fertilization on TF pasture did not influence molar proportion of acetate (Table 3.3). Although N fertilization of TF increased overall ADG of steers in the current study, it appears that no impacts on the carcass characteristics were detected.

Implications

Nitrogen fertilization is one of the most practical approaches to increase forage production and maintain high quality pastures for grazing animals. In this study, N fertilization improved DMI, ADG, and G:F ratio through increased ruminal fermentation of grazing steers. However, it is unclear the mechanism whereby N fertilization increases ruminal fermentation evidenced by increased VFA concentration, and thus this aspect needs to be investigated. Increased ruminal NH_3-N concentration by N fertilization on TF found in this study indicates that there is a need to supplement readily fermentable carbohydrates in TF pastures. Providing sufficient readily fermentable carbohydrates in the rumen is expected to improve dietary CP utilization by optimizing microbial protein synthesis, which may result in enhancing growth performance of grazing steers.

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Table 3.1. Nutrient concentration (% of DM) of tall fescue grass (n = 3)

	2010						2011					
Week and treatment[1]	DM, %	CP	EE[2]	NDF	ADF	NFC[3]	DM, %	CP	EE	NDF	ADF	NFC
Wk 4												
TF–NF	20.5	12.5	1.60	57.3[b]	30.1	14.4[a]	18.4	11.5[b]	3.69	54.3	30.3	17.5
TF+NF	19.1	13.0	1.65	59.3[a]	31.3	11.6[b]	16.4	12.7[a]	4.19	54.3	30.4	15.6
Average	19.8[f]	12.7[c]	1.63[d]	58.3[e]	30.7[e]	13.0[c]	17.4[e]	12.1[c]	3.94[d]	54.3[d]	30.3[d]	16.6[c]
Wk 8												
TF–NF	22.4	9.1	2.87	62.2	33.4	10.8	20.7	11.9[b]	4.23	56.0	32.3	11.8
TF+NF	22.8	10.2	2.95	63.2	34.1	10.1	19.3	13.3[a]	4.31	56.1	32.3	11.0
Average	22.6[e]	9.6[d]	2.91[c]	62.7[d]	33.7[d]	10.4[d]	20.0[d]	12.6[c]	4.27[d]	56.0[d]	32.3[c]	11.4[d]
Wk 12												
TF–NF	26.3	10.9	2.89	65.3[b]	35.6	6.4[a]	23.0	11.4[b]	5.41	59.2	32.5	8.6
TF+NF	24.0	12.1	3.14	67.0[a]	36.7	4.6[b]	22.0	12.5[a]	5.21	59.8	32.7	7.7
Average	25.1[d]	11.5[c]	3.02[c]	66.1[c]	36.1[c]	5.5[e]	22.5[c]	11.9[c]	5.31[c]	59.5[c]	32.6[c]	8.2[e]
Wk 16												

TF–NF	30.3	7.3	2.86	63.9	33.4	11.4[a]	20.6	7.6[b]	5.25	54.3	30.3	17.9
TF+NF	30.8	9.8	3.01	64.1	33.7	9.8[b]	19.9	9.1[a]	5.47	54.3	30.4	17.0
Average	30.6[c]	8.6[d]	2.94	64.0[d]	33.5[d]	10.6[d]	20.3[d]	8.4[d]	5.36[c]	54.3[d]	30.3[d]	17.5[c]
Pooled SEM	0.70	0.68	0.157[c]	0.51	0.41	0.40	0.69	0.31	0.167	0.82	0.50	0.72

[a,b]Within a column and week, means with different superscripts are different ($P < 0.05$).

[c-f]Within a column , averages with different superscripts are different ($P < 0.01$).

[1]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer.

[2]Ether extract.

[3]Non-fibrous carbohydrate = 100 – (CP + NDF + EE + ash).

Table 3.2. Effect of N fertilization of tall fescue on growth performance of grazing beef steers (n = 3)

	2010			2011		
Week and treatment[1]	DMI, g/kg BW	ADG, kg/d	G:F	DMI, g/kg BW	ADG, kg/d	G:F
Wk 4						
TF–NF	21.1	1.06	0.148	22.5	0.87[b]	0.097
TF+NF	22.3	1.06	0.158	23.6	1.22[a]	0.125
Average	21.7[c]	1.06[cd]	0.153[c]	23.1[c]	1.04[c]	0.111[c]
Wk 8						
TF–NF	19.1[b]	1.09	0.126	22.7	0.87	0.090
TF+NF	21.6[a]	1.23	0.143	23.0	1.05	0.102
Average	20.3[c]	1.16[c]	0.135[cd]	22.8[c]	0.96[c]	0.096[c]
Wk 12						
TF–NF	17.7	0.85	0.111	21.1	0.77	0.082
TF+NF	19.0	1.06	0.133	20.7	0.83	0.085
Average	18.3[d]	0.95[de]	0.122[d]	20.9[d]	0.80[cd]	0.084[cd]
Wk 16						

TF–NF	14.5	0.79	0.121	18.8	0.58	0.066
TF+NF	14.9	0.90	0.135	19.1	0.61	0.065
Average	14.7[e]	0.85[e]	0.128[cd]	19.0[e]	0.59[d]	0.065[d]
Wk 4–16[2]						
TF–NF	18.1	0.95[h]	0.126	21.3	0.77[h]	0.084
TF+NF	19.4	1.06[g]	0.142	21.6	0.93[g]	0.094
Pooled SEM	0.66	0.059	0.0101	0.66	0.084	0.0103

[a,b]Within a column and week, means with different superscripts are different ($P < 0.05$).

[c-f]Within a column , averages with different superscripts are different ($P < 0.05$).

[g,h]Within a column, means with different superscripts are different ($P < 0.05$).

[1]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer.

[2]Comparison between TF–NF and TF+NF in overall 16 wk of study.

Table 3.3. Effect of N fertilization of tall fescue on ruminal pH, VFA profile, and ammonia-N (NH_3-N) concentrations of grazing beef steers (n = 3)

	2010								2011							
Week and		Total	Individual VFA[3]					NH_3:		Total	Individual VFA					NH_3:
treatment[1]	pH	VFA[2]	A	P	B	A:P	NH_3-N[4]	VFA[5]	pH	VFA	A	P	B	A:P	NH_3-N	VFA
Wk 4																
TF–NF	7.15	58.0	68.7	18.3	8.37	3.80	6.03^{b}	0.061	6.99	58.4^{b}	71.4	15.3^{b}	9.66	4.68^{a}	5.56^{b}	0.056
TF+NF	7.15	60.8	67.8	18.3	8.78	3.71	8.47^{a}	0.082	6.85	69.4^{a}	70.7	17.2^{a}	9.10	4.12^{b}	7.80^{a}	0.066
Average	7.15	59.4^{cd}	68.3^{d}	18.3^{c}	8.58^{d}	3.76^{d}	7.25^{e}	0.071^{e}	6.92	63.9^{cd}	71.0	16.2	9.38^{c}	4.40	6.68^{d}	0.061^{d}
Wk 10																
TF–NF	7.21	55.0^{b}	71.3	16.7	7.90	4.27	8.89	0.096	6.89	63.6^{b}	70.4	16.0	8.32	4.47	10.6^{b}	0.098
TF+NF	7.10	60.3^{a}	70.5	17.5	7.41	4.04	10.4	0.101	6.96	68.8^{a}	71.0	16.4	8.33	4.33	12.5^{a}	0.107
Average	7.15	57.6^{d}	70.9^{c}	17.1^{d}	7.66^{d}	4.16^{c}	9.65^{d}	0.098^{d}	6.92	66.2^{c}	71.2	16.2	8.33^{cd}	4.40	11.6^{c}	0.103^{c}
Wk 16																
TF–NF	7.08	59.1^{b}	65.6	18.4^{b}	10. 8	3.57	11.6^{b}	0.115	7.16	56.3^{b}	71.4	16.3	8.21	4.40	9.97^{b}	0.104
TF+NF	7.11	66.3^{a}	66.4	19.6^{a}	9.32	3.44	13.9^{a}	0.123	7.02	66.0^{a}	70.4	17.3	8.01	4.06	12.1^{a}	0.108

Average	7.10	62.7[c]	66.0[e]	19.0[c]	10.1[c]	3.51[d]	12.7[c]	0.119[c]	7.09	61.2[d]	70.9	16.8	8.11[d]	4.23	11.0[c]	0.106[c]
Pooled SEM	0.058	1.06	0.74	0.33	0.449	0.103	0.596	0.0061	0.062	1.05	0.54	0.29	0.475	0.099	0.323	0.0040

[a,b]Within a column and week, means with different superscripts letter are different ($P < 0.05$).

[c-e]Within a column , averages with different superscripts letter are different ($P < 0.05$).

[1]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer.

[2]Expressed as m*M*.

[3]Expressed as mol/100 mol. A = acetate; P = propionate; B = Butyrate.

[4]Expressed as mg/100 mL.

[5]NH_3:VFA = NH_3-N (m*M*) ÷ total VFA (m*M*).

Table 3.4. Effect of N fertilization of tall fescue on carcass characteristics of grazing beef steers (n = 3)

	2010[2]			2011		
Treatment[1]	Back fat, cm	REA, cm^2	IM fat, %	Back fat, cm	REA, cm^2	IM fat, %
TF–NF	0.26	10.6	3.98	0.43	10.1	3.10
TF+NF	0.27	9.87	4.08	0.46	9.81	3.61
SEM	0.012	0.483	0.255	0.030	0.234	0.220
P-value	0.57	0.35	0.81	0.47	0.44	0.17

[1]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer.

[2]REA = ribeye area; IM fat = intramuscular fat.

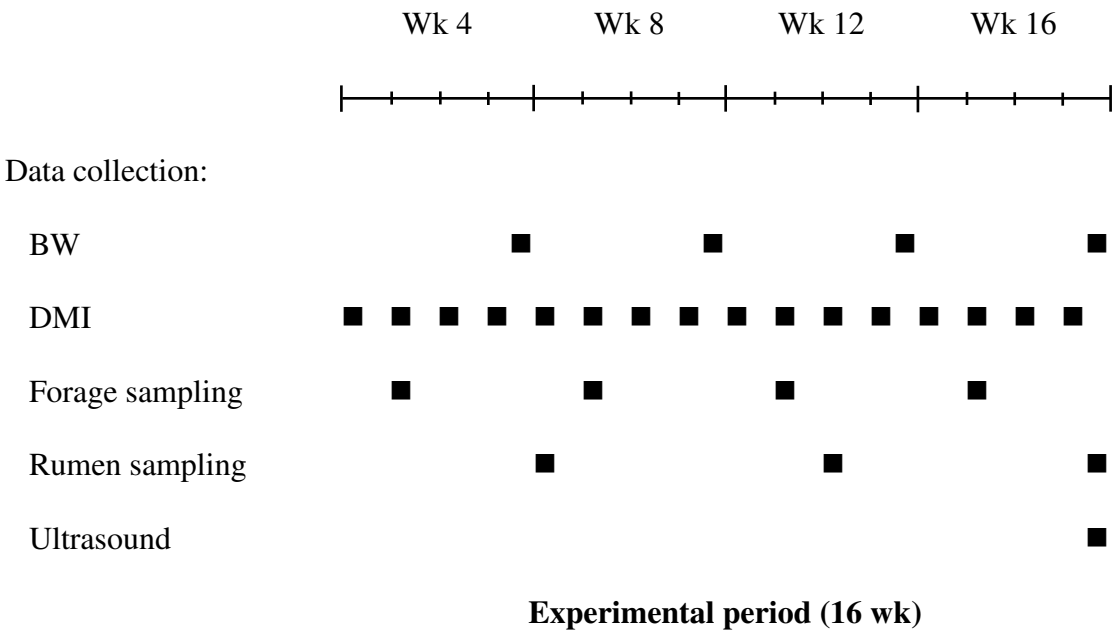

Figure 3.1. Experimental period and sampling schedule in 2010 and 2011

CHAPTER 4

FATTY ACID COMPOSITION IN ADIPOSE TISSUE OF PASTURE- AND FEEDLOT-FINISHED BEEF STEERS[1]

Introduction

The most common practice of finishing beef cattle in the United States is by feeding high grain diets in confinement feedlot, and it is very efficient in gaining BW in a short period. However, this practice also results in high SFA proportion of the beef, which is thought to confer negative effects on human health. Recent studies (Nuernberg et al., 2002; Realini et al., 2004; Fincham et al., 2009) demonstrated that beef of pasture-finished cattle has lower proportion of SFA, greater n-3 and less n-6 PUFA, and higher CLA compared to high grain-finished beef. Increased n-3 PUFA, especially C18:3 n-3, can reduce the risk of heart disease, hypertension, inflammation, and mammary cancer, and lower cholesterol concentration in blood (de Deckere et al., 1998; Tapiero et al., 2002). Ha et al. (1990) and Ip et al. (1994) have suggested that CLA isomers may be valuable in the human diet, due to their anti-carcinogenic properties in rodent model systems. There is evidence to suggest that CLA proportion in adipose tissue of pasture-fed beef steers were greater compared to those fed typical feedlot finishing diets (Basarab et al., 2007).

Tall fescue (**TF**; *Festuca arundinacea*) is a popular pasture grass in the Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and

[1] Noviandi, C. T., R. E. Ward, D. R. ZoBell, R. D. Stott, B. L. Waldron, M. D. Peel, and J.-S. Eun. 2012. Fatty acid composition in adipose tissue of pasture- and feedlot-finished beef steers. Prof. Anim. Sci. 28:184–193.

Nevada) due to its high adaptability to periodic drought, low fertility, and fluctuating seasonal temperatures. Although TF tolerates low fertility, N fertilization increases biomass production and N concentration of TF (Berg and Sims, 2000; Teuton et al., 2007) and positively affects beef cattle performance (Berg and Sims, 1995). However, there have been no studies to evaluate whether improvement in forage quality and beef cattle performance caused by N fertilization may influence the FA composition in adipose tissue of TF pasture-finished beef steers. Therefore, it was hypothesized that N fertilization would affect FA composition of adipose tissue in pasture-fed beef steers due to its potential impacts on nutrient and energy utilization. The objectives of this study were to determine if N fertilization on TF pasture would influence FA profiles of fat depots in adipose tissue. In addition, we were interested in beneficial effects of grazing steers by comparing the adipose tissue FA profiles between pasture- and feedlot-finished beef steers.

Materials and Methods

All procedures related to the animals used in the current study were accepted by the Institutional Animal Care and Use Committee at Utah State University, and the animals were cared for according to its guidelines.

Animals, Treatments, and Experimental Design

Twenty-seven Angus crossbred steers (initial BW = 394 ± 5.5 kg) were used in this study. The experiment was conducted at the Pasture Research Farm (Lewiston, UT) and the Utah State University Beef Research Farm (Wellsville, UT) from May through September 2010. All steers had been processed similarly prior to trial initiation by

receiving a Brucellosis vaccination, parasite treatment (Dectomax®, Pfizer Animal Health, Exton, PA), eight-way Clostridial vaccine (Pfizer Animal Health) and an intranasal respiratory product (BoviShield™, Pfizer Animal Health). In addition, animals were implanted with Ralgro® (36 mg of zeranol; Schering Plough, Madison, NJ).

The 18 steers on pasture were assigned to 1 of 2 treatments in a completely randomized design: TF without N fertilizer (**TF–NF**) and TF with N fertilizer (**TF+NF**). On each treatment, there were 3 replicated pastures (n = 3) with 3 steers per pasture. A total of 168 kg/ha N fertilizer was applied in 3 split applications at 56 kg/ha to the TF+NF. Each pasture was divided into 4 equal-size paddocks (51 × 23 m) with a single strand of polywire electrified by a battery-powered fence charger. During the experiment, grazing animals were assigned a pasture allowance of 215 kg of DM per group. Each paddock was grazed for 7 d, and then the same paddock was rested for 21 d until wk 12. Due to limitations in forage production starting on wk 13, each paddock was grazed for 3 to 5 d, and then it was rested for 9 to 15 d until the end of the study. All animals were rotated to new paddocks between 0900 and 1000 h on a weekly basis. All steers had ad libitum access to fresh water and mineral supplement (Right Now® Emerald, Cargill Animal Nutrition, Minneapolis, MN).

For feedlot TMR treatment (**FLT**), 9 steers were housed in 3 pens with 3 animals per pen (n = 3) and had ad libitum access to a finishing diet containing 5.0% alfalfa hay, 15.0% corn silage, 76.0% barley grain, and 4.0% feedlot vitamin and mineral supplement (DM basis). The FLT diet contained 11.0% CP, 28.1% NDF, 11.8% ADF, 1.40% crude fat, and 54.9% non-fibrous carbohydrate on DM basis.

Pasture or pen was considered as an experimental unit. The experiment was initiated at the same time at pasture and feedlot, and overall sampling procedures were exactly the same between the treatments.

Sample Collection

Adipose tissue samples were obtained at the end of wk 4, 12, and 16, beginning at approximately 0800 h. Biopsies of subcutaneous adipose tissue were obtained from the loin area on the left side adjacent the last rib ±10 cm down from the midline. The biopsy site was clipped and surgically prepared with 3 alternate scrubs of 70% isopropyl alcohol and 1% povidone-iodine solution (Betadine Surgical Scrub, The Purdue Frederick Co., Stamford, CT). Lidocaine (2% solution; total of 8 mL/animal; Vedco, St. Joseph, MO) was injected subcutaneously cranial to the site. A linear incision (approximately 5 cm) was made with a sterile scalpel through the skin. Approximately 1 g of adipose tissue was obtained. The incision was closed in a Ford interlocking pattern using #1 polyamid suture. Ceftiofur sodium (2 cc) was administered in the surgical site prior to closure to prevent infection.

Pasture forage and TMR samples were collected at 4-wk intervals throughout the study, beginning at approximately 0900 h. Forage samples were collected from the paddocks where the steers were grazing on 14-d intervals by clipping 6 of 0.102-m^2 quadrats in each plot during the grazing period. Clippings were made at the same time of the day, approximately 0900 h on the first day of grazing. Pastures were clipped to a height of 8 cm with the aid of battery-powered portable mower (SSC 1000, Black & Decker, Inc., Towson, MD), and care was taken to avoid soil contamination. Samples were placed in sealed plastic bags, placed in a cooler with dry ice, and immediately

transported to the laboratory to be frozen at -40°C to prevent oxidation and structural changes in FA. Pasture forage samples for FA analysis were freeze-dried (FreeZone 12 L Freeze Dry Systems, Labconco Corp., Kansas City, MO), and then ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA). Ground forage samples were composited within replication for the 28-d periods. Samples of TMR and refusal were obtained on weekly basis. Samples were immediately dried in a forced-air oven at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4), and composited within replication over 28-d periods. Samples of TMR were freeze-dried for FA analysis using the same procedure for pasture samples.

At the end of the grazing period, all steers were scanned using ultrasound (Aloka SSD-500V, Wallingford, CT) to determine the carcass characteristics (back fat, rib fat, ribeye area, and intramuscular fat) using proprietary analysis software (Brethour 1991, 1992).

Laboratory Analyses

Fatty acid extraction and methylation of feed samples were carried out according to procedures of Palmquist and Jenkins (2003). Approximately 0.4 g of samples were placed into a 15 mL screw-cap culture tubes in which 1 mL of internal standard (C19:0 in benzene) and 3 mL of 10% 3N methanolic HCl were added. The tubes were vortex-mixed for 30 s, and then incubated in 70°C water bath for 2 h. Then, the tubes were shaken vigorously for 5 s every 30 min during which the reaction content in the tubes was monitored. After removal from the water bath and cooled for 15 min, 7.5 mL of 6% K_2CO_3 and 1.5 mL of hexane were added and mixed using vortex for 10 s, and then

centrifuged at 600 × *g* for 5 min at room temperature. The hexane layers were transferred into GLC vials, capped, and then stored at -20°C until analysis.

Fatty acid extraction from adipose tissue samples was performed according to procedures of O'Fallon et al. (2007). Briefly, 100 mg of adipose tissue samples were placed into 15 mL screw-cap culture tubes in which 0.7 mL of 10 N KOH in water and 5.7 mL of MeOH were added. The tubes were incubated in a 55°C water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min to properly permeate, dissolve, and hydrolyze the sample. After cooling in a cold tap water bath for 15 min, 0.58 mL of 24 N H_2SO_4 was added. The tubes were mixed by inversion and incubated again in a 55°C water bath for 1.5 h with hand-shaking for 5 s every 20 min. After cooling the tubes in a cold tap water bath, 3 mL of hexane was added, and the tubes were vortex-mixed for 1 min. The tubes were centrifuged at 252 × *g* (Sorvall® RC-5B, DuPont Instrument, Wilmington, DE) for 5 min at 20°C, and the hexane layers were transferred into a GLC vial. The vial was capped and placed at -20°C until analysis.

Analysis of FA methyl esters was performed using a GLC equipped with an autoinjector, autosampler, and flame ionization detector (HP 6890N, Agilent Technologies Inc., Wilmington, DE). Samples containing methyl esters in hexane (1 µL) were injected through the split injection port (100:1) onto the column (100 m × 0.25 mm × 0.2 µm, CP-Sil 88, Varian, Lake Forest, CA). Oven temperature was set at 80°C and held for 10 min, then increased to 190°C at 12°C/min for 39 min. The temperature was then increased again to 218°C at 20°C/min and held for 21 min. Injector and detector were set at 250°C. Total run time was 71 min. Nonadecanoic acid methyl ester (C19:0) was used as a reference standard to determine recoveries and correction factors for

individual FA. Individual FA proportions were obtained by taking the specific FA area as mg of FA/100 mg of total FA.

Fatty acid identification and quantification were performed using ChemStation Software 10.01 (Agilent Technologies Inc.) by comparison with known standards (Nu-Chek Prep Inc., Elysian, MN). Total SFA were calculated by summation of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0. Total MUFA were calculated by summation of all FA with one carbon-carbon double bond, while total PUFA were calculated by summation of all FA with two or more carbon-carbon double bonds. The ratio of n-6 to n-3 FA was calculated by the sum of n-6 PUFA divided by the sum of n-3 PUFA.

Statistical Analyses

Data analyses were computed using the MIXED procedure of SAS (SAS Institute, Cary, NC). The effects of treatments (2 pasture treatments and FLT treatment) over time on FA compositions in pasture and FLT and adipose tissue were assessed using a one-way analysis of variance in a completely randomized design with repeated measures. The model included treatment, sampling time, and interaction between treatment and sampling time as fixed effects and the random effect of replication within treatment. Appropriate covariance structures for the repeated measures through time were selected based on information criteria. Pair-wise comparisons and contrasts were used to compare effects of pasture treatment and FLT. In all cases, significant effect was declared at $P < 0.05$, and trends were discussed at $P < 0.10$.

Results and Discussion

With the recent high cost of fuel and fertilizer, it may not be profitable to apply N fertilizer to pastures at the recommended application rates of 40 to 100 kg/ha of N (Poore et al., 2000; Lacefield et al., 2006). However, the practice may be still considered if it would beneficially shift FA composition in adipose tissue of steers, which motivated us to conduct the current study.

Fatty Acids Composition in Pasture Forages and FLT

Total fat concentration between the TF–NF and the TF+NF pasture treatments did not differ throughout the study (Table 4.1). Compared with TF pasture, a greater total fat concentration was measured in the FLT on wk 4, but no differences were observed on wk 12 and 16. As a cool-season grass, TF has reduced growth during hot and dry summer months (Roberts et al., 2009), which is similar to our study observed on wk 12 and 16. On this stage of growth, TF stores most of the excess nutrient in carbohydrates and lipids in leaves and stems, resulting in increased total fat concentration with progression of the grazing season.

The primary FA observed in TF was C18:3 n-3 (averaged 40.4%), whereas C18:2 n-6 was the primary FA found in the FLT (averaged 42.3%). Tall fescue had less proportion (mg/100 mg of total FA) of C16:0, C18:2 n-6, and SFA, but greater proportion of C18:3 n-3 compared with the FLT. Nitrogen fertilization increased C18:3 n-3 proportion in the TF+NF compared to the TF–NF in all weeks. This result is consistent with Boufaïed et al. (2003) who applied 120 kg N /ha on timothy grass which resulted in increased C18:3 n-3 proportion by 40%. The relatively low increase of C18:3 n-3

proportion in TF+NF when compared to TF−NF due to N fertilization (10.0, 9.0, and 4.3% increase on wk 4, 12, and 16, respectively) observed in our study may be due to the different responses to N fertilizer between TF and timothy grass. Because of its shallow and fibrous root system, timothy grass is more responsive to top-dress application of N fertilizer compared with TF that has an extensive and deep root system. In the current study, the C18:3 n-3 proportions in the pasture forages declined after wk 4 as compared with the first 4 wk. Fincham et al. (2009) reported similar results on the decline of the C18:3 n-3 proportion throughout grazing on triticale (*Triticosecale rimpaui*)/annual ryegrass (*Lolium multiflorum*), alfalfa (*Medicago sativa*)/orchardgrass (*Dactylis glomerata*), and a cool-season grass/legume mixture. Boufaïed et al. (2003) also reported that there was a positive relationship between total N concentration in the grass and C16:0, C18:2, and C18:3 FA percentages. Likewise, we previously reported that applying N fertilizer increased N concentration in TF (Noviandi et al., 2011). Nitrogen fertilization on plants increases the metabolic component, including chloroplast where the lipids are often localized, causing greater synthesis and accumulation of FA in the plant (Boufaïed et al., 2003).

Due to N fertilization, PUFA proportion increased in the TF+NF on wk 4 and 12 compared with the TF−NF, but no differences were observed on wk 16 (Table 4.2). Greater proportion of C18:3 n-3 in the TF+NF pasture resulted in increased PUFA proportion. Similarly, Boufaïed et al. (2003) reported that N fertilization increased PUFA proportion of timothy grass. Increased proportion of PUFA in the TF+NF also resulted in greater PUFA:SFA ratio in the TF+NF compared with the TF−NF throughout grazing.

Fatty Acids Composition in Adipose Tissue of Beef Steers

A major limitation in the current study is that we analyzed FA composition in adipose tissue of beef steers, but not in muscle, and the latter of which is the most commonly consumed beef product. Therefore, data reported in this study must be carefully extrapolated.

In general, consumption of TF+NF did not affect FA proportion (mg/100 mg of total FA) in adipose tissue of steers on wk 4 and 12 (Table 4.3). However, proportions of C18:0 and *cis*-9, *trans*-11 CLA in steers grazed in the TF+NF were greater compared with those in the TF–NF on wk 16. Increased *cis*-9, *trans*-11 CLA proportion in response to N fertilization of the TF pastures on wk 16 is worthy of discussion, as C18:3 n-3 proportion in pasture was greater in the TF+NF than the TF–NF starting on wk 4. Therefore, duration of N application to TF may impact *cis*-9 and *trans*-11 CLA deposition in adipose tissue of beef steers. It is likely that the increased *cis*-9, *trans*-11 CLA proportion on wk 16 may have resulted from gradual increase of C18:1 *trans*-11 FA during ruminal biohydrogenation in response to accumulative N fertilization, which in turn could provide increased C18:1 *trans*-11 in adipose tissue for the action of Δ^9-desaturase. Although not significant, C18:1 *trans*-11 in adipose tissue was numerically greater by animals grazed in the TF+NF compared to those in the TF–NF only on wk 16.

Proportion of C18:0 in adipose tissue of steers fed the FLT was lower than those that grazed TF throughout sampling weeks, whereas steers that grazed TF pasture had less C18:1 *cis*-9 compared to those fed the FLT (Table 4.3). This has important implications because C18:0 is a primary determinant of fat hardness (Wood et al., 2003; Chung et al., 2006), any dietary or production factor that enhances the conversion of C18:2 n-6 and

C18:3 n-3 to C18:0 will increase fat hardness, and then decrease palatability of beef. Kucuk et al. (2001) demonstrated that increasing dietary forage increased duodenal flow of C18:0 and C18:3 n-3, but decreased the duodenal flow of C18:1 *cis*-9 and C18:2 n-6. Feeding a high-grain diet typically decreases ruminal pH, and a prolonged reduction in ruminal pH also could cause reduction in the population of bacteria responsible for ruminal biohydrogenation (Vossenberg and Joblin, 2003; Fukuda et al., 2006; Wallace et al., 2006). Therefore, the decreased C18:0 proportion in adipose tissue of feedlot steers was likely due to direct impact of the acidic fermentative environment by feeding finishing TMR, resulting in less completion of ruminal biohydrogenation. Another possibility is that rate of digesta passage in the rumen of feedlot cattle was likely greater than that of grazing animals, resulting in less completion of ruminal biohydrogenation by the FLT. Decreased ruminal retention times on high concentrate diets would shorten the exposure time of dietary fats to ruminal microbes (Merchen, 1993), which could also contribute to reduced ruminal biohydrogenation (Kucuk et al., 2001).

Proportion of C18:1 *trans*-11 (*trans*-vaccenic acid) in the adipose tissue of steers was similar between TF pasture and the FLT on wk 4 and 12, but increased proportion was measured in adipose tissue of steers grazed on TF on wk 16 (2.43 vs. 1.68%). Proportion of *cis*-9, *trans*-11 CLA was greater in adipose tissue obtained from TF grazed steers than those fed the FLT on wk 4, 12, and 16 (0.42 vs. 0.25, 0.42 vs. 0.24, and 0.51 vs. 0.27%, respectively). There appear to be two routes of formation of the *cis*-9, *trans*-11 CLA: one route through the ruminal biohydrogenation process, and a second route, in which the precursor FA *trans*-vaccenic acid is converted to *cis*-9, *trans*-11 CLA by desaturation (removal of 2 hydrogens) at the ninth carbon of the FA by the enzyme Δ^9-desaturase.

However, endogenous synthesis of *cis*-9, *trans*-11 CLA appears to be the primary mechanism of the *cis*-9, *trans*-11 CLA production in ruminant products (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004). Therefore, maintaining increased proportions of *trans*-vaccenic acid in ruminal fluid, by pasture-finished cattle, is critical in optimizing *cis*-9, *trans*-11 CLA content in ruminant products. Ruminal fluid and serum *trans*-vaccenic acid are correlated with adipose tissue *trans*-vaccenic acid (Fincham et al., 2009). Therefore, sizable increase in the *cis*-9, *trans*-11 CLA proportion observed in beef steers grazed on TF pasture, particularly at the end of grazing, was likely resulted from elevated formation of *trans*-vaccenic acid during the ruminal biohydrogenation process. In addition, decreased ruminal retention time and its impacts on ruminal biohydrogenation due to feeding the FLT would influence the lower *cis*-9, *trans*-11 CLA proportion in the FLT compared with the grazing cattle as was previously discussed.

Although grazing steers on TF increased the *cis*-9, *trans*-11 CLA proportion in adipose, pasture-finished cattle generally have lower total FA concentration than feedlot-finished cattle; therefore, when CLA content of beef is calculated, the differences are less pronounced between pasture- and feedlot-finished cattle. Thus, it is important to consider the net CLA yield to the consumer rather than merely the concentration on per unit weight of fat (Mir et al., 2004).

Applying N fertilizer did not influence C18:2 n-6 proportion in adipose tissue of pasture-finished steers, but greater proportion of the C18:2 n-6 was found in the FLT steers compared to those on the TF pasture across all sampling weeks (averaged 1.29 vs. 1.12%, respectively). Basarab et al. (2007) and Duckett et al. (2009) reported higher proportion of C18:2 n-6 in subcutaneous fat from grain-finished cattle compared with

those finished in pasture. In this study, proportion of C18:2 n-6 in the FLT diet increased compared with those in TF pasture (averaged 43.3 vs. 8.32%), which caused greater proportion of C18:2 n-6 in adipose tissue of FLT steers. Although the FA in ruminants are at greater levels in muscle than adipose tissue (Pavan and Duckett, 2007; Wood et al., 2008), the proportion of FA in those locations are positively correlated (Basarab et al., 2007).

Adipose tissue proportion of C18:3 n-3 was similar between the TF–NF and the TF+NF treatment, but higher C18:3 n-3 proportion was observed in adipose tissue of steers fed TF pasture compared to those in the FLT on wk 4, 12, and 16. Similar to C18:2 n-6, the high proportion of C18:3 n-3 in adipose tissue of grazing steers reflects the unique FA composition in TF pasture and resultant greater intake of the C18:3 n-3. French et al. (2000) reported that steers offered grass increased intakes of n-3 PUFA because of the greater proportion of C18:3 in grass than in the concentrate.

Although de novo fatty acid biosynthesis appears to be the primary mechanism of SFA and MUFA production in cattle, grazing TF+NF pasture resulted in greater SFA and lower MUFA in adipose tissue of steers compared to those with TF–NF treatment on wk 12 and 16 (Table 4.4). Greater SFA and less MUFA proportions in adipose tissue were also observed in pasture-finishing steers compared to FLT steers on wk 4, 12, and 16. In this study, the dominant SFA in both TF and FLT treatments were C16:0 and C18:0, whereas the dominant MUFA was C18:1 *cis*-9. Pasture diets have been reported to cause more favorable ruminal pH, which may enhance microbial activity of *Butyrivibrio fibrisolvens* for isomerization and hydrogenation of PUFA into C18:0 (French et al., 2000; Jenkins et al., 2008). Increased forage intake also increases duodenal flow of C18:0

and SFA, while duodenal flow of C18:1 *cis*-9 and unsaturated FA is decreased (Kucuk et al., 2001). These FA are absorbed via the intestine into the blood stream, resulting in higher SFA and less MUFA proportions in adipose tissue.

Proportion of PUFA in adipose tissue from steers in the TF pasture increased on all sampling weeks compared to those in the FLT treatment by 11.8, 12.3, and 22.5%, respectively. French et al. (2000) and Realini et al. (2004) observed increased PUFA in intramuscular fat as a result of pasture finishing compared with grain finishing by 8 and 40%, respectively. In our study, increased C18:3 n-3 and *cis*-9, *trans*-11 CLA proportions caused the rise of PUFA proportion in adipose tissue. . However, because the *cis*-9, *trans*-11 CLA in adipose tissue comes mostly from endogenous synthesis involving the Δ^9 desaturase enzyme (Bauman et al., 2003), the effect of feed on the increasing proportion of this FA could be a minor.

Throughout all sampling weeks, no effects on the ratio of total PUFA:SFA were detected due to N fertilization or between TF pasture and the FLT treatments (Table 4.4). The PUFA:SFA ratio is mainly influenced by genetics (De Smet et al., 2004) and de novo synthesis (Bauman et al., 2003), and then much less by nutritional aspects. Thus, it is difficult in pasture-finished steers to achieve a high PUFA:SFA ratio through diet modification, because of extensive biohydrogenation of dietary PUFA, leading to production of SFA in the rumen and absorption in the small intestine. However, the ratio of total PUFA:SFA tended to be greater ($P = 0.06$) in the adipose tissue from pasture- than feedlot-finished steers on wk 16 (0.045 vs. 0.041).

Adipose tissue from grazing steers had lower n-6:n-3 ratio than those on the FLT treatment on all sampling weeks (averaged 2.74 and 5.11, respectively). Basarab et al.

(2007) and Duckett et al. (2009) reported lower n-6:n-3 ratio in subcutaneous fat from pasture- compared to grain-finished cattle. The decreased n-6:n-3 ratio is a consequence of increased C18:3 n-3 proportion and relatively constant proportion of C18:2 n-6 in adipose tissue from steers on TF pasture compared to FLT steers. Steers finished on high-grain diet showed a 2.5 times higher of n-6:n-3 ratio as those finished on pasture (Basarab et al., 2007; Duckett et al., 2009). However, the fold of increase in the n-6:n-3 ratio can be higher (i.e., between 9.3 and 10.5) when they were examined in beef muscles (Lorenz et al., 2002; Nuernberg et al., 2002).

Carcass Characteristics

Nitrogen fertilization on TF pasture had no effects on carcass characteristics measured (Table 4.5). We previously reported that there were no noticeable effects of N fertilization on TF nutrient proportion and animal performance (Noviandi et al., 2011). Therefore, it is not surprising that there were no effects on carcass characteristics between steers grazed on the TF−NF and the TF+NF pasture. Keane and Allen (1999) reported that level of N fertilizer (57 vs. 204 to 227 kg N/ha) did not affect herbage nutritive value, animal performance, and HCW, leading to no effects on carcass composition and meat quality traits of steers.

Compared to TF pasture-finished steers, steers on the FLT had larger back fat, rib fat, and ribeye area (0.51 vs. 0.22 cm, 0.65 vs. 0.22 cm, and 13.9 vs. 10.2 cm^2, respectively). This is a direct result of increased body size in steers fed TMR rather than grazed on TF (611 vs. 497 kg; $P < 0.05$; data not presented). Similarly, Realini et al. (2004), Kerth et al. (2007), and Faulkner et al. (2010) reported larger back fat, rib fat, and ribeye area of grain-finished steers compared to pasture-finished steers. In our study,

intramuscular fat percentage of steers grazed on TF pasture did not differ from that on the FLT, which is consistent with the result reported by French et al. (2003) who did not find any differences on intramuscular fat percentage of steers with grass or concentrate diet.

Implications

Beef producers continuously seek for better practices to improve nutritional values with beneficial FA composition to make it more attractive to consumers, and management strategies have very strong impacts on FA composition of beef. This study indicates that N fertilization on TF pasture increased *cis*-9, *trans*-11 CLA proportion on wk 16, whereas pasturing beef steers on TF increased *cis*-9, *trans*-11 CLA and lowered n-6:n-3 ratio in beef adipose tissue throughout grazing compared with feeding the FLT. Thus, 4 wk of grazing would be enough to observe significant differences on FA composition in the adipose tissue compared with typical feedlot finishing management. Although grazing on TF elicited positive FA composition in adipose tissue, consideration should be given to the impact on quality and grade of carcass, since TF pasture-finished steers had less back fat, rib fat, and ribeye area compared to those fed TMR.

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Table 4.1. Comparison of total fat, saturated, monounsaturated, and C18 fatty acid isomers in tall fescue pasture and feedlot finishing TMR (n = 3)

	Wk 4			Wk 12			Wk 16			
	Treatment[1]			Treatment			Treatment			
Item	TF–NF	TF+NF	FLT	TF–NF	TF+NF	FLT	TF–NF	TF+NF	FLT	SEM
Total fat, % DM	1.60	1.65	3.09	2.89	3.12	3.05	2.86	3.00	2.91	0.118
Fatty acids[2]										
C14:0	2.67	2.36	1.01	2.69	2.68	1.36	3.31	3.18	1.13	0.228
C16:0	8.70	9.42	20.4	10.4	10.8	21.4	6.46	7.70	21.0	0.241
C16:1 *cis*-9	0.17	0.18	0.18	0.27	0.28	0.11	0.16	0.20	0.09	0.026
C18:0	0.76	0.75	1.99	1.08	0.98	2.11	0.74	0.78	2.03	0.057
C18:1 *cis*-9	1.44	1.63	14.7	1.93	2.02	15.2	1.21	1.31	14.2	0.130
C18:1 *cis*-11	0.27	0.31	1.52	0.41	0.42	1.45	0.28	0.35	1.44	0.045
C18:2 n-6	7.40	8.25	43.0	10.3	10.5	40.3	6.44	7.04	43.5	0.435
C18:3 n-3	40.4	44.4	6.53	38.7	42.2	8.40	37.5	39.1	8.17	0.370

	Contrast[3], *P*-value											
	Wk 4				Wk 12				Wk 16			
	1	2	3	4	1	2	3	4	1	2	3	4
Total fat	0.74	< 0.01	< 0.01	< 0.01	0.17	0.40	0.69	0.69	0.41	0.74	0.62	0.83
C14:0	0.28	< 0.01	< 0.01	< 0.01	0.95	< 0.01	< 0.01	< 0.01	0.71	< 0.01	< 0.01	< 0.01
C16:0	0.01	< 0.01	< 0.01	< 0.01	0.21	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C16:1 *cis*-9	0.90	0.90	0.97	0.85	0.95	< 0.01	< 0.01	< 0.01	0.13	0.04	< 0.01	< 0.01
C18:0	0.95	< 0.01	< 0.01	< 0.01	0.16	< 0.01	< 0.01	< 0.01	0.49	< 0.01	< 0.01	< 0.01
C18:1 *cis*-9	0.20	< 0.01	< 0.01	< 0.01	0.53	< 0.01	< 0.01	< 0.01	0.52	< 0.01	< 0.01	< 0.01
C18:1 *cis*-11	0.48	< 0.01	< 0.01	< 0.01	0.84	< 0.01	< 0.01	< 0.01	0.23	< 0.01	< 0.01	< 0.01
C18:2 n-6	0.08	< 0.01	< 0.01	< 0.01	0.62	< 0.01	< 0.01	< 0.01	0.21	< 0.01	< 0.01	< 0.01
C18:3 n-3	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[1]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer; and FLT = feedlot finishing TMR.

[2]Fatty acid composition was expressed as mg/100 mg of total fatty acids.

[3]Contrast: 1 = TF–NF vs. TF+NF; 2 = TF–NF vs. FLT; 3 = TF+NF vs. FLT; and 4 = TF vs. FLT.

Table 4.2. Comparison of fatty acid families in tall fescue pasture and feedlot finishing TMR (n = 3)

	Wk 4			Wk 12			Wk 16			
	Treatment[2]			Treatment			Treatment			
Fatty acids[1]	TF–NF[2]	TF+NF	FLT	TF–NF	TF+NF	FLT	TF–NF	TF+NF	FLT	SEM
SFA	26.2	27.1	44.7	32.4	30.4	45.7	26.5	27.2	44.6	0.31
MUFA	31.7	27.6	18.5	26.9	26.5	17.6	32.3	32.1	18.1	0.33
PUFA	42.1	45.4	36.7	40.7	43.1	36.7	41.2	40.7	37.3	0.34
PUFA:SFA	1.61	1.67	0.82	1.26	1.42	0.80	1.55	1.50	0.83	0.025

Contrast[3], *P*-value

	Wk 4				Wk 12				Wk 16			
	1	2	3	4	1	2	3	4	1	2	3	4
SFA	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.05	< 0.01	< 0.01	< 0.01
MUFA	< 0.01	< 0.01	< 0.01	< 0.01	0.22	< 0.01	< 0.01	< 0.01	0.51	< 0.01	< 0.01	< 0.01
PUFA	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.24	< 0.01	< 0.01	< 0.01
PUFA:SFA	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.06	< 0.01	< 0.01	< 0.01

[1]Fatty acid composition was expressed as mg/100 mg of total fatty acids. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; and PUFA = polyunsaturated fatty acids.

[2]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer; and FLT = feedlot finishing TMR.

[3]Contrast: 1 = TF–NF vs. TF+NF; 2 = TF–NF vs. FLT; 3 = TF+NF vs. FLT; and 4 = TF vs. FLT.

Table 4.3. Effect of pasture or feedlot finishing treatments on saturated, monounsaturated, and C18 fatty acid isomers in adipose tissue of beef steers (n = 3)

	Wk 4			Wk 12			Wk 16			
	Treatment[2]			Treatment			Treatment			
Fatty acids[1]	TF–NF	TF+NF	FLT	TF–NF	TF+NF	FLT	TF–NF	TF+NF	FLT	SEM
C14:0	3.82	3.86	3.37	3.50	3.67	3.35	3.69	3.56	3.26	0.201
C16:0	29.6	29.8	29.9	28.1	28.6	29.8	27.5	28.6	28.3	0.52
C16:1 *cis*-9	5.12	4.85	4.78	5.04	4.71	5.38	4.76	4.84	5.26	0.312
C18:0	14.0	13.8	10.5	13.8	15.6	9.21	13.2	14.2	9.00	0.363
C18:1 *cis*-9	35.0	35.7	39.4	36.1	35.5	40.6	37.4	36.0	43.0	0.54
C18:1 *trans*-11	1.82	1.86	1.63	2.01	2.02	1.99	2.31	2.54	1.68	0.155
C18:2 n-6	1.15	1.10	1.29	1.08	1.11	1.29	1.20	1.07	1.28	0.062
cis-9, *trans*-11 CLA	0.43	0.41	0.25	0.41	0.42	0.24	0.44	0.57	0.27	0.021
C18:3 n-3	0.42	0.41	0.22	0.38	0.40	0.17	0.41	0.44	0.18	0.038

	Contrast[3], *P*-value											
	Wk 4				Wk 12				Wk 16			
	1	2	3	4	1	2	3	4	1	2	3	4
C14:0	0.88	0.30	0.30	0.01	0.69	0.69	0.38	0.19	0.69	0.30	0.38	0.05
C16:0	0.91	0.78	0.93	0.67	0.54	0.02	0.08	< 0.01	0.12	0.26	0.78	0.58
C16:1 *cis*-9	0.66	0.55	0.89	0.47	0.55	0.55	0.42	0.08	0.89	0.55	0.55	0.11
C18:0	0.74	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	< 0.01	< 0.01	< 0.01
C18:1 *cis*-9	0.25	< 0.01	< 0.01	< 0.01	0.37	< 0.01	< 0.01	< 0.01	0.04	< 0.01	< 0.01	< 0.01
C18:1 *trans*-11	0.37	0.88	0.31	0.41	0.56	0.31	0.59	0.26	0.31	< 0.01	< 0.01	< 0.01
C18:2 n-6	0.65	0.13	0.06	0.01	0.78	0.04	0.06	< 0.01	0.18	0.38	0.04	0.02
cis-9, *trans*-11 CLA	0.47	< 0.01	< 0.01	< 0.01	0.59	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:3 n-3	0.57	< 0.01	< 0.01	< 0.01	0.80	< 0.01	< 0.01	< 0.01	0.78	< 0.01	< 0.01	< 0.01

[1]Fatty acid composition was expressed as mg/100 mg of total fatty acids.

[2]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer; and FLT = feedlot finishing TMR.

[3]Contrast: 1 = TF–NF vs. TF+NF; 2 = TF–NF vs. FLT; 3 = TF+NF vs. FLT; and 4 = TF vs. FLT.

Table 4.4. Effect of pasture or feedlot finishing treatments on fatty acid families in adipose tissue of beef steers (n = 3)

	Wk 4			Wk 12			Wk 16			
	Treatment[2]			Treatment			Treatment			
Fatty acids[1]	TF–NF	TF+NF	FLT	TF–NF	TF+NF	FLT	TF–NF	TF+NF	FLT	SEM
SFA	50.8	50.7	47.2	49.2	50.5	45.2	48.0	48.9	43.0	0.33
MUFA	47.2	47.3	51.0	48.8	47.5	53.0	49.9	49.0	55.2	0.35
PUFA	2.09	1.99	1.83	1.98	2.00	1.77	2.15	2.16	1.76	0.098
PUFA:SFA	0.041	0.039	0.039	0.040	0.040	0.039	0.045	0.044	0.041	0.0020
n-6:n-3	2.72	2.71	5.91	2.84	2.81	7.39	2.89	2.46	7.14	0.273

Contrast[3], *P*-value

	Wk 4				Wk 12				Wk 16			
	1	2	3	4	1	2	3	4	1	2	3	4
SFA	0.79	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	< 0.01	< 0.01	< 0.01
MUFA	0.61	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	< 0.01	< 0.01	< 0.01
PUFA	0.46	0.08	0.22	0.03	0.97	0.13	0.12	0.03	0.97	0.03	0.30	< 0.01

PUFA:SFA	0.72	0.52	0.85	0.42	0.85	0.85	0.93	0.72	0.85	0.20	0.32	0.06
n-6:n-3	0.98	< 0.01	< 0.01	< 0.01	0.98	< 0.01	< 0.01	< 0.01	0.30	< 0.01	< 0.01	< 0.01

[1]Fatty acid composition was expressed as mg/100 mg of total fatty acids. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; and PUFA = polyunsaturated fatty acids.

[2]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer; and FLT = feedlot finishing TMR.

[3]Contrast: 1 = TF–NF vs. TF+NF; 2 = TF–NF vs. FLT; 3 = TF+NF vs. FLT; and 4 = TF vs. FLT.

Table 4.5. Effect of pasture or feedlot finishing treatments on carcass characteristics of beef steers (n = 3)

	Treatment[1]				Contrast[2], *P*-value			
Item	TF–NF	TF+NF	FLT	SEM	1	2	3	4
Back fat, cm	0.21	0.23	0.51	0.049	0.65	<0.01	< 0.01	< 0.01
Rib fat, cm	0.21	0.23	0.65	0.070	0.86	< 0.01	< 0.01	< 0.01
Ribeye area, cm²	10.6	9.87	13.9	0.562	0.24	< 0.01	< 0.01	0.04
Intramuscular fat, %	4.19	4.25	4.94	0.325	0.86	0.06	0.08	0.20

[1]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer; and FLT = feedlot finishing TMR.

[2]Contrast: 1 = TF–NF vs. TF+NF; 2 = TF–NF vs. FLT; 3 = TF+NF vs. FLT; and 4 = TF vs. FLT.

CHAPTER 5

EFFECTS OF ENERGY SUPPLEMENTATION IN PASTURE FORAGES ON IN VITRO RUMINAL FERMENTATION CHARACTERISTICS IN CONTINUOUS CULTURES[1]

Introduction

Finishing steers in a forage-based system has been extensively studied over the years as an alternative to high-grain finishing diet in a feedlot. However, cattle consuming diets solely comprised of forages are often unable to meet desired levels of production. The main challenge of high quality forage-fed beef production is the lack of energy and low N utilization efficiency in the rumen. Noviandi et al. (2012) reported that by the progression of grazing season, the non-fibrous carbohydrate (**NFC**) concentration of pasture forages decreased, causing increased ruminal ammonia-N (**NH_3-N**) concentration in grazing steers. The increased ruminal NH_3-N concentration may reflect reduced ruminal capture of CP in pasture forages for microbial protein synthesis due to the lack of energy in the rumen. Fieser and Vanzant (2004) stated that supplementing energy in forage-based steer diets can improve N utilization efficiency. In addition, energy supplementation in forage diet also improved the efficiency of feed utilization by increasing ruminal propionate proportion, decreasing acetate-to-propionate (**A:P**) ratio, and reducing methane (**CH_4**) production by the provision of readily fermentable energy sources for microbes during ruminal fermentation (Reis et al., 2001; Vibart et al., 2010).

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[1] C. T. Noviandi, J.-S. Eun, M. D. Peel, B. L. Waldron, B. R. Min, D. R. ZoBell, and R. L. Miller. Effects of energy supplementation in pasture forages on in vitro ruminal fermentation characteristics in continuous cultures. Manuscript submitted and in review process (Manuscript ID #: PAS-13-01218).

Energy supplementation in forage-based diets has been typically accomplished with corn grain (Reis et al., 2001; Pavan and Duckett, 2008; Duckett et al., 2009). For instance, corn grain supplementation on steers grazing tall fescue (**TF**; *Festuca arundinacea* Schreb.) increased ADG, carcass weight, and subcutaneous fat thickness in carcasses (Pavan and Duckett, 2008). However, feeding starch-based energy supplements, such as corn grain, has been shown to cause depressions in forage intake as well as negative associative effects on fiber digestibility (Pordomingo et al., 1991). Dried distillers grains with solubles (**DDGS**) is a by-product of the dry corn milling industry. Although low in starch, DDGS is high in digestible fiber and contains 11 to 12% fat (Lodge et al., 1997), and thus it may a viable source of supplemental energy to forage-based ruminant diets. Effects of DDGS supplementation on increases in ADG and carcass weight of the grazing heifers and steers have been well documented (MacDonald et al., 2007; Griffin et al., 2012).

Decreasing protein degradation in the rumen has been targeted on pasture forage-based diets for cattle to optimize dietary N utilization by ruminants. Condensed tannins (**CT**), polymers of flavanol units, can form complexes with numerous types of molecules, including proteins, polysaccharides, nucleic acids, and minerals (Haslam, 1989). The CT from forage species, including birdsfoot trefoil (**BT**; *Lotus corniculatus* L.), not only bind to protein by hydrogen bonding at near-neutral pH (pH 6.0 to 7.0) in the rumen to form CT-protein complexes, but also dissociate and release bound protein at pH less than 3.5 in the abomasum (Barry et al., 2001; Mueller-Harvey, 2006). Thus, CT-containing plants can protect dietary protein against degradation in the rumen and increase N utilization, resulting in reduced nitrogenous waste excretion and improved amino acid supply of

animal needs and nutritional status of the animal. In addition, there is a body of evidence indicating that feeding CT-containing forages decreases CH_4 production by ruminants (Woodward et al., 2001; Waghorn et al., 2002; Tavendale et al., 2005).

The objective of this study was to test a hypothesis that supplementation of corn grain or DDGS in grass monocultures [TF without (**TF−NF**) or with N fertilizer (**TF+NF**)] and low-CT [TF and alfalfa (*Medicago sativa*)] and high-CT grass-legume mixtures (TF and BT) would improve in vitro ruminal fermentation by increased N utilization and decreased CH_4 production in continuous cultures. The study focused on fermentative benefits by feeding grass-legume mixtures compared with the TF+NF and their interactions with energy supplementation.

Materials and Methods

Pasture Forage Harvest and Dietary Treatments

Pasture forages used in this study were TF−NF, TF+NF, TF-alfalfa mixture (**TF+AF**), and TF-BT mixture (**TF+BT**). The forages were planted in a randomized complete block design with four replications on August 4, 2010 at the Utah State University Intermountain Irrigated Pasture Project Farm (Lewiston, UT). Irrigation was used for establishment and during the growing season. Forages utilized for this study was harvested on July 8, 2011. Plots were harvested to a height of 8 cm with a sickle bar harvester (Swift Machine & Welding LTD, Swift Current, SK, Canada). This was the second harvest of the season, and therefore the TF was in the vegetative stage, while the BT was at approximately 5% bloom and the alfalfa late bud to 1% bloom. Forage samples were placed in sealed plastic bags, immediately cooled and transported to the

laboratory to be frozen at -40°C. Pasture forage samples for dietary treatments were freeze-dried (FreeZone 12 L Freeze Dry Systems, Labconco Corp., Kansas City, MO), and then ground to pass a 4.0-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ).

One control (no energy supplementation) and 2 types of energy supplementation (30% DM corn grain or 30% DM DDGS) were combined with 4 types of pasture forages (TF–NF, TF+NF, TF+AF, or TF+BT), resulting in 12 dietary treatments. The corn grain used in this study contained 10.4% CP, 9.12% NDF, 3.14% ADF, 73.5% starch, and 4.43% ether extract (**EE**), whereas the DDGS contained 28.4% CP, 34.3% NDF, 11.3% ADF, 3.55% starch, and 12.4% EE on a DM basis. The corn grain and the DDGS were ground to pass a 4.0-mm screen (standard model 4) in order to be supplemented in the pasture forages. A subsample of each dietary treatment was pooled, and ground to pass a 1.0-mm screen (standard model 4), and stored for nutritive value determination.

Continuous Culture Operation, Feeding Schedule, and Experimental Design

Rumen inoculum was taken from 3 ruminally fistulated beef cows fed forage diet of alfalfa and TF hay ad libitum. Care, handling, and sampling of the donor animals were approved by the Utah State University Institutional Animal Care and Use Committee.

Ruminal fluid was obtained from various locations within the rumen, placed in preheated insulated container, transported to the laboratory, and strained through polyester material (PeCAP, pore size 355 μm; B & SH Thompson, Ville Mont-Royal, QC, Canada). Approximately 700 mL of strained ruminal fluid was added to 8 continuous culture fermentors.

A dual-flow continuous culture system based on Teather and Sauer (1988) was used, and it consisted of 1 L gas tight fermentor vessels (Prism Research Glass, Inc., Research Triangle Park, NC). The design of the fermentors and the operation of the continuous cultures are described by Williams et al. (2011). Briefly, anaerobic condition in the fermentors was ensured by maintaining a constant flow of 20 mL/min CO_2. Artificial saliva (Slyter et al., 1966) was delivered at a rate of 0.78 mL/min using a pump (Model 323, Watson-Marlow Inc., Wilmington, MA) to maintain a fractional dilution rate of 6.3%/h. Cultures were continuously stirred by a central paddle attached to an electric motor.

Each independent run lasted 9 d. The first 7 d of each run allowed for adaptation to the diet, and the last 2 d was for data and sample collection. Each fermentor received a total of 15 g of DM/d according to the following protocol: 4 equal portions of pasture forages, fed at 0600, 1200, 1800, and 2400 h; and 2 equal portions of energy supplement, fed at 1200 and 2400 h. Diets were manually fed to the fermentor through a feed port on the fermentor vessel after the diets were gently mixed.

Dietary treatments were tested in a split-plot design with energy supplementation as a whole plot and pasture forage as a subplot with three independent runs as replicates (n = 3), and fermentor was an experimental unit.

Sampling and Chemical Analysis

Analytical DM and OM concentrations of dietary treatment samples were determined by oven drying at 105°C overnight and by ashing at 550°C for 5 h, respectively, while N concentration was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) according to AOAC (2000). Neutral detergent fiber and ADF

concentrations, both inclusive of residual ash, were determined according to Van Soest et al. (1991) and Van Soest and Robertson (1985), respectively, as modified for use with an ANKOM^{220} fiber analyzer (ANKOM Technology, Macedon, NY). Heat stable α-amylase and sodium sulfite were used in the NDF analysis, and NDF is expressed inclusive of residual ash. Ground (i.e., 1.0 mm screen) samples of 500 mg were weighed in duplicate into nylon bags with a 50 μm pore size and placed into the fiber analyzer for 75 min for NDF analysis, and subsequently for 60 min for ADF analysis. After each procedure, bags were rinsed in acetone. Ether extract was analyzed using ANKOM XT 10 Extractor (ANKOM Technology). Total extractable CT in forage samples and experimental diets was determined using a butanol-HCl colorimetric procedure (Terrill et al., 1992). Concentration of NFC was calculated using the formula: NFC (%) = 100 - (NDF % + CP % + EE % + ash %).

All data collection, sampling, and analysis of culture content from continuous fermentors were independently performed in each run. At d 8 and 9 of each run, ruminal culture pH was measured through a pH electrode connected to a pH meter (model 63, Jenco Instruments, Inc., San Diego, CA) at 1 and 2 h after the 1200- and 1800-h feeding times. At the same time points with pH measurement, CH_4 samples were collected from the headspace gas of each fermentor using a 10 μL gastight syringe (Hamilton Co., Reno, NV) and analyzed for CH_4 with a GLC (model CP-3900, Varian, Walnut Creek, CA). Daily CH_4 production (m*M*/d) was calculated as reported by Jenkins et al. (2003) using the equation: CH_4 proportion in fermentor headspace (m*M*/mL) × CO_2 gas flow through the fermentor headspace (20 mL/min) × 60 min × 24 h.

Immediately after CH_4 sampling at 1300 and 1900 h, 5 mL of culture content were taken and added to 1 mL of 25% meta-phosphoric acid and then stored at -40°C for VFA determination. Ruminal VFA were separated and quantified using a GLC (model 6890 series II, Hewlett Packard Co., Avandale, PA) with a capillary column (30 m × 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame ionization detection. The oven temperature was held at 170°C for 4 min, increased to 185°C at a rate of 5°C/min, then increased by 3°C/min to 220°C and held at this temperature for 1 min. The injector and the detector temperatures were 225 and 250°C, respectively, with helium as the carrier gas (Eun and Beauchemin, 2007). At the same time as VFA sample collection, another 5 mL of culture content were collected from each fermentor, mixed with 1% sulfuric acid, and stored frozen (-40°C) for NH_3-N analysis. Concentration of NH_3-N was determined as described by Rhine et al. (1998) using a plate reader (MRX^e, Dynex Technologies, Chantilly, VA).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute, 2012). The model used is described by:

$$Y_{ijk} = \mu + ES_i + Run_j(ES_i) + PF_k + ES_i \times PF_k + e_{ijk}$$

where,

Y_{ijk} = individual response variable measured,

μ = overall mean,

ES_i = fixed effect of energy supplement (i = 1 to 3),

$Run_j(ES_i)$ = whole plot error,

PF_k = fixed effect of pasture forage (k = 1 to 4),

$ES_i \times PF_k$ = fixed effect of interaction between energy supplement and pasture forage,

e_{ijk} = subplot error.

Energy supplements were compared with the whole plot error term, whereas pasture forages and interaction between energy supplements and pasture forages were tested using the subplot error term. Comparisons of means for energy supplement and pasture forage were done by contrast test with Tukey's HSD test when the effects of energy supplement and pasture forage ($P \leq 0.10$) were detected by the model. In all cases, significant effects were declared at $P \leq 0.05$, and trends were discussed at $P \leq 0.10$.

Results and Discussion

Nutrient Composition of Dietary Treatments

Concentration of CP in dietary treatments increased with DDGS supplementation but decreased with corn supplementation (Table 5.1). The greatest CP concentration was found in the TF+BT (averaged at 17.5%), while the TF+NF and the TF+AF had greater CP concentrations than the TF–NF (16.2 and 16.2 vs. 14.4%, respectively). Additionally, corn supplementation in pasture forages resulted in 2-fold increase in NFC concentration, whereas DDGS supplementation increased NFC concentration by 23% compared with no energy supplementation. Corn supplementation in pasture forages caused lower CP and greater NFC concentrations due to low CP and high starch concentration of corn grain. Meanwhile, greater concentrations of CP and EE in DDGS resulted in greater CP and EE concentrations in the diets supplemented with DDGS. Similar CP concentrations between the TF+NF and the TF-legume mixtures indicate that interseeding TF with alfalfa or BT resulted in a substitution effect for N fertilizer. Legumes fix atmospheric N through a

symbiotic relationship with bacteria that infect the roots forming nodules. As the nodules slough off and decay over time, some of the N that has been fixed is released for uptake by the associated grasses, which in turn increases yield and protein concentration of the grasses in mixed pasture. Barnett (2006) reported that 30% stand of legume in the pasture can supply 13 to 23 kg of N per year to the grasses in the pasture.

Supplementing either corn or DDGS decreased NDF and ADF concentrations, but increased EE concentration in the diets (Table 5.1). In general, DDGS contains 38 to 40% NDF (Schingoethe et al., 2009), and it is high in digestible fiber (62 to 71% digestible; Birkelo et al., 2004; Vander Pol et al., 2009) and contains approximately 11.5% EE (Lodge et al., 1997). Consequently, DDGS supplementation lowered NDF and ADF concentrations, but increased EE concentration compared with pasture forages with no energy supplementation. Regardless of energy supplementation, lower NDF concentration was noticed on the TF+AF and the TF+BT treatments compared with the TF–NF and the TF+NF, but ADF concentration was similar among pasture forages with 26.2% on average. The lower NDF concentrations of TF-legume mixture compared with those of TF monocultures was related to the fiber concentrations of alfalfa and BT. Owens et al. (2012) reported that NDF of alfalfa and BT were lower than TF (44.0 and 46.0 vs. 62.5%, respectively), resulting in lower NDF concentration when these legumes were mixed with TF in pasture.

As expected, only the TF+BT contained measurable CT concentration regardless of energy supplementation (1.55 to 2.57%; Table 5.1), but corn and DDGS supplementations diluted the CT concentration in the TF+BT pasture forage. Birdsfoot trefoil contains 0.5 to 4.70% CT for various cultivars (Barry and McNabb, 1999;

Scharenberg et al., 2007; Grabber, 2009), whereas the TF+BT without energy supplementation had 2.57% CT, indicating that the mixed culture in pasture would not interfere with CT biosynthesis of BT. In a 2-year study, Wen et al. (2003) found that CT concentrations were 55 to 100% greater when BT was grown in a mixture with TF compared with BT in a pure stand. The greater concentrations of CT in BT in mixed pastures compared with those in BT from monocultures may be associated with the changes in its morphology; in the mixed pasture, BT grew shorter than TF canopy. Bryant et al. (1992) reported that light limitation could affect the balance of N- and C-based secondary metabolites which may account for increased CT in BT in the TF+BT mixture.

Effects of Diets on Ruminal pH and VFA Production

Culture pH decreased when pasture forages were supplemented with corn (6.07 on average; $P < 0.01$; Figure 5.1), while it was similar between pasture forages. Supplementing corn grain in pasture forages resulted in the greatest total VFA concentration ($P < 0.01$; Figure 5.2). The increased total VFA concentration of the cultures with corn supplemented diets was attributed to high starch in the corn grain. Previous studies reported that grain supplementation in diets increased total VFA concentration (Reis et al., 2001; Lee et al., 2006; Fuentes et al., 2009), with no change in ruminal pH (Elizalde et al., 1998; Fieser and Vanzant, 2004). In contrast, DDGS supplementation decreased the total VFA concentration compared when no energy supplement was added in pasture forages. Similar decrease in total VFA concentration was reported by Leupp et al. (2009) when they partially replaced dry-rolled corn, sunflower meal, and urea in concentrate diets with 60% DDGS. Decreased total VFA

concentration in the cultures offered DDGS may be associated with changes of microbial populations in the cultures. For example, Callaway et al. (2010) observed that starch and fiber digesting bacterial population (i.e., *Succinivibrio* and *Ruminococcus*, respectively) decreased when concentrate ingredients in the diet was replaced with up to 50% DDGS, which may have resulted in lessened ruminal fermentation.

Regardless of energy supplementation, offering the TF+NF in cultures increased total VFA concentration ($P < 0.01$), which may have resulted from enhanced ruminal fermentation, particularly NDF fraction. Messman et al. (1991) and Valk et al. (1996) indicated that rate of degradation of cell walls increased with N fertilization. Peyraud et al. (1997) reported that a high level of N fertilization (80 kg N/ha/cut) to perennial ryegrass increased NDF concentration in pastures and digestibility by lactating dairy cows, which resulted in increased total VFA concentration. In addition, we reported in a 2-yr grazing study that N fertilization on TF increased total VFA concentration which positively influenced growth performances of finishing beef cattle grazed on TF pastures (Noviandi et al., 2012). Although the TF+AF and the TF+BT had relatively higher NFC concentration than the TF+NF (22.9 and 22.1 vs. 20.0%, respectively), increased fiber fermentation may result in increased total available energy from the TF+NF, causing greater total VFA concentration. The favorable change in cell wall degradation in the TF+NF maximized ruminal fermentation in cultures when corn was supplemented.

Either corn or DDGS supplementation in pasture forages decreased acetate concentration ($P < 0.01$) in the culture, but only corn supplementation increased propionate concentration compared with no energy supplementation ($P < 0.01$), resulting in lower A:P ratio ($P < 0.01$; Figure 5.2). The high readily fermentable carbohydrate in

corn grain increases ruminal fermentation with greater propionate concentration in the culture (Reis et al., 2001). Feeding the culture with the TF+NF diet resulted in the greatest concentrations of acetate and propionate compared with the other pasture forages ($P < 0.01$). Energy supplementation × pasture forage interactions were found on the concentrations of acetate and propionate; offering the TF+NF with corn supplementation sizably decreased acetate concentration, but increased propionate concentration, leading to the lowest A:P ratio with corn supplementation, with an energy supplementation × pasture forage interaction ($P < 0.01$). These findings are in agreement with Vibart et al. (2007) and Leupp et al. (2009) who reported a decrease in A:P ratio due to lower acetate and greater propionate concentrations in the ruminal fluid when corn or DDGS was added in the diets. Lower A:P ratio on ruminal fluid with energy supplementations indicate that corn or DDGS supplementations may enhance the carbohydrate utilization in the rumen. It is noteworthy to point out noticeable increases in total VFA and propionate concentrations due to corn supplementation in the TF+BT. Propionate is quantitatively the most important VFA precursor of glucose synthesis, and therefore has a major impact on hormonal release and tissue distribution of nutrients (Nagaraja et al., 1997), which is particularly important to rapidly growing cattle. Consequently, increased propionate concentration as a result of supplementing corn in the TF+BT would contribute to improving nutrient utilization by grazing cattle.

Effects of Diets on Ruminal N Metabolism

Lower NH_3-N concentration in cultures was detected by corn or DDGS supplementation in pasture forages compared with no energy supplementation ($P < 0.01$; Figure 5.3). Ruminal NH_3-N concentration is a result from a balance between production

(proteolysis) and assimilation (De Visser et al., 1997), and thus any efforts to maximize N utilization in the rumen should involve an optimal balance between the 2 metabolic processes. Yet, to optimize dietary N utilization, particularly pasture forages, in ruminants, protein degradation in the rumen should be decreased, whereas N use by ruminal microbes must be increased (Hoover and Stokes, 1991). It is believed that energy is the most limiting factor in microbial growth (Bach et al., 2005), and consequently increased NFC concentration due to corn supplementation in pasture forages tested in this study should contribute to decreased NH_3-N concentration. To the contrary, the decreased NH_3-N concentration with DDGS supplementation in pasture forages may have resulted from reduced microbial proteolysis. Given the fact that diets supplemented with DDGS contained the greatest CP concentrations (19.2% on average), it was expected that the DDGS supplementation in diets would increase NH_3-N concentration in cultures. As we already discussed on the potentially negative impacts of 30% DDGS supplementation in pasture forages on ruminal fermentation, the decreased NH_3-N concentration in response to DDGS supplementation further suggests detrimental effects of DDGS on microbial fermentation when supplemented in pasture forages. Nevertheless, most of the protein in DDGS is yeast cells that have been heated during distillation and concentration, and consequently the protein was denatured and resistant to lyses and microbial degradation (Klopfenstein et al., 2008). With RUP in DDGS ranged from 40.8 to 49.5% (Belyea et al., 2010), it is not surprising that the ruminal NH_3-N concentration was lower with DDGS supplementation compared with no energy supplementation.

Among the pasture forages, the reduction in NH_3-N concentration on the TF+BT is notable ($P < 0.01$; Figure 5.3). Similarly, Williams et al. (2010) reported that NH_3-N

concentration was reduced when replacing alfalfa hay with BT in 100% forage diets in continuous cultures. The N binding effects of CT have been well documented (Waghorn et al., 1987; Barry and McNabb, 1999; Beauchemin et al., 2007). The CT present in BT have been found to inhibit the growth of proteolytic bacteria and may also precipitate plant protein, making it less available for proteolysis (Min et al., 2000; Molan et al., 2001), thereby inhibiting ammonia production. When protein is rapidly degraded in the rumen, ammonia is produced more quickly than the microbes can utilize it for protein synthesis, resulting in more protein being degraded than synthesized (Broderick, 1995). The effect of cattle grazing a TF+BT pasture on retarding forage N degradation would enhance nutrient utilization by reducing N excretion in manure. Powell et al. (2009) reported a shift in N excretion routes by feeding CT-containing forages, because the ratio of N excreted in feces and urine was greatest for low-tannin and high-tannin BT treatments and lowest for alfalfa treatment. Reduced urinary N excretion would result in reduced environmental losses through nitrate leaching, ammonia volatilization, and nitrous oxide emissions.

Corn supplementation in pasture forages considerably decreased NH_3-N:VFA ratio due to decreased NH_3-N concentration and increased VFA concentration ($P < 0.01$; Figure 5.3). In contrast, decreased NH_3-N:VFA ratio with supplementing DDGS in pasture forages was resulted from decreased NH_3-N concentration with no net contribution by VFA concentration. Greater concentration of total VFA is associated with enhanced ruminal fermentation, whereas lower NH_3-N concentration is related to increased dietary N utilization efficiency. Under no energy or corn supplementation, the TF+NF and the TF+BT maintained similar NH_3-N:VFA ratios, which indicates that the

TF+BT was effectively fermented in cultures, resulting in similar efficiency of ruminal fermentation compared with the TF+NF.

Effects of Diets on CH_4 Production

Supplementation of corn grain in pasture forages decreased CH_4 production in cultures by 17% compared with no energy supplemented pasture forages ($P < 0.01$; Figure 5.4). This result corresponds to large reductions in A:P ratios with corn supplementation (Figure 5.2). Supplementing DDGS decreased CH_4 production due to reduced ruminal fermentation in pasture forages. In addition to the negative impacts of supplementing DDGS on ruminal fermentation, high lipid concentration of DDGS (12.4% DM) may contribute to the decreased CH_4 production. Supplementation of diets with lipids that are not protected from ruminal digestion is one strategy recognized to lower enteric CH_4 emissions (Boadi et al. 2004). McGinn et al. (2009) reported that addition of DDGS reduced CH_4 emissions by 19.9% when barley grain (35% of dietary DM) was replaced by corn DDGS in growing diet of beef cattle. Added fats decrease CH_4 production by lowering the quantity of OM fermented in the rumen, and by exerting toxic effects on cellulolytic bacteria, the activity of ruminal methanogens and protozoal numbers, and for lipids rich in unsaturated fatty acids, through the biohydrogenation process (Johnson and Johnson, 1995; Beauchemin et al., 2008).

In comparison to the other forages used in this study, cultures offered the TF+BT diets produced less CH_4 (5.93 m*M*/d; $P < 0.01$; Figure 5.4), while the TF+AF and the TF+NF had similar CH_4 production (6.79 and 6.66 m*M*/d, respectively). The reduced CH_4 production in the TF+BT diets was attributed to the high CT concentration from BT. In similar, Williams et al. (2011) reported decreased CH_4 production by 19% in

continuous cultures when BT-based dairy TMR was offered compared with alfalfa hay-based dairy TMR (7.32 vs. 9.02 m*M*/d). Although mechanisms for CT inhibition of methanogenesis are largely hypothetical, the reduction of methanogenesis in the TF+BT pasture forages may have resulted from the shift in the fermentation pathways as evidenced by clear pattern of A:P ratio in response to the TF+BT.

Beneficial effects on reduced CH_4 production due to CT have not been consistently observed (Patra and Saxena, 2010). One of the main reasons toward the discrepancies among different studies in response to feeding CT-containing forages or supplementing CT extracts is concentrations of CT in diets. Reliable and distinguishable effects of CT on CH_4 reduction can be expected only from CT concentrations > 2% DM, a threshold often not exceeded in current commercial feed supplementation with CT (Eun and Min, 2012). Hence, the challenge is to identify CT sources that can be feasibly added to the diet in a cost-effective manner that also result in a net reduction in CH_4 emissions. Therefore, further research is needed to determine the net effects of grazing cattle on the TF+BT without energy supplement (2.6% CT) or with corn grain (1.8% CT) on enteric CH_4 emissions.

Implications

While supplementation of by-products, such as DDGS, has been sought to improve nutrient utilization and animal performance of grazing cattle, interseeding legumes into grass stands in a highly efficient cropping system reduces N losses by eliminating application of N fertilizer. This study showed that DDGS supplementation decreased total VFA as well as NH_3-N concentrations in cultures, which indicates that 30% DDGS

supplementation in pasture forage diets has detrimental impacts on ruminal fermentation of pasture forages. In contrast, corn grain supplementation enhanced ruminal fermentation and lowered CH_4 production. Feeding the TF+NF or the TF+BT resulted in similar patterns of ruminal fermentation in cultures, implying that BT mixed with TF can eliminate N fertilization to TF by maintaining efficient ruminal fermentation. In addition to their ability to substitute N fertilizer on grass pastures, the TF+BT mixture also decreased CH_4 production due to bioactive CT. Overall results in this study indicate that corn supplementation in pasture forages can enhance ruminal fermentation, while grass and high CT-containing legume mixtures would be a sustainable component in grazing cattle systems to improve ruminal fermentation and nutrient utilization efficiency. Further animal trials are required to prove the results of in vitro fermentation profiles in comparison of pasture forages between the TF+NF and the TF+BT mixture on animal growth and environmental performance.

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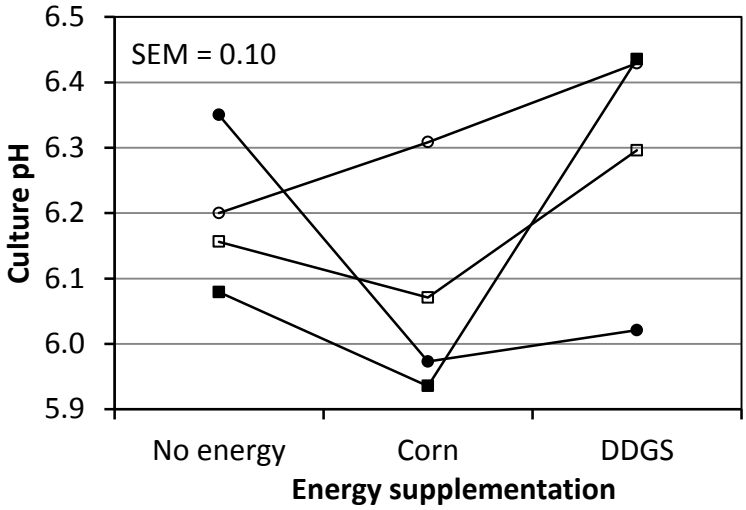

Figure 5.1. Culture pH as affected by energy supplementation and pasture forage (n = 3). No energy = full pasture forage diets without energy supplement (15 g DM pasture forage/d only); Corn = 70% DM pasture forage and 30% corn grain (10.5 g DM pasture forage/d and 4.5 g DM corn grain/d); DDGS = 70% DM pasture forage and 30% dried distilled grains with solubles (DDGS) (10.5 g DM pasture forage/d and 4.5 g DM DDGS/d); TF–NF (□) = tall fescue without N fertilizer; TF+NF (■) = tall fescue with N fertilizer; TF+AF (○) = tall fescue-alfalfa mixture; and TF+BT (●) = tall fescue-birdsfoot trefoil mixture. Significance of effect: energy supplementation ($P < 0.01$); pasture forage ($P = 0.10$); and interaction between energy supplementation and pasture forage ($P = 0.03$).

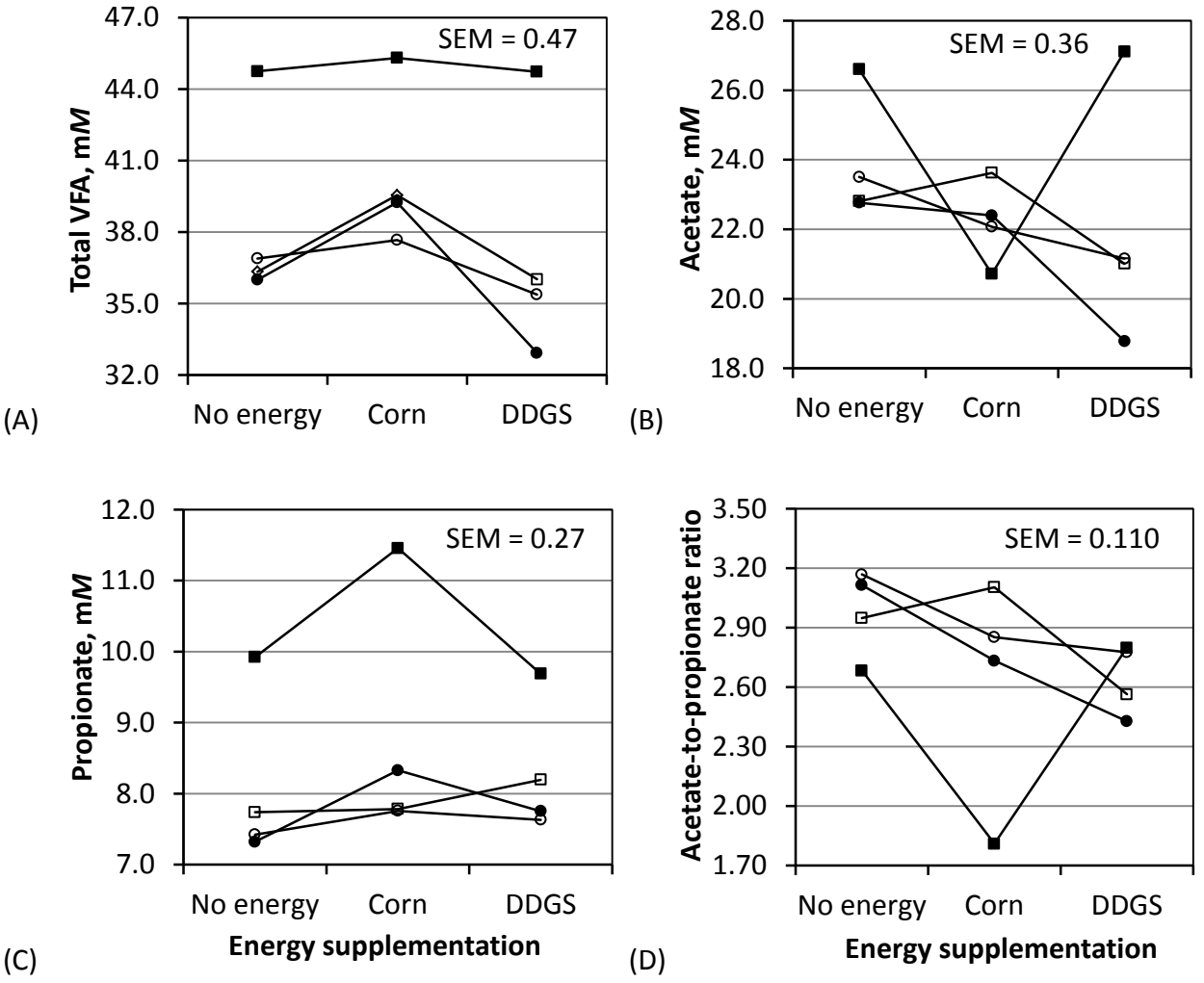

Figure 5.2. Concentration of total VFA (A), acetate (B), propionate (C), and acetate-to-propionate ratio (D) as affected by energy supplementation and pasture forage in continuous cultures (n = 3). No energy = full pasture forage diet without energy supplement (15 g DM pasture forage/d only); Corn = 70% DM pasture forage and 30% corn grain (10.5 g DM pasture forage/d and 4.5 g DM corn grain/d); DDGS = 70% DM pasture forage and 30% dried distilled grains with solubles (DDGS) (10.5 g DM pasture forage/d and 4.5 g DM DDGS/d); TF–NF (□) = tall fescue without N fertilizer; TF+NF (■) = tall fescue with N fertilizer; TF+AF (○) = tall fescue-alfalfa mixture; and TF+BT (●) = tall fescue-birdsfoot trefoil mixture. Significance of

effect: energy supplementation ($P < 0.01$); pasture forage ($P < 0.01$); and interaction between energy supplementation and pasture forage ($P < 0.05$).

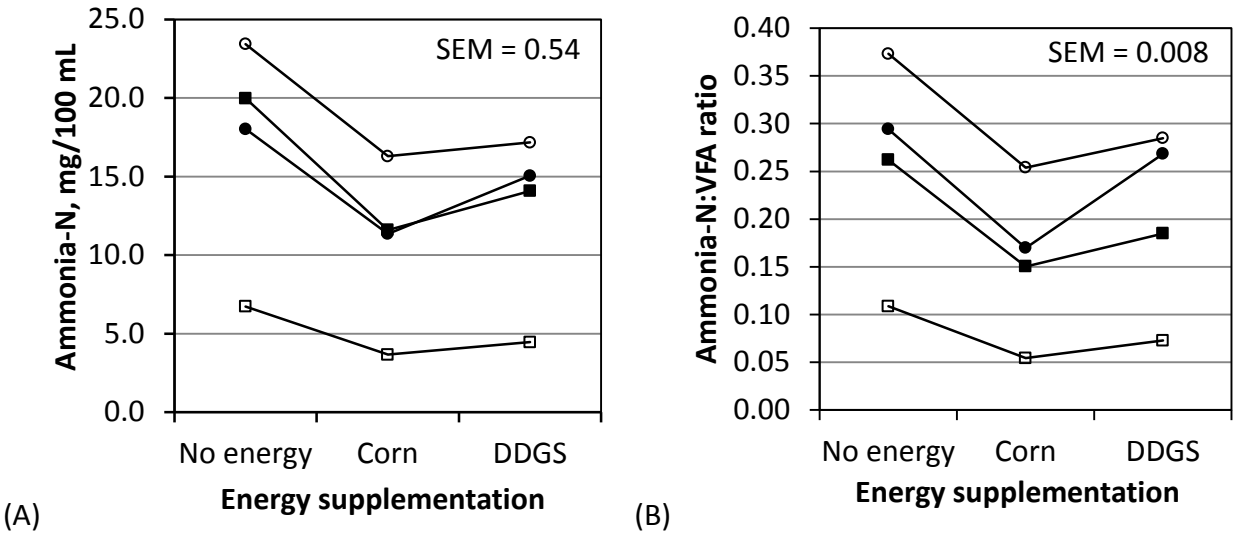

Figure 5.3. Concentration of ammonia-N (A) and ammonia-N:VFA ratio (B) as affected by energy supplementation and pasture forage in continuous cultures (n = 3). No energy = full pasture forage diet without energy supplement (15 g DM pasture forage/d only); Corn = 70% DM pasture forage and 30% corn grain (10.5 g DM pasture forage/d and 4.5 g DM corn grain/d); DDGS = 70% DM pasture forage and 30% dried distilled grains with solubles (DDGS) (10.5 g DM pasture forage/d and 4.5 g DM DDGS/d); TF–NF (□) = tall fescue without N fertilizer; TF+NF (■) = tall fescue with N fertilizer; TF+AF (○) = tall fescue-alfalfa mixture; and TF+BT (●) = tall fescue-birdsfoot trefoil mixture. Significance of effect: energy supplementation ($P < 0.01$); pasture forage ($P < 0.01$); and interaction between energy supplementation and pasture forage ($P < 0.01$).

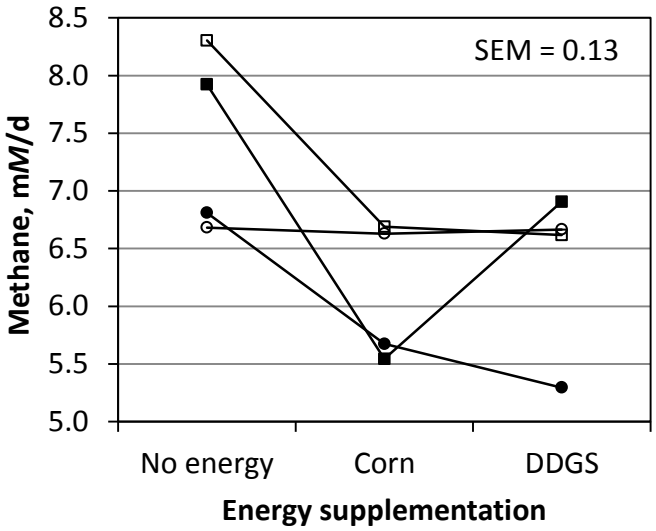

Figure 5.4. Methane production as affected by energy supplementation and pasture forage in continuous cultures (n = 3). No energy = full pasture forage diet without energy supplement (15 g DM pasture forage/d only); Corn = 70% DM pasture forage and 30% corn grain (10.5 g DM pasture forage/d and 4.5 g DM corn grain/d); DDGS = 70% DM pasture forage and 30% dried distilled grains with solubles (DDGS) (10.5 g DM pasture forage/d and 4.5 g DM DDGS/d); TF–NF (□) = tall fescue without N fertilizer; TF+NF (■) = tall fescue with N fertilizer; TF+AF (○) = tall fescue-alfalfa mixture; and TF+BT (●) = tall fescue-birdsfoot trefoil mixture. Significance of effect: energy supplementation ($P < 0.01$); pasture forage ($P < 0.01$); and interaction between energy supplementation and pasture forage ($P < 0.01$).

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Table 5.1. Nutrient composition (% DM) of dietary treatments provided to continuous cultures (n = 3)

	Diet[2]											
	No energy				Corn				DDGS			
Item[1]	TF–NF	TF+NF	TF+AF	TF+BT	TF–NF	TF+NF	TF+ AF	TF+BT	TF–NF	TF+NF	TF+ AF	TF+BT
OM	84.9	85.3	86.6	86.9	89.4	89.9	90.1	90.4	88.7	88.8	89.2	89.0
CP	13.5	15.5	16.3	17.8	12.1	13.5	14.0	14.6	17.7	19.6	19.4	20.2
NDF	56.8	56.7	51.4	52.6	45.7	45.4	43.6	42.6	51.8	50.5	49.8	49.3
ADF	31.7	31.4	30.1	31.4	21.9	21.2	23.3	22.0	25.6	24.2	26.6	25.1
EE	2.70	2.54	2.68	2.77	2.81	2.88	2.66	3.02	5.50	5.57	5.55	5.74
NFC	13.6	12.1	18.0	15.5	30.6	30.1	31.6	32.2	18.3	17.8	19.1	18.5
CT	0.95	0.47	0.60	2.57	0.67	0.82	0.74	1.77	0.55	1.17	0.92	1.55

[1]EE = ether extract; NFC = non-fibrous carbohydrate (100 – CP – NDF – ether extract – ash); and CT = condensed tannins.

[2]No energy = full pasture forage diet without energy supplement; Corn = 70% DM pasture forage and 30% corn grain; DDGS = 70% DM pasture forage and 30% dried distilled grains with solubles; TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer; TF+AF = tall fescue-alfalfa mixture; and TF+BT = tall fescue-birdsfoot trefoil mixture.

CHAPTER 6

EFFECTS OF GRASS-LEGUME MIXED PASTURE FORAGES WITH DIFFERENT COMPOSITION RATIOS ON MICROBIAL FERMENTATION IN CONTINUOUS CULTURES[1]

Introduction

Tall fescue (*Schedonorus arundinaceus* Schreb.; **TF**) is the most widely planted grass species in the humid pasture region of the United States. However, the nutritive quality of TF decreases with the progression of grazing season (Noviandi et al., 2012a). Mixed grass-legume pastures may be a good pasture management option to improve the nutritive value of pastures and animal performance. Birdsfoot trefoil (*Lotus corniculatus* L.; **BT**) in mixed grass-legume pastures not only increases CP concentration and total yield of pasture, but also increases total weight gain per hectare of steers (Wen et al., 2002). In addition, an in vitro study reported by our group showed that TF and BT mixed diets improved nutrient utilization by decreasing ammonia-N (**NH_3-N**) and methane (**CH_4**) production (Noviandi et al., 2012b).

Despite some advantages of TF-legume mixed pastures, there are still some limitations on their application to grazing cattle. One of the largest limitations is legume's poor stand persistence during the grazing season. Wen et al. (2002) found that there was a rapid decline in total yield of BT monoculture during spring to fall grazing season, which led to decreased proportion of BT in the TF and BT mixed pasture from 31.5 to 13.5%. In

[1] C. T. Noviandi, K. Neal, J.-S. Eun, M. D. Peel, B. L. Waldron, D. R. ZoBell, and B. R. Min. Effects of grass-legume mixed pasture forages with different composition ratios on microbial fermentation in continuous cultures. Manuscript submitted and in review process (Manuscript ID#: PAS-13-01259).

contrast, observations by producers and plant geneticists indicate an increased proportion of legumes in TF-legume mixed pasture during spring to fall season (May to September) in northern Utah. To our knowledge, no research has evaluated the effects of varying ratios of TF-legume mixed diets using different legume species. Thus, it was hypothesized that TF-legume mixed diets with greater proportion of legume would improve in vitro ruminal fermentation characteristics. Additionally, condensed tannins (**CT**) in BT would result in beneficial effects by reducing NH_3-N concentration and CH_4 production in continuous cultures. Therefore, the objectives of this study were to determine in vitro ruminal fermentation profiles in response to feeding various TF-legume mixed diets and assess how the dietary treatments would affect the ruminal fermentation under varying TF-legume ratios using continuous culture system. As legume pasture forages, we chose alfalfa (*Medicago sativa* L.; **AF**), BT, and cicer milkvetch (*Astragalus cicer*; **CM**) for this study.

Materials and Methods

Pasture Forages, Dietary Treatments, and Experimental Design

Forages assessed in this study were planted in a randomized complete block design with 4 replications on August 4, 2010 at the Utah State University Intermountain Irrigated Pasture Project Farm in Lewiston, UT. Irrigation was used for establishment of the pastures and during production. Nitrogen fertilizer was applied to TF monocultures at 68 kg N/ha in 3 applications during the growing season, and the applications were made the first week of April prior to plant growth and following the first (June 4th) and third harvest (July 31st). All pasture forages tested in the current study were harvested in July

2, 2012. Plots were harvested to a height of 8 cm with a Swift Current sickle bar harvester (Swift Machine & Welding LTD, Swift Current, SK, Canada). This was the second harvest of the season, and therefore the TF was in the vegetative stage, while AF, BT, and CM were at approximately 5% bloom, late bud to 1% bloom, and mid to late bud development, respectively. The nutrient composition of the forages is presented in Table 6.1.

After fresh forage samples were collected, they were immediately cooled, transported to the laboratory, and freeze-dried (FreeZone 12 L Freeze Dry Systems, Labconco Corp., Kansas City, MO). Forage samples for dietary treatments were ground to pass a 4.0-mm screen (Standard Model 4; Arthur H. Thomas Co., Swedesboro, NJ), while those for proximate and fatty acid analyses were ground to pass a 1.0-mm screen (Standard Model 4).

Nine treatments were randomly applied to a 8-unit dual-flow continuous culture fermentor system using a split-plot design with 3 TF-legume ratios as a whole plot and 3 TF-legume mixed diets as a subplot. The continuous culture fermentors were considered as experimental units, while 3 independent runs were used as replicates (n = 3). The ratios of TF:legume were 75:25 (**G75L25**), 50:50 (**G50L50**), and 25:75 (**G25L75**), while TF-legume mixed dietary treatment consisted of TF and AF (**TF+AF**), TF and BT (**TF+BT**), and TF and CM (**TF+CM**) mixtures. Because in practical situations it is not possible to maintain the exact TF-legume ratios, the mixed diets were prepared by combining pasture forages from monocultures to mimic the mixed pasture forages in ratios what we chose to test. The dietary treatments were made based on forage DM

(Table 6.1) to achieve pre-determined ratios according to TF-legume mixed pasture forage diets.

Continuous Culture Operation

Eight 1000 mL dual-flow continuous culture fermentors (Prism Research Glass, Inc., Research Triangle Park, NC) designed according to Teather and Sauer (1988) were used in 3 replicated periods of 8 d (5 d of adaptation and 3 d of data and sample collection). Each fermentor was inoculated with pooled ruminal fluid obtained from 3 rumen-fistulated beef cows fed a forage diet (AF and TF hay) ad libitum. Care, handling, and sampling of the donor animals were approved by the Utah State University Institutional Animal Care and Use Committee. Ruminal fluid was collected from various locations within the rumen, strained through a polyester material (PeCAP, pore size 355 μm; B & SH Thompson, Ville Mont-Royal, QC, Canada) into preheated insulated containers, and transported to the laboratory. Approximately 700 mL of strained ruminal fluid was added into each continuous culture fermentor.

Anaerobic condition in the fermentors was maintained by infusion of CO_2 at a rate of 20mL/min. Artificial saliva prepared according to Slyter et al. (1966) was continuously infused into fermentors at a rate of 0.78 mL/min using a pump (Model 323, Watson-Marlow Inc., Wilmington, MA) to maintain a fractional dilution rate of 6.3%/h. To mimic rumen motility, cultures were continuously stirred by a central paddle attached to an electric motor. Each fermentor received a total of 15 g of DM/d that was fed in 4 equal portions at 0600, 1200, 1800, and 2400 h.

Sample Collection

All data collection, sampling, and analysis of culture content from continuous fermentors were independently performed in each run. At d 6 and 7 of each run, ruminal culture pH data and 2 sets of 5 mL culture fluid samples for VFA and NH_3-N analysis were collected. Culture pH was measured hourly through a pH electrode connected to a pH meter (Model 63, Jenco Instruments, Inc., San Diego, CA). At 0600, 0900, 1200, 1500, and 1800 h, CH_4 samples were collected from the headspace gas of each fermentor using a 10 μL gastight syringe (Hamilton Co., Reno, NV) and analyzed for CH_4 with a GLC (Model CP-3900, Varian, Walnut Creek, CA). Daily CH_4 production (m*M*/d) was calculated as reported by Jenkins et al. (2003) using the equation: CH_4 proportion in fermentor headspace (m*M*/mL) × CO_2 gas flow through the fermentor headspace (20 mL/min) × 60 min × 24 h.

Immediately after CH_4 sampling at 0900 and 1500 h, 5 mL of culture contents were taken, added to 1 mL of 25% meta-phosphoric acid, and stored at -40°C for VFA determination. At the same times as VFA sample collection, another 5 mL of culture content was collected from each fermentor, mixed with 1% sulfuric acid, and stored frozen (-40°C) for NH_3-N analysis.

On the final day of each run (d 8), the total volume of fermentor contents was collected and blended using a blender (Master Prep, EURO-PRO Operating LLC, Boston, MA) for 1 min. The remaining ruminal fluid was filtered through polyester material (PeCAP, pore size 355 μm). For fatty acid analysis, approximately 150 mL of filtrate was collected, freeze-dried (FreeZone 12 L Freeze Dry Systems), and ground with a mortar and pestle. Another 500 mL of filtrate were centrifuged at 800 × *g* for 15 min at 4°C to

remove solids, and then the supernatant fraction was centrifuged at 27,000 × *g* for 30 min at 4°C to obtain a bacterial pellet (Yang et al., 2004). The pellets were freeze-dried and ground using a ball mill (Mixer Mill MM2000; Retsch, Haan, Germany) at 25 MHz for 4 min to a fine powder for determination of N content using an organic elemental analyzer (Flash 2000 N/Protein Analyzer, Thermo Scientific, Cambridge, UK).

Chemical Analysis

Analytical DM concentration of samples was determined by oven drying at 105°C for 3 h (AOAC, 2000; method 930.15), and OM was determined by ashing at 550ºC for 5 h (AOAC, 2000; method 942.05). Concentration of N was determined using an organic elemental analyzer (Flash 2000 N/Protein Analyzer, Thermo Scientific, Cambridge, UK). Concentrations of NDF and ADF were sequentially determined using an ANKOM200/220 Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treated with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO). Ether extract was measured (AOAC, 2000; method 2003.05) using an AnkomXT20 Fat Analyzer (ANKOM Technology). Total extractable CT concentration in forage samples and experimental diets was determined using a butanol-HCl colorimetric procedure (Terrill et al., 1992). Concentration of non-fibrous carbohydrates (**NFC**) was calculated using the formula: NFC, % = 100 - (% NDF + % CP + % ether extract + % ash).

Culture VFA were separated and quantified using a GLC (Model 6890 series II, Hewlett Packard Co., Avandale, PA) with a capillary column (30 m × 0.32 mm i.d., 1 μm

phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame ionization detection. The oven temperature was held at 170°C for 4 min, increased to 185°C at a rate of 5°C/min, then increased by 3°C/min to 220°C and held at this temperature for 1 min. The injector and the detector temperatures were 225 and 250°C, respectively, and the carrier gas was helium (Eun and Beauchemin, 2007). Concentration of NH_3-N was determined as described by Rhine et al. (1998) using a plate reader (MRX[e], Dynex Technologies, Chantilly, VA).

Fatty acid extraction of diets was performed according to procedures of O'Fallon et al. (2007), while extraction of ruminal fluid fatty acids was done based on a one-step methylation method (Sukhija and Palmquist, 1988; Jenkins, 2010). Analysis of fatty acid methyl esters was performed using a GLC equipped with an autoinjector, autosampler and flame ionization detector (HP 6890N, Agilent Technologies Inc., Wilmington, DE). Samples containing methyl esters in hexane (1 µL) were injected through the split injection port (25:1) onto the column (HP 88, Agilent Technologies Inc.). Oven temperature was set at 35°C and held for 2 min, then increased to 190°C at 12°C/min for 39 min. The temperature was then increased again to 218°C at 20°C/min and held for 16 min. Injector and detector were set at 250°C. Total run time was 66 min. Individual fatty acid proportions were obtained by taking the specific fatty acids area as a percentage of total fatty acids, and were reported as g/100 g total fatty acids. Fatty acid identification and quantification were performed using Agilent Chem Station Software (Agilent Technologies Inc.) by comparison with GLC-603 standards (Nu-Chek Prep Inc., Elysian, MN).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst., Cary, NC) using the model described below:

$$Y_{ijk} = \mu + GLR_i + Run_j(GLR_i) + MPF_k + GLR_i \times MPF_k + e_{ijk}$$

where, Y_{ijk} is the individual response variable measured; μ is the overall mean, GLR_i is the fixed effect of forage ratio between TF and legume; $Run_j(GLR_i)$ is the whole plot error; MPF_k is the fixed effect of TF-legume mixed diets; $GLR_i \times MPF_k$ is the fixed effect of interaction between TF-legume ratio and TF-legume mixed diets; and e_{ijk} is the subplot error.

The TF-legume ratio was compared with the whole plot error term, whereas TF-legume mixed diets and interaction between TF-legume ratio and TF-legume mixed diets were tested using the subplot error term. Comparison of TF-legume ratio and TF-legume mixed diets means were done by contrast test with Tukey's HSD test, when the effect of TF-legume ratio and TF-legume mixed diets ($P \leq 0.10$) was detected by the model. Significant effects were accepted when $P \leq 0.05$, and trends were discussed when $P \leq 0.10$.

Results and Discussion

Nutrient Composition of Diets

In comparison with TF, legumes contain greater CP and less fiber concentrations (Table 6.1). Thus, when TF-legume mixed diets contained a greater proportion of legume, CP concentration increased, while NDF and ADF concentrations decreased (Table 6.2). Regardless of TF-legume ratio, the TF+AF and the TF+CM had greater CP

concentrations compared with the TF+BT (averaged 14.2 and 14.3% vs. 12.7%, respectively), whereas fiber concentrations of the TF+CM were the lowest (43.9% NDF and 26.9% ADF on average). The differences in nutrient composition among TF-legume mixed diets were caused due to legume type; AF and CM showed similar CP concentration, but BT had the lowest concentration (averaged 19.4, 18.8 vs. 16.4%, respectively). Additionally, CM had the lowest NDF and ADF concentrations (averaged 25.9 and 20.4%, respectively). Similar CP concentrations between AF and CM with less fiber concentration in CM have been reported by others (Chang et al., 2002; Acharya et al., 2006; Williams et al., 2011). Differences in fiber concentrations between CM and the other 2 legumes (AF and BT) can be accounted for by the different leaf-to-stem ratio of the plants; CM has high leaf-to-stem ratio, which is 40% greater than that of AF (Baldridge and Lohmiller, 1990).

A noticeable CT concentration was only detected in BT among pasture forages (3.25% DM; Table 6.1). Consequently, dietary treatments containing BT had accountable concentrations of CT ranging from 0.91 to 2.43% (Table 6.2), and the CT concentration increased with increasing proportion of BT in the diet. The CT concentration in BT has been reported at 0.97 to 7.31% DM (Scharenberg et al., 2007; Williams et al., 2010; Lyman et al., 2012).

Primary fatty acids observed in all diets were C18:3 n-3 followed by C16:0 and C18:2 n-6 at 48.3, 20.3, and 13.4 g/100 g of total fatty acids on average, respectively. The C18:2 n-6 proportion slightly increased with increasing proportion of legume, but no noticeable differences were detected in the other fatty acid profiles due to different TF-legume ratios or TF-legume mixed diets. The increased C18:2 n-6 proportion in diets

with greater legume proportion was related to the differences of fatty acid profiles between grass and legume; it was reported that legumes normally comprised greater amounts of C18:2 n-6 than grass species (Boufaied et al., 2003; Dierking et al., 2010).

Culture pH and VFA Production

Although culture pH was affected by TF-legume ratio and mixed diet (Table 6.3), all cultures maintained a pH of at least 6.10, and the largest difference in culture pH was 0.19 unit between the TF+AF in the G50L50 and the TF+CM in the G75L25. Therefore, biological importance of the effect of culture pH in response to dietary treatments would be minimal.

None of dietary treatments influenced concentrations of total VFA, acetate, and butyrate. Similarly, Tavendale et al. (2005) and Williams et al. (2010) reported that there was no effect on total VFA concentration in vitro between AF and BT diets. In contrast, propionate concentration increased when fermentors were fed with a greater proportion of legumes in our study. The increased concentration of propionate in cultures given greater proportions of legumes may have resulted from NFC concentration of legumes. As shown in Table 6.2, the NFC concentration increased with increasing legume proportion in the mixed diets. In this study, the greatest concentration of NFC was detected in CM followed by BT and AF (averaged 40.5, 39.8, and 34.6%, respectively; Table 6.1). Regardless of TF-legume ratio, feeding the TF+CM consistently elicited increased propionate concentration compared with the TF+AF and the TF+BT (11.9 vs. 9.94 and 10.6 m*M*, respectively), resulting in decreased acetate-to-propionate ratio. The differences in propionate concentration due to TF-legume mixed diets was related to their NFC concentration which was greater in the TF+CM than the TF+AF and TF+BT

(averaged 25.1, 18.2, and 20.2%, respectively; Table 6.2). In an in vitro experiment using 5 different forage-based diets, Williams et al. (2011) reported that fermentors offered CM-based diet had greater propionate concentration and lower acetate-to-propionate ratio.

Nitrogen Metabolism

Within TF-legume ratios tested, the G25L75 resulted in the greatest NH_3-N concentration followed by the G50L50 and the G75L25 (18.9, 16.9, and 13.7 mg/100 mL on average, respectively; Table 6.3). The increased NH_3-N concentration due to greater legume proportion was associated with the greater CP concentration in the corresponding diets. As shown in Table 6.2, CP concentrations of the G75L25, the G50L50, and the G25L75 increased with increasing legume proportion in the diets (averaged at 11.4, 13.1, and 16.7%, respectively). Ruminal NH_3-N concentration is a result of a balance between production (proteolysis) and assimilation (De Visser et al., 1997). Thus, increased ruminal NH_3-N concentration due to high legume proportion in the diets may reflect an imbalance between dietary protein degradation and ruminal capture of NH_3-N for microbial protein synthesis. Although increasing legume proportion in the diets increased NFC concentration, it is likely that the increased NFC concentration would not be sufficient to incorporate the NH_3-N into microbial protein. Increased NH_3-N concentration-to-VFA concentration ratio due to increased legume proportion in the diets supports the inefficient utilization of NH_3-N for microbial protein synthesis. It is believed that energy is the most limiting factor in microbial growth (Bach et al., 2005), and thus increasing NFC as a proportion of carbohydrates typically has positive impacts on carbohydrate utilization for microbial protein synthesis. However, Lykos et al. (1997)

found that inclusion of NFC in the range of 35 to 42% DM was needed to increase energy density in the diets. In our study, the NFC concentrations were between 15 and 26% DM, and therefore it is unlikely that the NFC concentrations would affect NH_3-N utilization.

Feeding the TF+BT resulted in lower NH_3-N concentration compared with the TF+AF and the TF+CM (14.7 vs. 18.0 and 16.8 mg/100 mL on average, respectively; Table 6.3). However, the TF+BT did not consistently increase microbial N concentration. Therefore, the reduced NH_3-N concentration in cultures offered the TF+BT may have resulted from inhibitory effects of CT on the proteolysis of dietary soluble protein rather than an increase in microbial protein synthesis. Condensed tannins affect ruminal NH_3-N concentration in 2 ways: 1) reducing dietary protein degradation via formation of insoluble tannin-protein complexes or decreasing the solubility of protein (Tanner et al., 1994; Min et al., 2000); and 2) inhibiting proteolytic bacteria and/or proteolytic enzymatic activity (Patra et al., 2012). Under typical cattle feeding conditions, manipulation of ruminal protein degradation or the efficiency of N utilization in the rumen is the most effective strategy to reduce N losses (Tamminga, 1996). Using data obtained from continuous culture studies, Bach et al. (2005) reported that as efficiency of N utilization increases, NH_3-N accumulation in the fermentors decreases ($R^2 = 0.78$). Thus, the reduction in the NH_3-N concentration through CT in the TF+BT can contribute to improving utilization of dietary N in ruminal fermentation and reducing N excretion.

Although the TF+AF and the TF+CM had similar CP concentrations (averaged 14.2 and 14.3%, respectively; Table 6.2), the TF+CM produced less NH_3-N concentration than the TF+AF (averaged 16.8 vs. 18.0 mg/100 mL, respectively; Table 6.3). In a study using 6 different legumes, Lees et al. (1982) observed that CM had a thicker epidermal layer

containing smaller epidermal cells, which may increase CM resistance to mechanical damage occurred during microbial digestion. Furthermore, the authors also reported that the venation pattern and vein structure in CM were very effective in restricting digestion by rumen microbes (Lees et al., 1982). In addition, Broderick and Albrecht (1997) reported that rate of ruminal CP degradation was slower in CM than AF. Thus, the slower ruminal degradation of CM may limit the availability of dietary N in the rumen, resulting in less NH_3-N concentration in the rumen.

Cultures fed the TF+BT with the greatest portion of legume (G25L75) showed a lower increase of NH_3-N concentration than those with the G50L50 (7.29 vs. 21.8%), which resulted in an interaction between TF-legume ratio and TF-legume mixed diet (Table 6.3). As shown in Table 6.2, the TF+BT under the G25L75 contained 2.43% CT, while the TF+BT under the G50L50 contained 1.65% CT. The results in our study suggest that inhibitory effects of CT on dietary protein degradation may occur when the CT concentration was at 1.65% and could further decrease the protein degradation at 2.43% CT. It has been reported that the minimum concentration of CT needed to decrease protein degradation was about 0.4% in in vitro studies (Aerts et al., 1999; Molan et al., 2000; Williams et al., 2010), while in in vivo studies the required CT concentration was between 2.0 to 3.2% (Min et al., 2002; Al-Dobaib, 2009).

Within the TF-legume ratio treatments, the G75L25 showed the lowest NH_3-N:VFA ratio, while no difference was detected between the G50L50 and the G25L75. The lowest NH_3-N:VFA ratio in the G75L25 was resulted from lower dietary concentration of CP and resultant lower NH_3-N concentration in addition to lower VFA concentration, and therefore it does not imply an improvement of dietary N utilization by the G75L25.

Regardless of TF-legume ratio, fermentors fed the TF+BT or the TF+CM resulted in lower NH_3-N:VFA ratios compared with the TF+AF (averaged 0.18 vs. 0.21, respectively). The low NH_3-N:VFA ratios due to the TF+BT and the TF+CM were related to their lower NH_3-N concentrations. The lower NH_3-N concentration in the TF+BT may be a direct effect of CT, while in the TF+CM it may have resulted from thick epidermal layer of CM that can improve its resistance to ruminal microbial digestion. This finding also suggests that both the TF+BT and the TF+CM may have a similar efficiency in N utilization in the rumen.

Methane Production

As the legume proportion in the diets increased, CH_4 production decreased (Table 6.3). Methane generated in the rumen is formed primarily from hydrogen produced during the fermentation of feed, particularly of high fiber diets. The amount of CH_4 produced is therefore dependent upon the amount of hexose fermented and the amount of individual VFA produced. Changing the fermentation stoichiometry to produce more propionate at the expense of acetate and butyrate typically results in less CH_4 from fermentation. In this study, AF, BT, and CM contained greater concentrations of NFC than TF (34.6, 39.8, and 40.5 vs. 19.0%, respectively; Table 6.1), and consequently a greater proportion of legume in diets led to greater propionate concentration and less CH_4 production. It has been reported that soluble sugars yield less CH_4 than plant fiber (Moss et al., 1995; Hindrichsen et al., 2004), and therefore increasing concentrations of soluble sugars in forage plants can be a way of reducing ruminal CH_4 production.

Cultures fed CT-containing diets (TF+BT) produced less CH_4, while there was no difference in CH_4 production between the TF+AF and the TF+CM. There is a body of

evidence to demonstrate that feeding CT-containing forages or supplementing CT extracts decreases CH_4 production in vitro (Huang et al., 2011; Tan et al., 2011; Williams et al., 2011) and in vivo (Woodward et al., 2004; Animut et al., 2008; Sun et al., 2012). The inhibitory effects of CT on rumen methanogenesis have been attributed to direct effects of reducing methanogenic archaea (Finlay et al., 1994; Patra and Saxena, 2009, 2011) and indirect effects through a depression of fiber digestion in the rumen (Patra et al., 2012). In the current study, VFA concentration was similar across dietary treatments, and we did not observe any detrimental effect on ruminal fermentation. Therefore, the decreased CH_4 production may have resulted from direct impacts of CT on methanogenesis rather than indirect effects of depression of ruminal fiber digestion in cultures.

When cultures were fed with an increased dietary portion of BT in diets, reduction of CH_4 production was more apparent, resulting in an interaction between TF-legume ratio and TF-legume mixed diet (Table 6.3). In this study, the TF+BT under the G25L75 contained a greater concentration of CT (2.43%) than the other diets (Table 6.2), but the TF+BT under the G50L50 had CT at 1.65%, leading to no effect on CH_4 production. Eun and Min (2012) stated that reliable and distinguishable effects of CT on CH_4 reduction can be expected only from CT concentrations greater than 2.0% DM.

Fatty Acid Profiles of Ruminal Culture Contents

Overall, C16:0 (ranged from 9.27 to 10.1 g/100 g of total fatty acids) and C18:0 (ranged from 25.4 to 30.9 g/100 g of total fatty acids) composed major proportions of SFA in cultures (Table 6.4). While C14:0 proportion decreased with decreasing proportion of legume in the diets, there was no effect on the other fatty acid profiles. It

has been reported that CT inhibit ruminal biohydrogenation, thus leading to the accumulation of C18:1 *trans*-11 (*trans* vaccenic acids; **TVA**) at the cost of C18:0 production (Khiaosa-Ard et al., 2009; Vasta et al., 2009; Lee et al., 2010); however, it may require a minimum CT concentration at 9.35% to elicit noticeable effects of CT on the ruminal fatty acid profiles (Vasta et al., 2009). In our study, the greatest CT concentration in the diets was 2.43% in the TF+BT under the G25L75.

A greater TVA proportion was achieved when the TF+BT was fed to the cultures, while the TF+AF and the TF+CM maintained a similar proportion of TVA. In contrast, feeding the TF+BT decreased C18:0 proportion, but there was no effect on C18:0 proportion when the TF+AF and the TF+CM were fed. These results indicate that incomplete ruminal biohydrogenation may occur due to the presence of CT, particularly in the last step of the hydrogenation process. It was not expected to observe the shift in the ruminal biohydrogenation process due to a relatively low CT concentration in the current study (a maximum of 2.43% in the TF+BT under the G25L75). The *Butyrivibrio* group are the most active species among the group A bacteria, which form TVA from CLA and/or C18:2 *trans*-11 *cis*-15 (Harfoot and Hazlewood, 1997), while few species of bacteria such as *Fusocillus* spp. and *Clostridium proteoclasticum* (group B) convert TVA to C18:0 (Maia et al., 2007; Paillard et al., 2007; Durmic et al., 2008). Min et al. (2002) reported that CT from BT reduced the proliferation of *C. proteoclasticum*. Durmic et al. (2008) tested the inhibitory power upon biohydrogenating bacteria in response to CT and found that the minimum dose of plants needed to inhibit the proliferation of *B. fibrisolvens* was much greater than the dose needed to inhibit *C. proteoclasticum*, suggesting that *C. proteoclasticum* would be more sensitive to CT than *B. fibrisolvens*

(Durmic et al., 2008). In our case, therefore, a relatively low CT concentration in the diets would inhibit *C. proteoclasticum*, resulting in increased TVA proportion without affecting overall biohydrogenation process.

Implications

Mixed grass-legume pastures have been shown to improve the nutritive quality of pastures as well as animal performance. However, over competition by the grass and reduced persistence of legumes when grown together warrants the need of information on proper grass-to-legume ratios. In addition, there is a need to identify which grasses and legumes are most compatible for growing together and proper grazing management. Overall results in the current in vitro study showed that high proportions of legume in TF-legume mixed diets had beneficial effects on ruminal fermentation by producing more propionate and less CH_4. Among TF-legume mixed diets tested, feeding the TF+BT sizably reduced NH_3-N concentration and CH_4 production, but increased TVA proportion in cultures. In addition, feeding the TF+CM also resulted in improvement on microbial N concentration and similar N utilization efficiency compared with the TF+BT. Therefore, the TF+BT and the TF+CM may have a potential to improve ruminal fermentation and nutrient utilization efficiency of grazing cattle, which can contribute to sustainable ruminant production on pasture. Because TVA is the main precursor to form CLA in the adipose tissue and the mammary gland, the increased TVA proportion in cultures offered the TF+BT through CT may induce beneficial fatty acid profiles in animal products. Overall beneficial effects of feeding the TF+BT and TF+CM with a relatively higher

legume proportion need to be confirmed in in vivo experiment with focus on ruminal fermentation and animal performance.

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Table 6.1. Nutrient composition of pasture forages used for mixed dietary treatments

Item, % DM	Pasture forage			
	Tall fescue	Alfalfa	Birdsfoot trefoil	Cicer milkvetch
DM, %	28.7	19.6	19.3	14.3
OM	83.8	89.5	89.2	88.0
CP	8.70	19.4	16.4	18.8
NDF	53.5	32.9	30.9	25.9
ADF	29.6	25.1	23.2	20.4
Ether extract	2.62	2.60	3.06	2.86
Nonfiber carbohydrates	19.0	34.6	39.8	40.5
Condensed tannins	0.12	0.19	3.25	0.44

Table 6.2. Nutrient composition and fatty acid profiles of dietary treatments provided to continuous cultures

	Diet[1]								
	G75L25			G50L50			G25L75		
Item	TF+AF	TF+BT	TF+CM	TF+AF	TF+BT	TF+CM	TF+AF	TF+BT	TF+CM
Nutrient composition, % DM									
OM	83.7	83.6	83.8	85.3	85.1	84.7	87.2	87.2	86.9
CP	12.1	10.5	11.7	13.7	11.8	13.8	17.0	15.8	17.3
NDF	54.1	53.0	44.5	50.4	50.2	45.9	46.5	47.2	41.3
ADF	32.3	31.7	26.1	29.6	31.0	28.6	31.0	28.9	26.0
Ether extract	2.58	1.72	1.68	2.89	2.71	2.06	2.48	2.41	2.01
Non-fibrous carbohydrates	15.0	18.4	25.9	18.3	20.4	22.9	21.2	21.8	26.4
Condensed tannins	0.14	0.91	0.20	0.16	1.65	0.28	0.17	2.43	0.36
Fatty acid profile, g/100 g of total fatty acids									
C14:0	0.57	0.58	0.48	0.60	0.60	0.46	0.61	0.62	0.39
C16:0	20.6	20.0	20.5	21.6	19.5	20.3	21.5	18.5	20.2
C16:1 *trans*-9	0.86	0.84	0.78	0.82	0.80	0.79	1.23	0.78	0.79

C16:1	2.16	2.10	1.97	2.15	2.15	2.06	2.14	2.14	2.12
C18:0	1.99	1.70	2.14	2.41	1.84	2.23	2.64	1.89	2.41
C18:1 *cis*-9	2.60	2.69	2.79	2.36	2.40	2.64	1.98	2.18	2.52
C18:2 n-6	12.7	12.3	12.5	14.5	13.4	12.5	15.4	14.3	12.9
C18:3 n-3	47.8	49.9	48.7	45.7	50.3	49.1	42.8	50.6	49.6
C20:0	0.63	0.57	0.73	0.78	0.67	0.79	0.87	0.73	0.90

[1]G75L25 = 75% grass and 25% legume in a DM basis; G50L50 = 50% grass and 50% legume in a DM basis; G25L75 = 25% grass and 75% legume in a DM basis; TF+AF = tall fescue-alfalfa mixed diet; TF+BT = tall fescue-birdsfoot trefoil mixed diet; and TF+CM = tall fescue-cicer milkvetch mixed diet.

Table 6.3. In vitro ruminal fermentation characteristics as affected by grass-legume mixed diet with their varying TF-legume ratios

	Diet[1]												
	G75L25			G50L50			G25L75				*P*[2]		
Item	TF+AF	TF+BT	TF+CM	TF+AF	TF+BT	TF+CM	TF+AF	TF+BT	TF+CM	SEM	GLR	MPF	INT
Culture pH	6.17^{abcd}	6.11^{ab}	6.10^{a}	6.29^{e}	6.24^{de}	6.12^{abc}	6.28^{e}	6.19^{cde}	6.20^{cde}	0.031	< 0.01	< 0.01	0.25
Total VFA, m*M*	48.8	48.6	50.5	50.0	47.8	55.4	53.2	52.4	56.1	2.79	0.16	0.16	0.89
Individual VFA, m*M*													
Acetate (A)	31.7	30.7	31.9	31.5	29.7	34.5	33.3	32.3	34.9	2.21	0.49	0.31	0.94
Propionate (P)	9.36^{a}	10.1^{ab}	10.5^{abc}	9.91^{ab}	10.1^{ab}	12.5^{cd}	10.5^{abc}	11.5^{bcd}	12.8^{d}	0.68	0.03	< 0.01	0.68
Butyrate	5.47	5.71	5.66	5.87	5.63	5.56	6.13	5.95	5.11	0.357	0.92	0.39	0.53
A:P	3.42^{b}	3.05^{ab}	3.05^{ab}	3.20^{ab}	2.94^{ab}	2.77^{a}	3.15^{ab}	2.82^{a}	2.72^{a}	0.176	0.17	0.03	0.99
NH_3-N, mg/100 mL	14.6^{bc}	12.5^{a}	13.9^{b}	18.7^{e}	15.3^{c}	16.8^{d}	20.6^{g}	16.4^{d}	19.5^{f}	0.26	< 0.01	< 0.01	< 0.01
NH_3-N:VFA	0.18^{ab}	0.15^{a}	0.16^{a}	0.22^{d}	0.19^{bc}	0.18^{ab}	0.23^{d}	0.19^{bc}	0.21^{cd}	0.009	< 0.01	< 0.01	0.51
Microbial N, % DM	7.64^{a}	8.40^{bc}	8.32^{abc}	8.08^{ab}	8.33^{abc}	9.00^{c}	9.01^{c}	8.73^{bc}	8.96^{c}	0.236	<0.01	0.04	0.13
microbial biomass													
CH_4 production,	9.00^{d}	8.45^{c}	9.68^{e}	8.23^{c}	8.27^{c}	8.16^{c}	8.34^{c}	7.25^{a}	7.68^{b}	0.133	< 0.01	< 0.01	< 0.01

m*M*/d

[a-g]Means in the same row with different superscripts differ at $P < 0.05$.

[1]G75L25 = 75% grass and 25% legume in a DM basis; G50L50 = 50% grass and 50% legume in a DM basis; G25L75 = 25% grass and 75% legume in a DM basis; TF+AF = tall fescue-alfalfa mixed diet; TF+BT = tall fescue-birdsfoot trefoil mixed diet; and TF+CM = tall fescue-cicer milkvetch mixed diet.

[2]GLR = effect of TF-legume ratio between grass and legume; MPF = effect of mixed diet; and INT = interaction between GLR and MPF.

Table 6.4. Fatty acid profile (g/100 g of total fatty acids) of ruminal culture contents as affected by TF-legume mixed diet with their varying TF-legume ratios

	Diet[1]												
	G75L25			G50L50			G25L75				*P*[2]		
Fatty acid	TF+AF	TF+BT	TF+CM	TF+AF	TF+BT	TF+CM	TF+AF	TF+BT	TF+CM	SEM	GLR	MPF	INT
C14:0	1.93^{bc}	1.97^{c}	1.78^{bc}	2.00^{c}	1.81^{bc}	1.62^{ab}	1.81^{bc}	1.79^{bc}	1.33^{a}	0.107	0.03	<0.01	0.45
C15:0	1.89	1.88	1.75	1.85	1.58	1.62	1.68	1.51	1.63	0.118	0.07	0.25	0.75
C16:0	9.64	10.0	9.39	10.1	9.69	9.72	9.56	9.68	9.27	0.340	0.49	0.41	0.80
C17:0	0.52	0.44	0.48	0.48	0.44	0.50	0.42	0.40	0.46	0.033	0.12	0.11	0.72
C18:0	29.4^{cd}	25.9^{a}	27.9^{bc}	27.2^{ab}	27.2^{ab}	29.9^{d}	27.5^{ab}	26.9^{ab}	27.5^{ab}	0.56	0.23	<0.01	0.06
C18:1 *trans*-11 TVA	6.98^{a}	7.41^{a}	6.92^{a}	6.77^{a}	9.02^{b}	7.11^{a}	6.85^{a}	9.31^{b}	6.90^{a}	0.367	0.13	<0.01	0.06
C18:1 *cis*-9	1.19	1.36	1.16	1.22	1.24	1.32	1.24	1.16	1.36	0.092	0.92	0.70	0.31
C18:2 n-6	0.79	0.78	0.58	0.84	0.76	0.70	0.79	0.84	0.83	0.081	0.34	0.27	0.57
cis-9 *trans*-11 CLA	0.53	0.50	0.47	0.47	0.51	0.49	0.47	0.49	0.44	0.023	0.17	0.32	0.42
C18:3 n-3	1.55	1.75	1.40	1.73	1.81	1.70	1.54	1.96	1.77	0.146	0.24	0.12	0.64

[a-f]Means in the same row with different superscripts differ at $P < 0.05$.

[1]G75L25 = 75% grass and 25% legume in a DM basis; G50L50 = 50% grass and 50% legume in a DM basis; G25L75 = 25% grass and 75% legume in a DM basis; TF+AF = tall fescue-alfalfa mixed diet; TF+BT = tall fescue-birdsfoot trefoil mixed diet; and TF+CM = tall fescue-cicer milkvetch mixed diet.

[2]GLR = effect of TF-legume ratio between grass and legume; MPF = effect of mixed diet; and INT = interaction between GLR and MPF.

CHAPTER 7

SUMMARY AND CONCLUSIONS

Pasturing cattle offers various benefits for consumers, producers, animals, and the environment. For many farmers, feeding cattle on pasture is an important part of ensuring that the animals have comfortable, healthy living conditions in ecologically- and environmentally-friendly operations, while still lowering the input costs. However, one of the major limitations of grazing ruminants is their inefficiency in utilizing dietary nutrients. For instance, it is well reported that protein in high-quality fresh forage is rapidly degraded in the rumen, resulting in a greater NH_3-N concentration in the rumen, thus increasing N waste in the environment. Any efforts to increase ruminal N utilization can consequently minimize the loss of N from diets and reduce N pollution into the environment. The 2-year grazing research was performed to determine the effects of fertilizing tall fescue (**TF**) with N on growth performance, ruminal fermentation, and fatty acid composition of beef steers. In addition, a series of in vitro experiments were conducted to evaluate the effects of energy supplementation on pasture forage and grass-legume ratio on microbial fermentation in continuous cultures.

As the grazing season progresses, the quality of pasture forages decreases. The decrease in quality of pasture forages can be reduced by fertilizing pasture with N fertilizer. Nitrogen fertilizer not only improves the protein concentration of forages, but also increases crop yield, thus improving animal performance. The main objective of this 2-year grazing study was to evaluate the effects of finishing beef steers grazing TF with N fertilizer on growth performance, ruminal fermentation, and carcass characteristics. There were no noticeable effects detected on nutrient quality when TF was fertilized with

168 kg N/ha in the first year of the study. However, in the second year, fertilized TF showed a greater CP concentration than non-fertilized TF. As the consequence of increasing CP concentration in fertilized TF, steers grazing fertilized TF showed an increase in DMI, ADG, and G:F ratio, but no effects were detected on carcass characteristic of the steers. Nitrogen fertilizer on TF also affected ruminal fermentation characteristic by increasing total VFA and NH_3-N concentrations in the rumen. However, the mechanism whereby N fertilization increased VFA concentration is still unclear, thus further research is needed to investigate how N fertilization influences microbial dynamics to improve fiber fermentation. Greater ruminal NH_3-N concentration of steers grazing on fertilized TF indicates that high-quality grasses are lacking of readily fermentable carbohydrates, and therefore, energy supplementation is required to optimize ruminal fermentation. By providing sufficient readily fermentable carbohydrate in the rumen, an improvement in dietary N utilization for microbial protein synthesis may be expected, which may enhance growth performance of grazing beef steers.

Recently, there has been an increased interest in the effects of feeding pasture forages on fatty acid profiles of finishing steers' tissues. In many studies, pasture-finishing beef has been proven for having a healthier animal product for consumers, as the meat is lower in saturated fatty acids and higher in n-3 polyunsaturated fatty acids. In the second study, steers grazing fertilized TF had greater *cis*-9, *trans*-11 conjugated fatty acid (**CLA**) proportion in their adipose tissue than those grazing non-fertilized TF. In comparison with steers fed a high-grain diet, pasture-finished steers showed greater proportions of C18:0, *cis*-9, *trans*-11 CLA, and C18:3 n-3, but lowered n-6:n-3 ratio in beef adipose tissue throughout the grazing season. Therefore, it was concluded that

finishing beef steers on fertilized TF pasture would give positive impacts on fatty acid compositions in adipose tissue by increasing *cis*-9, *trans*-11 CLA and lowering n-6:n-3 ratio. However, this study also showed that finishing steers on TF pasture resulted with less back fat, rib fat, and ribeye area compared to feedlot-finished steers. Thus, these unfavorable findings should be accounted for when considering grazing pasture as a finishing system for steers.

Compared with N fertilizer application, interseeding legumes into grass-dominated pasture has been reported to reduce N losses into the environment. Additionally, corn grain supplementation in high-quality forage diets has demonstrated enhanced nutrient utilization and performance of grazing cattle. The third study was focused on fermentative benefits by feeding grass-legume mixtures [TF and birdsfoot trefoil (**BT**)] compared with the TF with N fertilizer (**TF+NF**) and any associative effects with energy supplementation on in vitro mixed cultures. Supplementing dried distillers grains with solubles (**DDGS**) in pasture forage diets at 30% had detrimental impacts on ruminal fermentation by decreasing total VFA and NH_3-N concentrations in cultures. In contrast, the greatest total VFA and propionate concentrations were detected when corn was supplemented in pasture forages, implying that corn grain supplementation enhanced ruminal fermentation by providing more energy for microbial protein synthesis in the cultures. Corn supplementation also resulted in less methane (**CH_4**) production, which indicates less energy loss in the cultures. Feeding the TF+NF or the TF and BT mixed diets (**TF+BT**) into cultures elicited similar patterns of ruminal fermentation in the cultures, implying that the TF+BT can maintain efficient ruminal fermentation. In addition, the TF+BT also decreased CH_4 production due to bioactive condensed tannins

(**CT**) in BT. Overall results of this in vitro study indicate that corn supplementation in pasture forages can enhance ruminal fermentation, while grass and high CT-containing legume mixtures would be beneficial in grazing cattle systems to improve ruminal fermentation, nutrient utilization efficiency, and reduce environment pollution. However, availability of N and OM and their utilization efficiencies may be different between in vitro and in vivo conditions due to N recycling, rates of digestion, and forage intake. Thus, further animal trials are required to prove the results of in vitro fermentation profiles in comparing pasture forages between the TF+NF and the TF+BT pastures on animal growth and environmental performance.

Although the third study indicates that grass-legume mixed pastures can improve nutrient quality of mixed diets and ruminal fermentation in in vitro, the persistence of legumes may be decreased due to weather changes and competition with grass when legumes are grown together with grass. Additionally, due to their differences in nature, different legumes would show different response when combined with grasses. Thus, the last study was performed to determine the proper grass-to-legume ratios and find out which legumes would be most beneficial for mixing with grass pasture on in vitro fermentation. Increased propionate concentration and less CH_4 production were detected, as the legume proportion in the diet increased. Beneficial effects of CT in BT were also shown when the TF+BT mixed diet was fed into cultures. These beneficial effects included lower NH_3-N concentration, less CH_4 production, and increased C18:1 *trans*-11 (*trans* vaccenic acids; **TVA**) proportion in cultures. Similar N utilization efficiency in cultures fed the TF+BT and TF and cicer milkvetch mixed diet (**TF+CM**) was detected, with further increase in microbial N concentration in cultures fed the TF+CM. These

findings suggest that the TF+BT or the TF+CM may improve ruminal fermentation and nutrient utilization efficiency on grazing cattle. Increased TVA proportion in cultures offered the TF+BT mixed diet may imply that finishing steers grazing the mixed pasture may have greater proportion of CLA in their meat. However, further in vivo studies focusing on ruminal fermentation and animal performance are needed to confirm these beneficial effects of feeding the TF+BT and the TF+CM mixed pasture diets.

Throughout this project, we found that N fertilization on TF pasture increased CP concentration of TF, thus improving growth performance of grazing steers as well as increasing *cis*-9 *trans*-11 CLA proportion in the adipose tissue. However, energy supplementation is required to maximize ruminal fermentation and reduce N waste from the rumen. Improvement in ruminal fermentation also occurred when high CT-containing legume, BT was included in forage-based diets. An additional ecological advantage of CT-containing forage mixture diets may be achieved by reducing CH_4 emissions in the environment. Therefore, finishing beef steers on grass-legume mixed pasture can create nutritive and environmental benefits with improved nutrient utilization efficiency and animal performance.

APPENDIX

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I am requesting your permission to include the paper titled:

1. Growth performance, ruminal fermentation profiles, and carcass characteristics of beef steers grazing tall fescue without or with nitrogen fertilization,
2. Fatty acid composition in adipose tissue of pasture- and feedlot-finished beef steers,
3. Effects of energy supplementation in pasture forages on in vitro ruminal fermentation characteristics in continuous cultures, and
4. Effects of grass-legume mixed pasture forages with different composition ratios on microbial fermentation in continuous cultures,

of which you are coauthors. Your contribution will be acknowledged in a footnote to the chapter title.

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- Enhancement of forage utilization by ruminants.

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M.Anim.St., Animal Nutrition with emphasis in Feed Technology, Faculty of Natural Resources, Agriculture and Veterinary Studies, University of Queensland, Australia, July 2005; Thesis title: **The Effect of Chemical Treatments on the Chemical Composition and In Vitro Digestibility of Tropical Forages**; Advisor: Gordon McL. Dryden, Ph.D.

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- Indonesian Association of Nutrition and Feed Science, 2001 – present.
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PUBLICATIONS:

Refereed Journal Articles

Noviandi, C. T., K. Neal, J.-S. Eun, M. D. Peel, B. L. Waldron, D. R. ZoBell, and B. R. Min. Effects of grass-legume mixed pasture forages with different composition ratios on microbial fermentation in continuous cultures. Manuscript submitted and in review process (Manuscript ID#: PAS-13-01259).

Noviandi, C. T., J.-S. Eun, M. D. Peel, B. L. Waldron, B. R. Min, D. R. ZoBell, and R. L. Miller. Effects of energy supplementation in pasture forages on in vitro ruminal fermentation characteristics in continuous cultures. Manuscript submitted and in review process (Manuscript ID #: PAS-13-01218).

Noviandi, C. T., B. L. Waldron, J.-S. Eun, D. R. ZoBell, R. D. Stott, and M. D. Peel. 2012. Growth performance, ruminal fermentation profiles, and carcass characteristics of beef steers grazing tall fescue without or with nitrogen fertilization. Prof. Anim. Sci. 28:519–527.

Noviandi, C. T., R. E. Ward, D. R. ZoBell, R. D. Stott, B. L. Waldron, M. D. Peel, and J.-S. Eun. 2012. Fatty acid composition in adipose tissue of pasture- and feedlot-finished beef steers. Prof. Anim. Sci. 28:184–193.

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Dschaak, C. M., C. T. Noviandi, J.-S. Eun, V. Fellner, A. J. Young, D. R. ZoBell, and C. E. Israelsen. 2011. Ruminal fermentation characteristics and lactational performance of Holstein dairy cows fed whole safflower seeds. J. Dairy Sci. 94 (E-Suppl. 1):178 (Abstr.).

Vera, J. M., C. T. Noviandi, Arief, J. S. Eun, and D. R. ZoBell. 2010. Effects of supplementing an exogenous proteolytic enzyme on growth performance in growing beef steers. J. Anim Sci. 88 (E-Suppl. 2):140 (Abstr.).