BEEF AVERAGE DAILY GAIN AND ENTERIC METHANE EMISSIONS ON BIRDSFOOT TREFOIL, CICER MILKVETCH AND MEADOW BROME PASTURES

by

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ABSTRACT

Beef Average Daily Gain and Enteric Methane Emissions
on Birdsfoot Trefoil, Cicer Milkvetch
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by

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This thesis project explored the use of perennial legumes, including the non-bloating birdsfoot trefoil (BFT; *Lotus corniculatus* L.) for beef production. In 2011 and 2012, cattle averaged approximately 300 kg at the beginning of the grazing season, and approximately 450 kg in 2013. Average daily gain on pastures ranged from a low of 0.63 kg d^{-1} on cicer milkvetch (CMV; *Astragalus cicer* L.) in 2011 and 2013 to a high of 1.03 kg d^{-1} on Norcen BFT in 2013. Feedlot gains ranged from 1.14 to 1.57 kg d^{-1}. Blood plasma fatty acids did not differ when feeding treatments were imposed, but at the end of each grazing season saturated and omega-6 fatty acids were elevated in feedlot-fed cattle compared with pasture-fed cattle, while *trans*-vaccenic acid (TVA) and omega-3 fatty acids were elevated in pasture-fed cattle. The ratio of omega-6 to omega-3 fatty acids was always higher in feedlot-fed cattle at the end of the grazing season, but in 2013, when all cattle were nearing slaughter weight, the omega-6 to omega-3 ratio was 50% higher for feedlot-fed than for BFT-fed cattle and double that of grass-fed cattle. Digestive (enteric) methane (CH_4) production of beef cows was lower when cattle grazed BFT and CMV pastures (167 and 159 g CH_4 per cow per d, respectively) compared to cows on meadow bromegrass (MB; *Bromus riparus* Rehmann) (355 g CH_4 per cow per d). Meadow bromegrass has more fiber than legume forages, which will increase the proportion of acetate to propionate created by microbial digestion in the rumen, increasing the production and release of CH_4. Perennial legume forages fix nitrogen, eliminating the need for chemical nitrogen fertilizer, and tannin-containing legumes can be grazed without risk of bloat. These forages will play an important role in developing more environmentally and economically sustainable agricultural production systems.

(89 pages)

PUBLIC ABSTRACT

Beef Average Daily Gain and Enteric Methane Emissions on Birdsfoot Trefoil, Cicer Milkvetch and Meadow Brome Pastures

Lance Pitcher

Conventional production of meat products from ruminant animals in the United States requires inputs including the cultivation and nitrogen fertilization of annual grains such as corn and barley, and transportation of cattle and grain to feedlots. Consumers have concerns about the impact of feedlot conditions on animal health, and about the implications of pharmaceutical inputs such as growth hormones and antibiotics on the environment and human health. These concerns have led to a growing interest in pasture-finished meat production by consumers. Such smaller-scale livestock production systems can be healthier and lower-stress for animals, are integrated into local food systems and are more transparent to consumers, and have higher potential profitability for producers than traditional ruminant production methods.

There is a strong market for pasture-finished beef products, and prices for naturally or organically raised beef have remained well above feedlot-produced product prices. There is also concern about the impact of ruminant production on the environment, including air and water pollution from feedlot production and greenhouse gasses that are emitted from ruminant animals during feed digestion. This thesis project explored the potential of a beef production system based on perennial legumes, including the non-bloating legume birdsfoot trefoil (BFT; *Lotus corniculatus* L.) for producing meat products from cattle while reducing concentrate feeding and methane production. The condensed tannins that are produced by BFT bind proteins in the rumen but allow them to be digested in the abomasum and intestines, which in turn leads to better utilization of forage nutrients during the finishing period and higher gains or milk production. The higher digestibility of legumes compared with grasses reduces methane emissions in cattle both through higher digestibility of the forage and through direct impacts on methanogens operating in the rumen.

As reported in this thesis, steers finished on BFT gained significantly more weight per day than steers fed another perennial forage legume, cicer milkvetch, but did not gain as rapidly as feedlot-fed steers. At the end of summer grazing, the blood plasma of pasture-fed steers was lower in saturated and omega-6 fatty acids and higher in *trans*-vaccenic and omega-3 fatty acids than the blood plasma of feedlot-fed steers. When beef cows grazed grass and legume pastures, enteric methane emissions were lower on the legume pastures than the grass pasture. These results demonstrate that, compared with other feed sources, perennial legume pastures used for cattle production can improve cattle gains and reduce environmental impacts.

DEDICATION

Dedicated to my parents, my wife and kids for giving me the strength and support to continue my education

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Lance R. Pitcher

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Cool season grasses are the most widely used forages for pastures because seed is readily available, inexpensive, grasses are relatively easy to establish and highly persistent (e.g., Volesky et al., 2010). Forage grasses are adapted to a range of soil pHs, many can use dormancy to survive periods of drought, and the morphology of grasses, which includes rhizomes, stolons, prostrate growth, and the placement of growing points near or below the soil surface, allows grasses to survive frequent defoliation as well as wide temperature extremes. Many grass species are well adapted to production under irrigation in the Mountain West (Lauriault et al., 2005), including tall fescue (S*chedonorus arundinaceus* (Schreb.) Dumort.), orchardgrass (*Dactylis glomerata* L.), smooth brome (*Bromus inermis* Leyss.), meadow bromegrass (*Bromus riparius* Rehmann) and perennial ryegrass (*Lolium perenne* L.), although perennial ryegrass is more shallow-rooted and non-dormant than the other species. Quackgrass [*Elymus repens* (L.) Gould] is a very common species in irrigated pastures in the Mountain West, and while it is listed as a noxious weed in many western states, it provides good yield and nutrition if managed well (Abaye et al., 2009).

Broadleaf weeds can be controlled easily in pure grass stands with herbicides such as 2,4-D, but chemical weed control is costly and requires significant time, while the profit margin for livestock production is highly variable (Holmgren and Pace, 2013). There are benefits in having greater forage plant species diversity for both pasture and livestock production. Legumes that fix their own nitrogen transfer this benefit to companion plant species, particularly under rotational grazing, eliminating the need for nitrogen fertilization and the negative environmental impacts of its production and application (Mosier et al., 2005). The crude protein content of legumes is greater than that of grasses, and legumes are lower than grasses in NDF and can therefore be digested more readily than grasses, increasing intake (Smith et al., 1972). Including non-leguminous forbs such as narrow-leaf plantain (*Plantago lanceolata* L.)., chicory (*Cichorium intybus* L.), or small burnet (*Sanguisorba minor* Scop.) can increase the rooting diversity, benefit soil microbial ecology, and provide a range of beneficial secondary compounds not found in grasses to ruminants.

Legumes, Condensed Tannins and Livestock Production

Legumes are well-adapted to cultivation under irrigation in the soils and climate of the Mountain West as demonstrated by the prominence of commercial alfalfa (*Medicago sativa* L.) production in this region (Putnam et al., 2000). However, if grass persistence is poor, legumes can dominate mixed pastures in the West, leading to bloat (MacAdam et al., 2006). Therefore, the use of non-bloating legumes either in mixtures or in pure stands for pastures intended is recommended in this region. Birdsfoot trefoil can be as productive in mixtures as white clover and can greatly improve quality and production compared with monoculture grass pastures (MacAdam et al., 2006). A recent irrigated variety trial that included 14 cultivars of BFT demonstrated that cultivars from a wide range of geographical origins are productive and persistent in the Mountain West (MacAdam and Griggs, 2013).

Recent studies have found that grazing legumes monocultures can result in rapid ruminant gain (MacAdam et al., 2011; Wen et al., 2002) while requiring no additional nitrogen fertilizer, which is less detrimental to the soil microbial community and the environment than adding nitrogen fertilizer to a grass pasture (Geisseler and Scow, 2014). BFT contains the secondary plant compound condensed tannins (CT), which are water soluble compounds shown to bind proteins in the rumen (Min et al., 2003). In the case of the CT produced by BFT, the tannins disassociate at the lower pH of the abomasum, freeing proteins for digestion and absorption in the small intestines (Mueller-Harvey, 2006; Waghorn et al., 1987) and resulting in greater ruminant meat and milk production (Min et al., 2003; Waghorn, 2008). While many other forage legumes produce CT, only the tannins from sainfoin (*Onobrychis viciifolia* Scop.), sulla (*Hedysarum coronarium* L.) and BFT have been shown to improve ruminant production, and only BFT CT consistently improve ruminant production (Mueller-Harvey, 2006). The relatively low concentration of CT in BFT will not reduce intake due to astringency or slow the rate of digestion in the rumen (Waghorn, 2008). The protein content of BFT in pastures is similar to that of alfalfa, and higher than the protein requirement of ruminants, and BFT digestibility and intake has been found in numerous studies to be greater than that of alfalfa (McGraw and Marten, 1986; Mowat et al., 1969; van Soest, 1965).

In a typical feedlot setting, average daily gain (ADG) for cattle ranges from 0.79 to 2.43 kg/d for 90 to 120 days (Reinhardt et al., 2012). Studies examining cattle ADG on BFT showed spring gains of 1.26 to 1.53 kg/d on pure stands of BFT in Missouri (Wen et al., 2002) and MacAdam et al. (2011) showed gains of 1.30 to 1.64 kg/d on pure stands of BFT for 61 to 77 days in the Mountain West. These unusually high gains for pasture-based production may be explained by the activity of BFT CT. Waghorn and others (1987) demonstrated that proteins bound to BFT CT in the rumen were not used for energy in the rumen, and were more available for digestion when they reached the abomasum than proteins bound to the CT of other species. This resulted in higher feed efficiencies and increased absorption of amino acids in BFT-fed ruminants.

Other benefits of including BFT in ruminant diets include reduction in methanogenic rumen microbes (Min et al., 2003), improved ruminant nitrogen use efficiency (Woodward et al., 2009) and reduced dietary energy loss to CH_4 (Waghorn, 2008; Woodward et al., 2004). The enteric CH_4 that is expelled by ruminants is produced in the rumen through digestion of feed materials by rumen microbes in an anaerobic environment. Through this digestion process, microbes produce the volatile fatty acids acetate, butyrate and propionate; these are the main energy sources used by the ruminants (Martin et al., 2010). Increases in crude fiber result in an increase in the ratio of acetate to propionate. Acetate is the main component in methane production in the rumen, and with lower quality forages such as many varieties of grasses, the amount of crude fiber is significantly higher than that of legumes (Hook et al., 2010; Mountfort et al., 1982). Forages such as perennial legumes, with lower NDF concentrations, should therefore produce less methane during digestion.

Methane represents a loss of energy that could have been used by the ruminant, and is dependent on the quality, intake and composition of the feed that is provided. These forage characteristics can, in turn, have a direct impact on methanogenesis (Hook et al., 2010). Other plant secondary metabolites can affect CH_4 production in ruminant livestock, including saponins, tannins, alkaloids and essential oils. They act on rumen microbes and the resulting production of acetate, butyrate and propionate, and if used as additives, are generally viewed by the public as safe and natural alternatives to chemical additives (Cieslak et al., 2013; Martin et al., 2010).

Sustainable agriculture has been legally defined (US Code Title 7, Section 3103) to be an integrated system of plant and animal production practices having a site-specific application that will over the long-term: (1) satisfy human food and fiber needs, (2) enhance environmental quality and the natural resource base upon which the agriculture economy depends, (3) make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls, (4) sustain the economic viability of farm operations, and (5) enhance the quality of life for farmers and society as a whole (USDA, 2009). The use of high-quality perennial pastures, especially on agricultural land that has limitations reducing its suitability for annual cropping, is a more sustainable approach to ruminant production than the current system of cow-calf production on low quality grasslands followed by the feeding of annual cereal grains in feedlots. Birdsfoot trefoil has been identified as the foremost example of the type of forage plant species that could support a more sustainable approach to ruminant production (Gurian-Sherman, 2011).

Livestock and Methane Emissions

Beef and dairy farming operations produce the greatest amount of CH_4 from human-related activities (Lassey, 2007), so methane generated by ruminant production systems and its effects on global climate change is a cause of concern worldwide (Martin et al., 2010). In the United States, CH_4 represented 14% of the total greenhouse gas emitted in 2007 and 7% of this methane was due to agriculture (U.S. EPA, 2013). Enteric CH_4 emissions can be measured on single animals or small groups in metabolism chambers, while feeding stations that collect discrete methane samples can measure many individuals from the same treatment over longer time periods. Metabolism chambers and the sulfur hexafluoride (SF_6) tracer gas technique are the most widely used and accepted methods to determine CH_4 emission rates from ruminants (Johnson et al., 2007). Data from well-maintained metabolism chambers can be very precise but these studies can be limited due to the fact that trained animals are necessary, the forages have to be harvested and fed to the animal, animal movement is confined to small area, preventing their use for grazing studies, and the chambers are expensive to install, use and maintain (Johnson et al., 2007). The SF_6 technique was created to address the limitations of metabolism chambers and is the only accurate method for taking integrated daily enteric CH_4 emission data from individual ruminants fed in a feedlot or grazing on range or in a pasture setting (Lassey et al., 2011).

The SF_6 method produces similar results to metabolism chambers. Johnson et al. (2007) compared metabolism chambers and individual pens, Boadi et al. (2002) used respiration hoods and individual stalls, and Grainger et al. (2007) looked at the SF_6 technique while cattle were kept in metabolism chambers. These three studies showed very comparable values for the SF_6 method and metabolism chambers (Pinares-Patiño et al., 2011). The SF6 technique will be used in this study to compare the enteric CH_4 emissions of beef cows grazing different pasture forages. We expect BFT to have more rapid digestion and more efficient forage utilization because of its greater digestibility and tannin content; therefore, BFT should produce lower CH_4 emissions than the grass forage used in this study, meadow bromegrass (MB). We also expect lower CH_4 emissions per unit of forage intake on CMV than on MB because the intake of legumes is higher than grasses. Meadow brome has higher NDF digestibility than other grasses, which may help reduce enteric methane emissions. In an in vitro study comparing CMV and BFT with alfalfa (ALF), CMV and BFT produced significantly lower CH_4 compared with ALF, and CMV produced less CH_4 per unit of digested fiber than either ALF or BFT (Williams et al., 2011).

For beef producers willing to market directly to the public, consumers are willing to pay a premium for locally raised pasture-finished beef from cattle that were not fed hormones or antibiotics (Conner and Oppenheim, 2008). The urban-rural interface typical of the Mountain West supports irrigated perennial pastoral ruminant production systems, while agricultural production that depends on annual cropping, or drylot ruminant production systems that include waste storage, are less attractive to non-farming suburban neighbors. The public has rejected corporate agricultural production systems that degrade the environment and undermine the health and well-being of agricultural workers and livestock (Lusk and Fox, 2002; U.S. EPA, 2005), while embracing agricultural production systems that work for the well-being of the consumer, the livestock and the producer while sustaining or improving the environment (Brown, 2003). Increasing the use of high-quality legume forages, particularly as an alternative to the use of nitrogen-fertilized grass and cereal for ruminant feeding, is an important element in the development of more sustainable ruminant production systems.

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CHAPTER 2

AVERAGE DAILY LIVEWEIGHT GAIN OF STEERS GRAZING NON-BLOATING LEGUMES

INTRODUCTION

The concentration of fiber is greater in grasses than in legume forages and the cell wall digestion rate of legumes is greater than the cell wall digestion rate of grasses (Smith et al., 1972). This can result in greater digestibility and intake of legumes, and greater ruminant productivity of forage legumes (Crampton et al., 1960). Legume pastures have the further advantage of biological nitrogen fixation, producing their own nitrogen as needed. Unfortunately, the incidence of bloat increases with the rate of rumen digestion (Howarth et al., 1991) and the most commonly cultivated temperate legumes – alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pretense* L.) – are all bloat-causing. The perennial legume cicer milkvetch (*Astragalus cicer* L.; CMV) is nonbloating because of the epidermal and vein structure of its leaves (Lees et al., 1982), and the most commonly used tannin-containing temperate legume forages – birdsfoot trefoil (*Lotus corniculatus* L.; BFT) and sainfoin (*Onobrychis viciifolia* Scop.) – are non-bloating because tannins bind to proteins and precipitate them in the rumen. Depending on their type and concentration, tannins can reduce the nutritive value of forages. Birdsfoot trefoil tannins are present in a low concentration (1-3%), and while they bind excess plant proteins in the rumen, they release proteins at the lower pH of the abomasum (Waghorn et al., 1987). A review by Waghorn (2008) concluded that the condensed tannins synthesized by BFT increased utilization of proteins in the abomasum, resulting in better feed efficiency and increased absorption of amino acid compared with other tannin-containing forages. Greater meat and milk production have been reported for ruminants grazing BFT compared with alfalfa (Barker et al., 1999; Douglas et al., 1995; Marten et al., 1987).

While BFT does not persist well in humid regions like Missouri (Wen et al., 2002), it is highly productive and persistent when grown under irrigation in the Mountain West, which is characterized by a cool, dry climate and high pH soils (MacAdam and Griggs, 2006). A preliminary study demonstrated higher gains on BFT than on CMV (MacAdam et al., 2011). The goal of the present study was to determine the ADG of cattle grazing CMV and BFT in comparison with cattle fed on concentrates. Two cultivars of BFT were used in this study, ‘Norcen’ which has a low (10 g kg^{-1}) tannin concentration and ‘Oberhaunstadter’ which has a medium (20 g kg^{-1}) tannin concentration (Marley et al., 2006; Marshall et al., 2008). Our objectives were to document forage production and forage nutritive value, BFT tannin concentration, ADG of both younger fall-born (300 kg) and older spring-born (450 kg) steers, and to document the blood plasma fatty acid composition of both feedlot-fed cattle and cattle grazing pure stands of forage legumes with and without tannins.

MATERIALS AND METHODS

Pasture Design and Establishment

This study was carried out at the USU Caine Dairy Research Center in Wellsville, Utah (latitude 41°39' N, longitude 111°54' W) from 2011 to 2013, and a grass treatment was also assessed at the Intermountain Irrigated Pasture Project in Lewiston, Utah in 2013. The Wellsville site was designed as a randomized complete block with six replications (Fig. 2.1). Each replication consisted of three contiguous pastures totaling 1.15 ha; pastures within each replication measured approximately 0.38 ha and were randomly assigned to one of three pasture treatments: ‘Monarch’ CMV, Norcen BFT or Oberhaunstadter BFT. Cicer milkvetch was seeded at 30 kg ha^{-1} pure live seed (PLS) and BFT was seeded at 18 kg ha^{-1} PLS in the early autumn of 2010. Pastures were watered with a K-Line pod sprinkler irrigation system (K-Line Irrigation/North America, St. Joseph, MI 49085), which served from 1 to 5 pastures; see riser locations in Figure 2.1. Cattle herds comprised of three steers (n=2 to 4 herds per treatment per year) were rotated among replications to accommodate the irrigation system. In early autumn of 2012, five grass pastures each approximately 0.36 ha were established at the USU Intermountain Irrigated Pasture Project in Lewiston, Utah (latitude 41°56' N, longitude 111°52' W). Each pasture was seeded with ‘Cache’ meadow bromegrass (*Bromus riparius* Rehman) at 37 kg ha^{-1} PLS. These pastures were irrigated with hand lines, and pairs of cattle were rotated within each pasture during the grazing study period in 2013.

Grazing Management

Experiments and procedures involving livestock were carried out following review and approval by the USU Institutional Animal Care and Use Committee. In 2011, 48 fall-born Angus steers with a mean weight of 302 kg ± 7 kg SEM (665lbs. ± 15lbs. SEM) were sorted into four treatment groups of 12 cattle with similar body weigh distributions. One of these groups was assigned to each of the three pasture treatments at the Wellsville site, and the fourth group was assigned to separate pens at the USU feedlot. All four treatment groups were subdivided into four herds of three cattle, each with a similar distribution of body weight. In 2012, 36 fall-born Angus steers with a mean weight of 321 kg ± 4 kg SEM (707 lbs. ± 9lbs. SEM) were sorted into four treatment groups of nine cattle each. One of these groups was assigned to each of the three pasture treatments at the Wellsville site, and the fourth group was assigned to the USU feedlot. All four treatment groups were subdivided into three herds of three cattle, each with a similar distribution of body weight. In 2013, 34 spring-born Angus steers with a mean weight of 451 kg ± 5 kg SEM (994 lbs. ± 11 lbs. SEM) were sorted into five treatment groups with similar body weight distributions. Ten steers were assigned to the grass treatment at the Lewiston site and the remaining 24 steers were sorted into four treatment groups of six cattle each. One of these groups was assigned to each of the three pasture treatments at the Wellsville site, and the fourth group was assigned to the USU feedlot. These four treatment groups were subdivided into two herds of three cattle, each with a similar distribution of body weight. Each year at the Wellsville site, cattle in all herds grazed paddock subdivisions of their assigned treatment within a single rep during each week of the study, and at the USU feedlot each herd was confined to its own pen.

The Wellsville pastures were subdivided at the beginning of each week into eight, six or four paddocks in 2011, 2012 and 2013, respectively. Herds of three cattle were randomly assigned to one paddock, and every second paddock was unoccupied. After 3.5 days, herds were moved to a previously ungrazed paddock within the same replication. At the Lewiston site, pairs of cattle were assigned to each of the five grass pasture replications, and rotationally grazed within that pasture until the end of the grazing season. Lewiston pastures were subdivided into 6 paddocks (Fig. 2.2), and pairs of steers were moved to a fresh paddock every 3.5 days. For the first 4 weeks of the study period in 2013, cattle assigned to the grass treatment continuously grazed an endophyte-free tall fescue pasture while the MB pasture enclosures were being completed; these steers were moved to MB pastures the first week of July 2013. Every occupied paddock was supplied at all times with fresh water and a trace mineralized salt block (Morton iOFIXT T-M). Cattle in this study were not implanted with growth hormones or treated withcother growth-stimulating inputs. In 2011 and 2012, cattle were treated every 4 (2011) or 3 (2012) weeks with a liquid spray to reduce flies, and in 2013 were given Permethrin® ear tags (Y-Tex GardStar Plus) at the beginning of the grazing season.

Data Collection

Cattle were shrunk overnight and weighed on two consecutive days at the beginning and end of the study each year, and once every 28 days in 2011 or once every 21 days in 2012 and 2013, to determine average daily liveweight gain (ADG). Blood samples for plasma fatty acid composition were also taken at the beginning and end of the grazing period each year. Blood was collected from the caudal vein of each animal in vacuum tubes lined with EDTA and immediately placed on ice. Blood samples were centrifuged at 1,200 × *g* for 10 min at 4°C, and 3 0.5-mL aliquots of plasma were stored at -80°C until analyzed.

Before cattle were moved to a new pasture replication (or a new paddock at Lewiston) each week, ungrazed pastures of the next replication or paddock were sampled for forage DM and nutritive value. In 2012 and 2013, pure samples of the seeded plant species were also collected for tannin analysis from pre-grazing pastures. Forage DM was estimated non-destructively using a rising plate meter calibrated for each plant species. At least 30 rising plate meter measurements were averaged to estimate pre- and post-grazing DM, and calibration samples were collected from each replication of each pasture each week. Forage nutritive value samples were clipped by walking a transect across an ungrazed paddock and clipping a sample of grazable forage every few steps. These samples were dried to constant weight at 55°C, weighed, ground with a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ) to pass a 1 mm screen, and assayed using near infrared spectroscopy (NIRS; FOSS Model 6500, FOSS NIRSystems Inc., Eden Prairie, MN). Samples for tannin assay were frozen in the field between blocks of dry ice, then freeze dried and ground to pass a 0.5 mm screen before assay.

Laboratory Analyses

Forage samples were analyzed for condensed tannins using the butanol-HCl-acetone method of Grabber et al. (2013). Briefly, triplicate 0.030 g dry matter of ground plant tissue was suspended in 15 mL of tannin assay solution and heated for 2.5 h in a 70 °C water bath. Samples were mixed periodically during heating. Standard and blank solutions and check samples were also included in each run. Tannin assay solution contained 0.15% w/v ammonium iron (III) sulfate dodecahydrate, 3.3% v/v water, 5% v/v concentrated HCl, 41.7% v/v butyl alcohol, and 50% v/v acetone. After tubes cooled, they were centrifuged at 5,000 *x g* for 10 minutes and the absorbance of the supernatant was determined at 554 nm.

Tannin standards for the spectrophotometric assay were isolated from a single sample of Oberhaunstadter cv. of *L. corniculatus* as described by Hagerman (2011).

Briefly, a suspension of 5% w/v finely ground plant material in 1% v/v acetic acid, 24% v/v water and 75% v/v acetone was sonicated for 30 minutes with periodic mixing. Mixtures were centrifuged for 10 minutes at 3000 *x g* and the supernatant filtered through a coarse fritted disk; plant material was extracted a total of three times and supernatants combined. The supernatant was mixed with an equal volume of ethyl ether and the aqueous layer was retained; the supernatant was extracted a total of three times with equal volumes of ethyl ether. The acetone and ethyl ether remaining in the aqueous solution were removed by rotary evaporation. The aqueous solution was mixed with Sephadex LH 20 resin equilibrated in a 4:1 v/v ethyl alcohol:water solution, rinsed with 95% ethyl alcohol, and extracted with a 3:1 v/v acetone:water solution. The acetone was removed by rotary evaporation, and the aqueous solution was frozen and freeze-dried.

Blood fatty acids were analyzed according to the method of O'Fallon et al. (2007) with slight modifications. Briefly, 100 µL of plasma or forage extract was added to a screw cap tube along with 100 µL of internal standard (0.5 mg/mL C19:1 triglyceride in chloroform), 700 µL of 10 M KOH and 5.3 mL of methanol. Samples were incubated for 1.5 h at 55°C in a shaking water bath, after which tubes were cooled on ice before 570 µL of 12 M H_2SO_4 was added. Tubes were inverted several times to mix thoroughly and then placed back in the shaking water bath at 55°C for 1.5 hrs. Tubes were cooled on ice, 3 mL of hexane was added and samples were vortexed for 30 sec. Samples were centrifuged for 5 min at 1,000 × *g* to separate phases, and 1 mL of the hexane layer was pipetted into a sample vial for gas chromatographic analysis.

Fatty acid methyl esters were analyzed using a Shimadzu GC2010 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD) equipped with an HP-

88 capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Agilent Technologies, Santa Clara, CA) and a flame ionization detector. The injector was maintained at 250°C and a head pressure of 206.7 kPa, and 1 μL of sample was injected at a split ratio of 10:1. Hydrogen was used as the carrier gas at a linear flow rate of 31.1 mL/min. The oven program was as follows: initial temperature 35°C for 2 min, ramp at 40°C/min to 175°C, hold 4 min, ramp at 3.5°C/min to 250°C and hold for 25 min. The flame ionization detector was operated at 250°C, with air and hydrogen supplied at 400 and 39 mL/min, respectively. A standard mixture of fatty acids (GLC-663; NuChek Prep, Elysian, MN) was used for analyte identification by retention time and for the generation of response factors. Fatty acid data are expressed as a percentage of all fatty acids detected (g/100 g fat).

Statistical Design and Analysis

Herds of three cattle that moved through all six replications at weekly intervals served as the experimental unit at the Wellsville site, and herds of two cattle along with the pasture to which they were assigned served as the experimental unit at the Lewiston site. Forage pre-grazing and post-grazing dry matter, allowance, utilization and tannin concentration were analyzed using mixed model with year, species as classified fixed effects, week and pre-grazing dry matter (for post and utilization only) as covariates and with grazing units as random blocks. Animal weights were analyzed using a mixed model in which year and treatment were classified as fixed effects and days in study as a continuous regressor. Treatment and year were tested at the herd level, and ADG was tested at the animal level using a random coefficients model with animals as the subjects.

Average daily gains were the slope estimates of days in study under different year and treatment combinations. The estimated coefficient for initial weight was 0.99±0.01; therefore, initial weights were chosen as an appropriate offset for the ADG model. The covariance structure AR(1) was chosen for the residuals within each animal to account for the correlations among repeated measurements. A separate analysis was carried out for the year 2013 to allow inclusion of a grass treatment. Blood fatty acid components of the animals were analyzed using a mixed model with year, treatment and date (start, end) as classified fixed effects. Year and treatment were tested at herd level and date was tested at animal level. The residual covariance structure was compound symmetry to account for the repeated measures on each animal. Each of the seven blood fatty acid components was analyzed separately. All analyses were conducted using PROC GLIMMIX of SAS/STAT 13.2 (SAS Institute, Cary, NC). We used the ESTIMATE statement in the procedure to compare the slopes for ADG and to compare the LSMeans of interest for blood fatty acid components.

RESULTS

Pasture dry matter is reported for the pastures (Wellsville) or paddocks (Lewiston) at the time they were subject to grazing (Fig. 2.3-2.6). From 2011 to 2012, mean pre-grazing and post-grazing dry matter increased ($P \leq 0.01$), and the trend continued into 2013 (Table 2.1). Forage allowance in pastures (Table 2.2; forage disappearance reported as a function of steer live body weight) also increased for legume pastures over the course of the study. The dry matter remaining after grazing varied but was generally above 2000 kg ha^{-1} with the exception of the meadow brome pastures, where mean post-grazing biomass averaged 1733 kg ha^{-1}. Mean post-grazing pasture dry matter was maintained between 1733 and 2898 kg ha^{-1} throughout the study (Table 2.1) by adjusting stocking density so that forage availablility would not limit intake. Low stocking density is demonstrated by the high forage allowance and low forage utilization (Fig. 2.3-2.6), which ranged between 49 and 57% of available forage (Table 2.3). In all three years, pastures were grazed twice during the season.

In 2012 and 2013, pastures were sampled for tannin concentration. The tannin concentration was measured in all forages, including CMV and MB (Fig. 2.4) which are not reported to contain condensed tannins. In both 2012 and 2013, mean tannin concentrations in Norcen and Oberhaunstadter BFT ranged between 10 and 14 g kg^{-1} DM (Table 2.4) and were not significantly different for the two BFT cv.

Mean ADG for all pasture treatments are reported in Table 2.5, along with the ADG of control steers in the feedlot. Change in steer weights at 3- or 4-week intervals are illustrated in Fig. 2.8, where the reported rate of gain is the slope of the steeper linear regressions that followed adjustment to pastures. Mean gain on pastures of the two BFT cv. was always higher than on CMV pastures even though forage allowance on CMV was numerically higher than on BFT pastures (Table 2.2), and lower than for feedlot-fed cattle. Gains were higher on Oberhaunstadter than Norcen BFT in 2011, not significantly different in 2012, and higher on Norcen than Oberhaunstadter in 2013

Blood plasma fatty acid variables did not differ among treatments at the beginning of the study in any year (Tables 2.5-2.7), but initial blood fatty acid composition did differ among years due to the feed regimen immediately preceding the pasture study. Steers were fed a mixture of alfalfa hay and corn silage before the start of the grazing study in 2011 and 2013, but in 2012, no corn silage was fed and this is reflected in the higher omega-3 and lower omega 6 concentrations in their blood plasma at the beginning of grazing. At the end of each grazing season, all steers on pasture diets had favorable omega-6 to omega-3 (n-6:n-3) ratios in their blood plasma, between 1.5 and 2.8, while feedlot cattle had higher n-6 levels and lower n-3 levels after 12 weeks on concentrates (Tables 2.2-2.8).

DISCUSSION

Forage Production

Factors that influenced forage production during this study included the normal development of perennial forages in the years following planting, and the number and weights of cattle assigned to pastures. In a Utah BFT variety trial (MacAdam and Griggs, 2013) forage production increased for the first two years after planting. Values reported in Missouri by Wen et al. (2002) for spring-grazed BFT pastures were 3,304 and 4,266 kg/ha in successive years, a 29% increase, and we also found an increase in pre-grazing DM of 34% in Norcen and 31% in Oberhaunstadter between 2011, the year following planting, and 2012.

Condensed Tannins

Meadow brome and CMV were not expected to contain tannins (Broderick and Albrecht, 1997), so minimal assayed values are likely due to plant phenolics other than tannins. The tannin concentrations of Norcen and Oberhaunstadter were consistent with literature reports showing both to have relatively low concentrations of tannins, but with Oberhaunstadter ranking higher than Norcen (Marshall et al., 2008).

Average Daily Gains

Average daily gains ranged from 0.63 kg d^{-1} on CMV in 2011 and 2013 to 1.03 kg d^{-1} for Norcen BFT in 2013 (Table 2.4 and Fig. 2.8). Wen reported spring gains of 1.26 to 1.53 kg d^{-1} on pure stands of BFT in Missouri and MacAdam et al. (2011) reported gains of 1.30 to 1.64 kg d^{-1} on pure stands of BFT for 61 to 77 days. Since the morphology, chemical composition and nutritive value of CMV and BFT are not identical, the higher gain on BFT cannot be attributed to tannins, but BFT tannins have been demonstrated to improve the efficiency of protein utilization in forage legumes (Waghorn, 2008). In 2013, cattle assigned to the grass treatment lost weight for four weeks on tall fescue pastures, so the rate of gain on meadow brome for the final 6 wk of the season likely included compensatory growth. Gains reported by other studies for cool-season perennial grass pastures have been as high as 0.84 kg d^{-1} for tall fescue (Hess et al., 1996) and smooth bromegrass (*Bromus inermis* Leyss.; Barker et al., 1999). On grass-legume mixtures, gains of 0.87 kg d^{-1} for tall fescue-BFT (Wen et al., 2002), 0.97 kg d^{-1} for smooth brome-BFT pastures (Barker et al., 1999), and 1.81 kg d^{-1} for early-season irrigated white clover-orchardgrass (*Dactylis glomerata* L.) pastures (Hull et al., 1961). Feedlot gains in the current study ranged from 1.14 to 1.57 kg d^{-}1. In a typical feedlot setting, ADG for cattle are reported to range from 0.79 to 2.43 kg d^{-1} for 90 to 120 days (Reinhardt et al. 2012), so our gains were reasonable, especially considering that the feedlot-fed cattle received no growth-promoting inputs.

In 2011, the cattle used in the study had been on range and did not adjust well to rotational stocking management. In 2012, cattle of similar initial weight were more settled and expressed steady responses to treatments for the 12 weeks of the study. In 2013, cattle of higher initial weights were used so they could be slaughtered at the end of the grazing season. Even the feedlot cattle did not gain well during the first three weeks of the study, but treatment separation was better than in previous years: during the 9 weeks following the adjustment period, Norcen-fed cattle gained 0.2 kg d^{-1} more than Oberhaunstadter-fed cattle, which gained 0.2 kg d^{-1} more than CMV-fed cattle. Cattle on MB gained at about the same rate as cattle on Oberhaunstadter, but as noted earlier, this rate of gain may have been influenced by the 4 weeks of weight loss these cattle experienced on tall fescue pastures.

Blood Plasma Fatty Acids

Following grazing in 2011 and 2012, when younger cattle were used, there were few differences in fatty acid concentrations among legume treatments, and differences between cattle fed the two BFT cultivars were not consistent. The other very striking difference between pasture-finished and feedlot-finished steers in 2013 is the n-6 to n-3 ratios in blood plasma. These characteristics differed in younger cattle, but n-6 fatty acids were 50% higher than BFT-fed cattle and double that of grass-fed cattle in 2013, and n-3 fatty acids were one-third to one-quarter that seen in pasture-finished cattle. The higher final weights in 2013, when cattle were slaughtered after finishing, and suggests that as feedlot-fed cattle accumulate body fat, the omega-6 to omega-3 fatty acid ratio increases, in this study from 7.6 in 2012, to 11.4 in 2013, when feedlot-fed cattle averaged 567 kg at the end of the grazing season.

In studies of other tannin-containing legumes, the fatty acid composition of blood plasma predicted trends in the fatty acid composition of intramuscular fat (Vasta et al., 2009). At the end of the 2013 grazing season, the blood plasma of cows on the grass treatment was significantly higher in *trans*-vaccenic acid (TVA), the precursor of conjugated linoleic acid (CLA; 18:2c9t11) in intramuscular fat. *trans*-Vaccenic acid is produced in the rumen via biohydrogenation of mono- and polyunsaturated substrates, including both linoleic and linolenic acids; linolenic acid is more efficiently converted to TVA than linoleic acid (Bauman et al., 2003). In cattle on a feedlot diet in 2013, TVA was significantly lower than for steers on any pasture treatment. Cattle fed on pastures and in the feedlot in 2013 were slaughtered, and subjected to carcass evaluation. The meat from these cattle was used in sensory panels and was evaluated for quality characteristics. The results of these studies will be reported in other publications.

CONCLUSIONS

Across the three years of the study, steers gained 0.94 kg d^{-1} during a 3-month grazing period on Norcen BFT and 0.83 kg d^{-1} on Oberhaunstadter BFT compared with 0.69 kg d^{-1} on cicer milkvetch and 1.31 kg d^{-1} in the feedlot. This study also demonstrated that the blood fatty acid concentration of saturated and omega-6 fatty acids was reduced and omega-3 fatty acids was increased for all pasture-fed compared with feedlot-fed cattle.

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Table 2.1 Mean forage dry matter in pre- and post-grazed pastures.

	Pasture Dry Matter									
	2011		2012		2013			Mean		
Grazing Period Treatment	Pre	Post	Pre	Post	Pre		Post	Pre	Post	
					kg/ha					
Grass					4058		1733			
CMV	4448	1890	5169	2165	6347[†]	a	2898	5322	2284	b
Norcen	4075	2046	5445	2456	5517	b	2682	5012	2445	ab
Ober	4540	2270	5476	2535	5752	ab	2782	5256	2514	a

[†] Values in columns followed by different letters are statistically different at $P \leq 0.05$.

Table 2.2 Forage DM allowance of pasture treatments.

	Forage Allowance			
Grazing Period Treatment	2011	2012	2013	
	% of BW/d[†]			
MB			4.52	
CMV	3.48	5.48	6.81[‡]	a
Norcen	3.31	4.98	5.49	b
Ober	2.95	4.92	5.91	b

[†] Values are means of pre-graze minus post-graze forage DM divided by cow live body weight (BW) and days per grazing unit * 100.

[‡]Values in columns followed by different letters are statistically different at $P \leq 0.05$.

Table 2.3 Percent forage utilization, or disappearance of pre-grazing forage dry matter.

	Forage Utilization[†]				
Grazing Period Treatment	2011	2012	2013	Mean	
	% of Pre-grazing DM				
MB			57	57	
CMV	57	57	54	56	a
Norcen	50	53	51	51	ab
Ober	49	51	51	50	b

[†] Values in columns followed by different letters are statistically different at $P \leq 0.05$.

Table 2.4 Condensed tannin concentrations of pasture forages in 2012 and 2013.

Grazing Period Treatment	Condensed Tannin Concentration 2012		Condensed Tannin Concentration 2013	
	g kg^{-1} dry matter			
MB			2.48	b
CMV	2.45†	b	1.72	c
Norcen	10.65	a	12.92	a
Ober	12.54	a	13.60	a

†Values in columns followed by different letters are statistically different at $P \leq 0.05$.

Table 2.5 Mean steer average daily gain.

	Average Daily Gain						
Grazing Period Treatment	2011		2012		2013		Mean
	kg/d						
MB					0.88	bc	
CMV	0.64[†]	d	0.81	c	0.63	c	0.69
Norcen	0.80	c	0.98	b	1.03	b	0.94
Ober	0.95	b	0.88	bc	0.65	c	0.83
Feedlot	1.14	a	1.21	a	1.57	a	1.31

[†] Values in columns followed by different letters are statistically different at $P \leq 0.10$.

Table 2.6 2011 cattle blood plasma fatty acid composition at the beginning (23 June) and end (9 Sept) of a 78-d grazing period on perennial legume pastures containing different concentrations of tannins, or in a feedlot.

		Grazing Period Treatment			
Fatty acid component	Date	CMV	Norcen	Ober	Feedlot
		g/100 g fat			
Saturated fatty acids	Start	35.1	34.3	35.0	34.3
	End	34.9[†] b	35.4 b	34.3 b	37.8 a
trans-Vaccenic acid	Start	0.907	0.890	0.972	0.771
	End	0.600 b	1.094 a	0.721 b	0.374 c
Monounsaturated fatty acids	Start	14.6	14.0	14.8	13.8
	End	12.2 a	10.7 b	9.1 c	8.6 c
Polyunsaturated fatty acids	Start	43.9	45.5	43.2	45.5
	End	47.7	47.3	50.6	47.9
Total omega-6 fatty acids	Start	32.5	34.2	32.0	34.4
	End	30.6 b	31.5 b	33.2 b	37.9 a
Total omega-3 fatty acids	Start	11.1	11.0	10.9	10.7
	End	16.8 a	15.7 a	17.2 a	9.7 b
Omega-6:omega-3 ratio	Start	2.93	3.10	2.95	3.22
	End	1.83 b	2.00 b	1.93 b	3.93 a

[†] Values in rows followed by different letters are statistically different at $P \leq 0.05$.

Table 2.7 2012 cattle blood plasma fatty acid composition at the beginning (31 May) and end (23 August) of an 84-d grazing period on perennial legume pastures containing different concentrations of tannins, or in a feedlot.

		Grazing Period Treatment			
Fatty acid component	Date	CMV	Norcen	Ober	Feedlot
		g/100 g fat			
Saturated fatty acids	Start	35.3	35.4	36.2	35.2
	End	33.7	33.3	33.4	34.2
trans-Vaccenic acid	Start	0.99	1.00	1.05	0.93
	End	1.24[†] a	1.24 a	1.27 a	0.56 b
Monounsaturated fatty acids	Start	12.5	12.5	12.1	12.4
	End	11.0 ab	9.8 b	9.9 b	11.7 a
Polyunsaturated fatty acids	Start	44.3	44.4	43.9	44.2
	End	48.6	50.3	50.4	48.9
Total omega-6 fatty acids	Start	24.8	24.5	24.3	23.8
	End	30.5 b	31.3 b	32.3 b	43.1 a
Total omega-3 fatty acids	Start	19.1	19.6	19.1	20.0
	End	17.8 a	18.8 a	18.0 a	5.6 b
Omega-6:omega-3 ratio	Start	1.30	1.25	1.27	1.19
	End	1.71 b	1.67 b	1.80 b	7.71 a

[†] Values in rows followed by different letters are statistically different at $P \leq 0.05$.

Table 2.8 2013 cattle blood plasma fatty acid composition at the beginning (31 May) and end (23 August) of an 84-d grazing period on perennial grass or legume pastures containing different concentrations of tannins, or in a feedlot.

		Grazing Period Treatment				
Fatty acid component	Date	Grass	CMV	Norcen	Ober	Feedlot
		g/100 g fat				
Saturated fatty acids	Start	34.6	34.7	33.9	35.5	33.9
	End	32.4	33.2	31.2	31.9	31.8
trans-Vaccenic acid	Start	0.594	0.562	0.611	0.609	0.561
	End	2.198[†] a	0.853 b	1.040 b	1.162 b	0.132 c
Monounsaturated fatty acids	Start	20.7	20.9	18.4	19.5	21.0
	End	20.0 a	18.9 ab	15.8 c	16.8 bc	14.9 c
Polyunsaturated fatty acids	Start	39.5	39.4	41.9	39.6	40.1
	End	38.0 c	42.1 b	47.2 a	45.7 ab	49.2 a
Total omega-6 fatty acids	Start	29.3	29.4	30.8	29.0	29.6
	End	22.6 d	27.2 c	29.9 b	29.9 b	45.1 a
Total omega-3 fatty acids	Start	10.0	9.8	11.0	10.4	10.3
	End	14.9 b	14.7 b	17.1 a	15.6 ab	4.0 c
Omega-6:omega-3 ratio	Start	2.92	3.01	2.81	2.79	2.87
	End	1.51 c	1.85 b	1.74 b	1.91 b	11.32 a

[†] Values in rows followed by different letters are statistically different at $P \leq 0.05$.

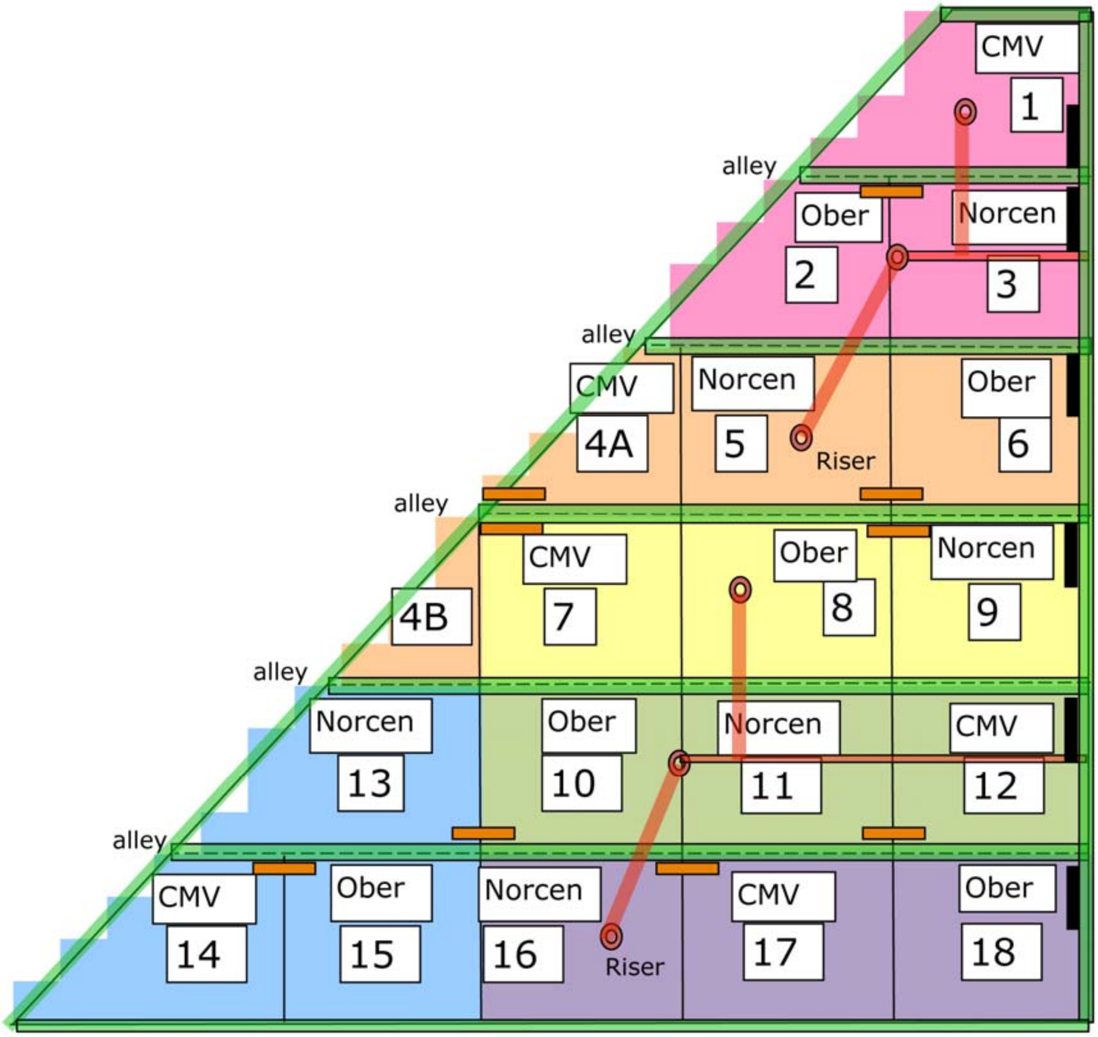

Figure 2.1 Pastures with assigned treatments at the Caine Dairy in Wellsville, Utah. Each replication was comprised of a group of three pastures; replications are indicated with different colors.

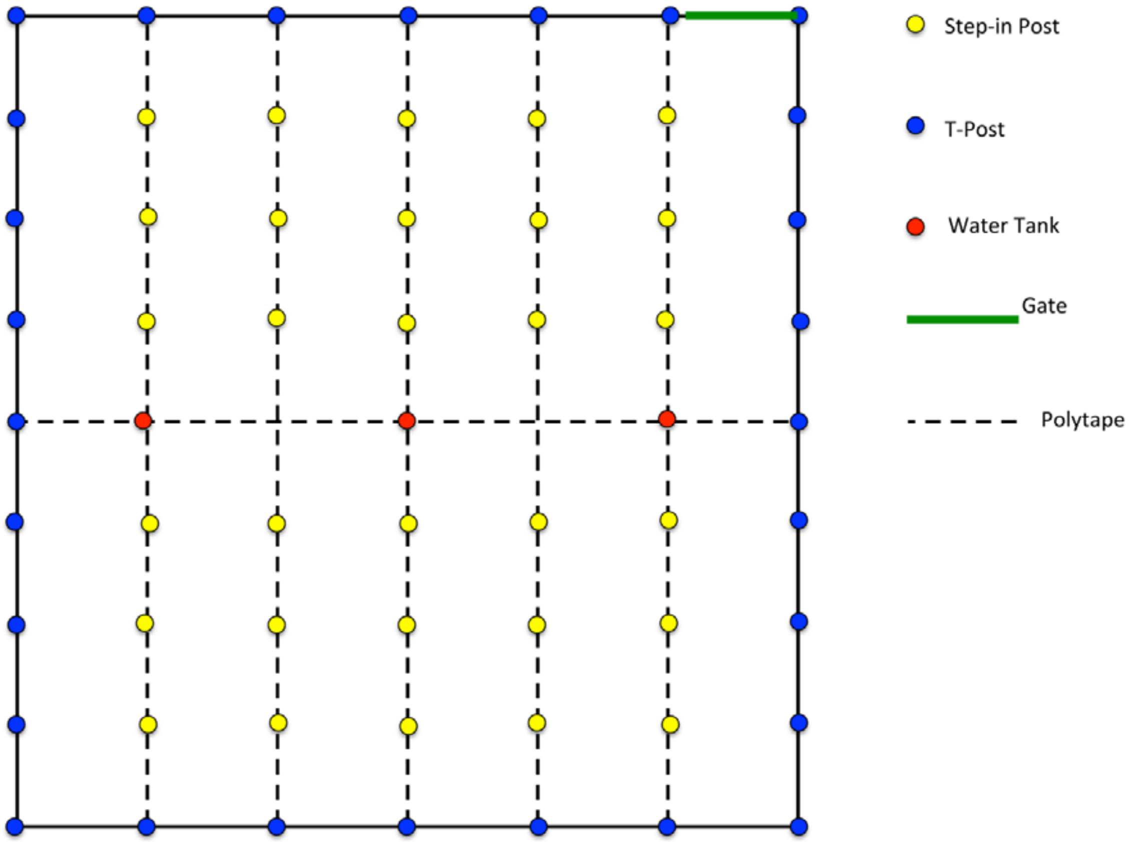

Figure 2.2 Lewiston meadow brome (MB) pasture design demonstrating the 12 paddocks that were each grazed for 3.5 days during each rotational grazing cycle.

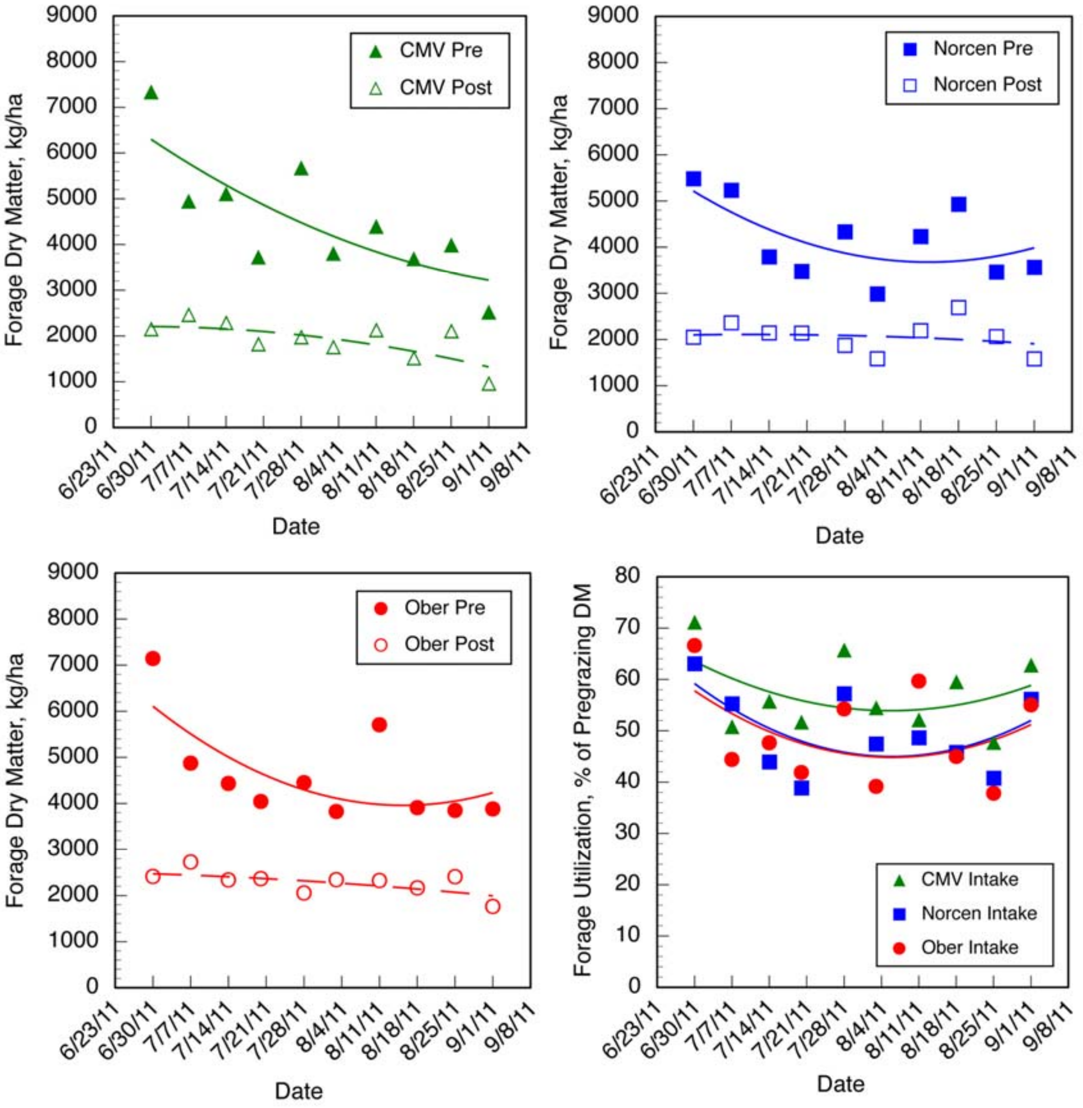

Figure 2.3 2011 forage dry matter before (blue) and after (red) grazing of CMV and BFT (Norcen and Ober) pastures, and intake measured as the disappearance of forage.

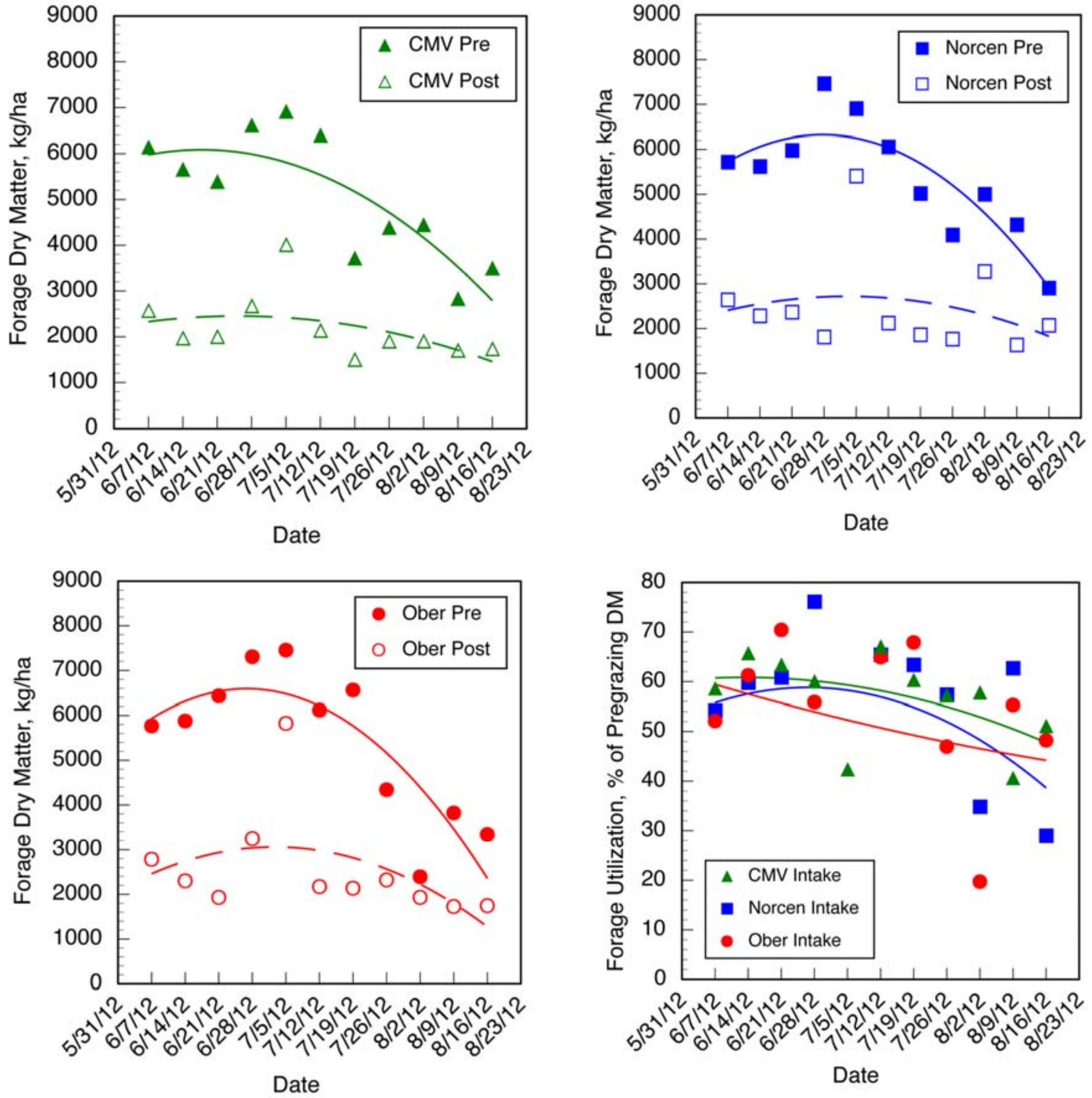

Figure 2.4 2012 forage dry matter before (blue) and after (red) grazing of CMV and BFT (Norcen and Ober) pastures, and intake measured as the disappearance of forage.

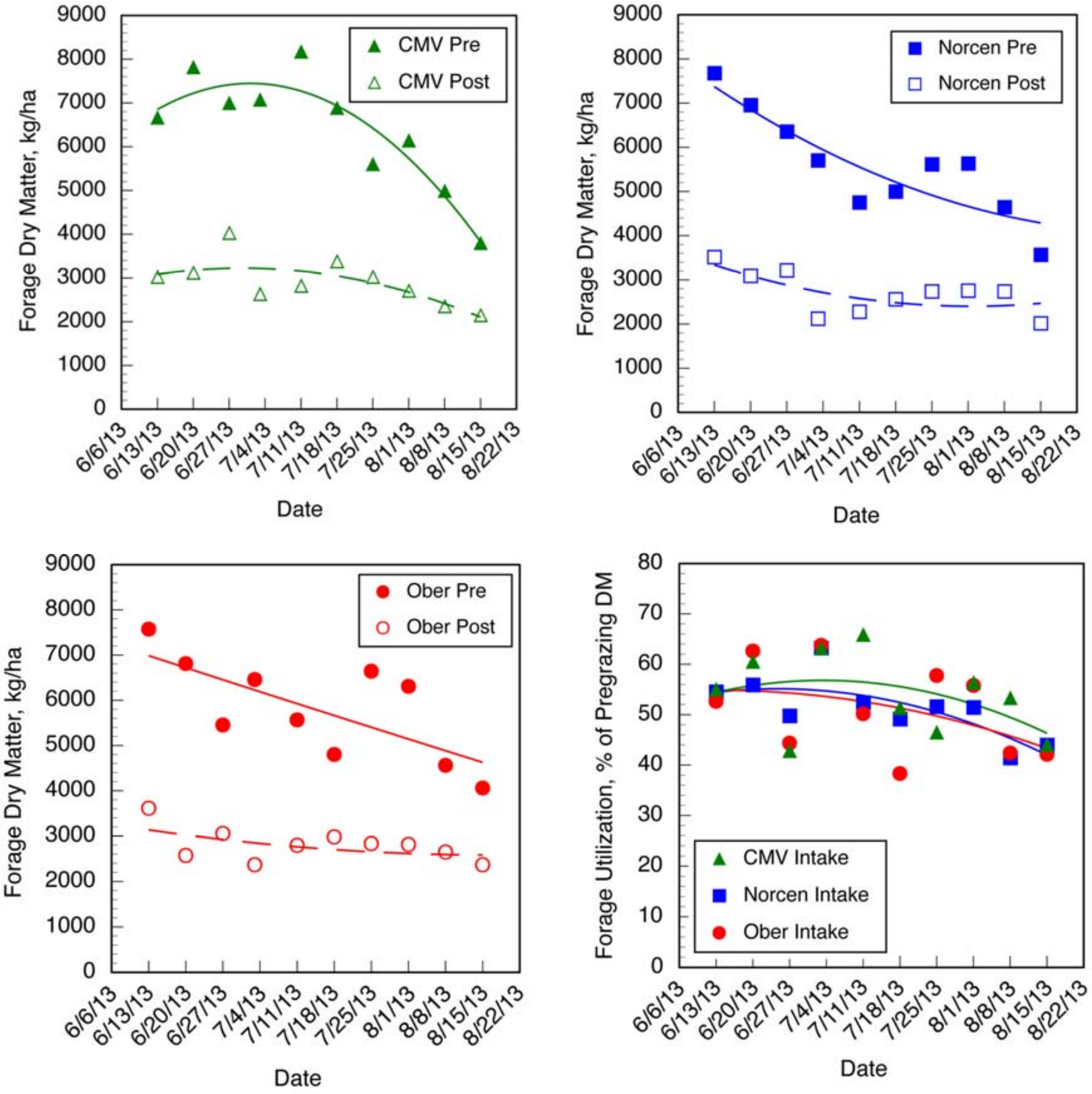

Figure 2.5 2013 forage dry matter before (solid symbols) and after (open symbols) grazing of CMV and BFT (Norcen and Ober) pastures, and intake measured as the disappearance of forage.

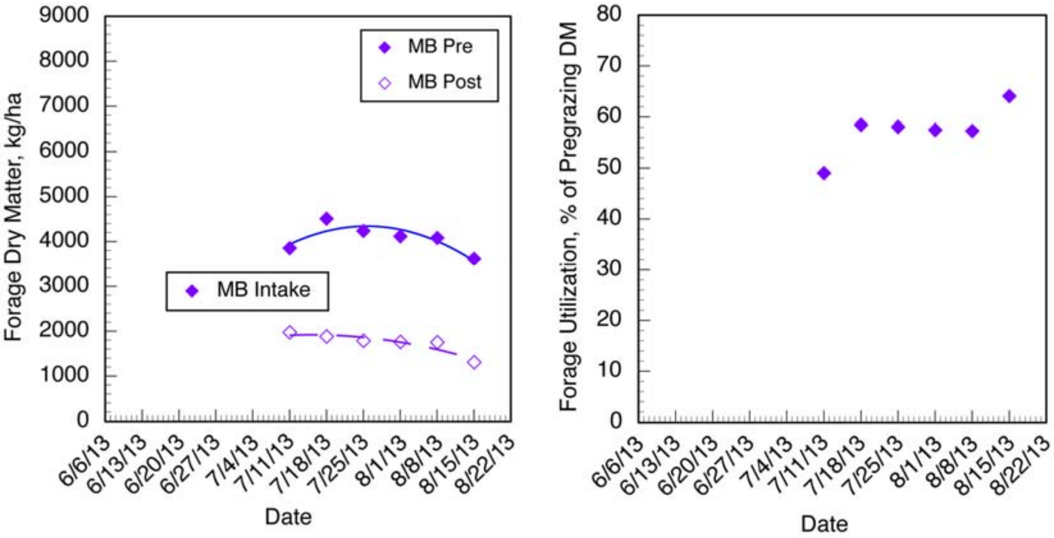

Figure 2.6 2013 forage dry matter in pastures before (solid symbols) and after (open symbols) grazing of MB, and intake measured as the disappearance of forage.

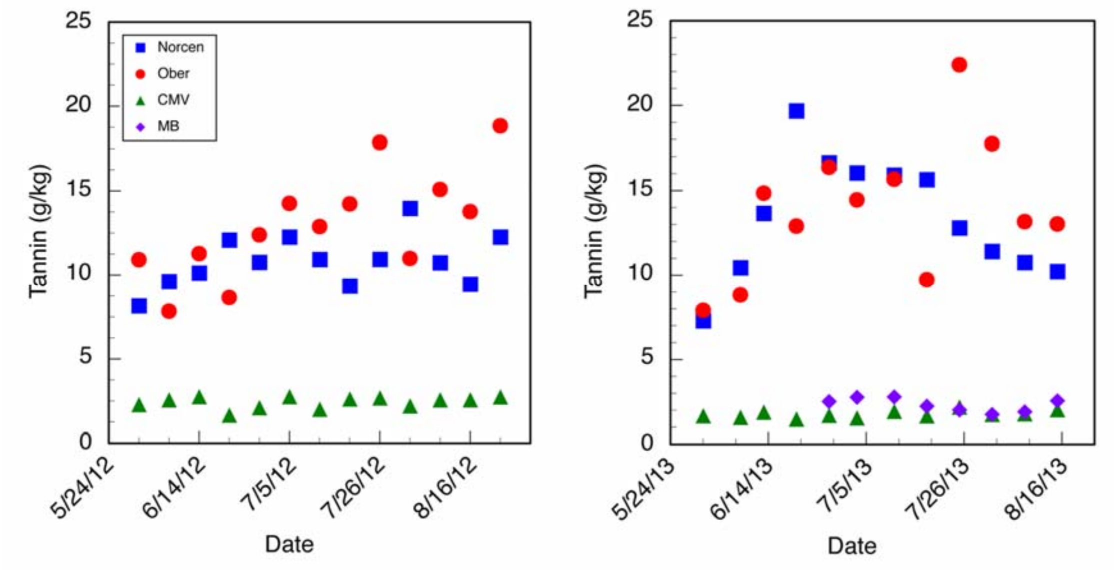

Figure 2.7 2012 and 2013 tannin concentrations in pre-grazed pastures. In 2012, tannins in BFT (Oberhaunstadter and Norcen cultivars) appeared to increase over time, while in 2013 tannin followed patterns of seasonal change seen in other studies of BFT regrowth under rotational stocking management, increasing through mid-summer and decreasing later in the season.

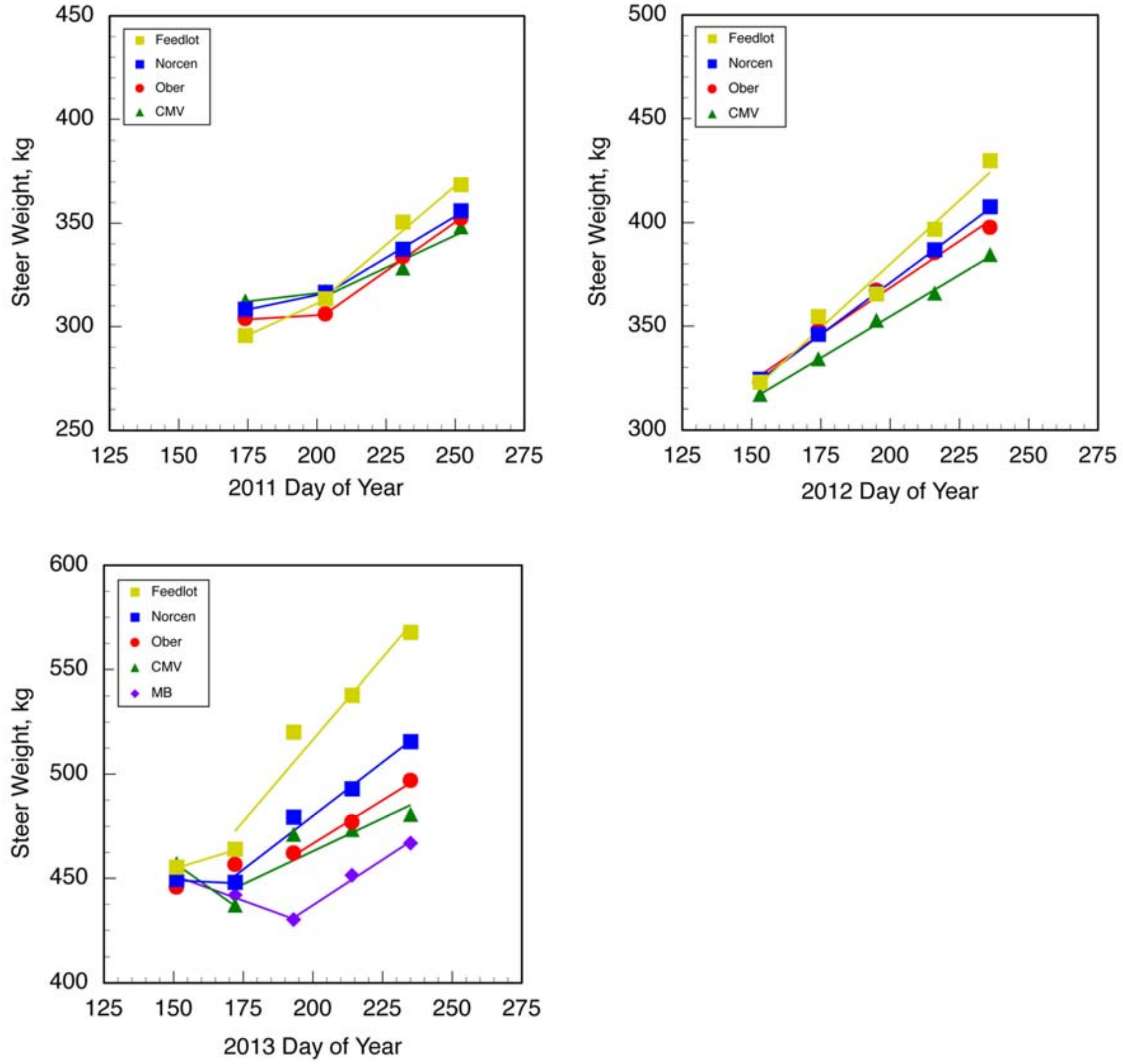

Figure 2.8 Steer average daily gains. Rates of gain reported in Table 2.3 are based on the linear increase in live body weight following adjustment to pastures.

CHAPTER 3

METHANE EMISSIONS AND THE SF_6 METHOD

INTRODUCTION

Methane (CH_4) produced from livestock and its effects on global climate change are a cause of concern on a worldwide scale (Martin et al., 2010). In the United States, CH_4 represented 14% of the total greenhouse gas emitted in 2007 and agriculture was responsible for roughly seven percent of this CH_4 (U.S. EPA, 2013). Ruminant livestock farming operations produce the greatest CH_4 from human-related activities (Lassey, 2007), and enteric CH_4 produced by ruminant livestock represents one third of the US emissions due to agriculture (U.S. EPA, 2013).

Rumen Methanogenesis

Roughly 90% of CH_4 from ruminants is discharged through the mouth and nose (Hook et al., 2010). The enteric CH_4 that is expelled by ruminants is produced in the rumen through digestion of feed materials by rumen microbes in an anaerobic environment. Through this digestion process, microbes produce the volatile fatty acids acetate, butyrate and propionate that are the main energy source for the ruminant (Martin et al., 2010). When acetate is metabolized in the rumen it provides a methyl group for methanogenesis. As ruminants digest greater amounts of crude fiber, the ratio of acetate to propionate increases, resulting in higher amounts of CH_4 produced in the rumen that is then expelled by the animal (Hook et al., 2010; Mountfort et al., 1982).

The CH_4 that is expelled from the rumen represents a loss of gross energy. This loss can be anywhere from 2 to 12% of feed energy and depends on feed quality, feed intake and feed composition, which have a direct impact on rumen microbes and the concentrations of acetate, butyrate and propionate that they produce (Hook et al., 2010). Over the last several decades there has been an increasing interest in plant oils and secondary compounds and their ability to reduce CH_4 generation in ruminants (Martin et al., 2010). These compounds include saponins, tannins, alkaloids and essential oils, and their effects on rumen microbes and the production of acetate, butyrate and propionate have been studied (Cieslak et al., 2013). Research using extracts and plant material from birdsfoot trefoil (*Lotus corniculatus* L.; BFT), a condensed tannin-containing legume, have shown a reduction of CH_4 production of up to 30% in sheep and goats (Martin et al., 2010). Another study by Woodward et al. (2004) in New Zealand showed that the condensed tannins in BFT lowered CH_4 production by about 13% in dairy cows. Similarly, when condensed tannins were provided at 2-4% dry matter they reduced the amount of CH_4 produced by rumen microbes an in vitro study (Williams et al., 2010).

Previous studies have shown that CH_4 production is primarily determined by the quantity and more importantly the quality of the forage that is being fed. Feeds with higher digestibility yielded lower CH_4 emissions when compared to those with poor digestibility. For feedlot cattle that are fed concentrate diets, roughly 2% of their intake energy is converted to CH_4, while in cattle that graze in poor quality rangeland or pasture settings, as much as 12% of their intake energy is converted to CH_4 (Johnson et al., 2007).

Methane Collection

Many systems exist today that can measure CH_4 emissions from livestock. Some can only measure single animals while others can measure several animals in the same treatment at one time. Metabolism chambers and the sulfur hexafluoride (SF_6) tracer gas technique are the most widely used to determine CH_4 emission rates of individual ruminants (Johnson et al., 2007). Data from well-maintained metabolism chambers can be very precise but studies are limited in scope by the fact that trained animals are necessary, forages must be harvested and fed, animal movement is reduced and the systems require high labor input and are expensive, limiting the number of animals that can be tested (Johnson et al., 2007).

The SF_6 technique was created to address the limitations of metabolism chambers and is the only accurate method for measuring integrated daily CH_4 emissions from individual animals grazing on range or pastures, or in a feedlot setting (Lassey et al., 2011). Methane emissions measured using the SF_6 tracer method have shown similar results to those obtained from metabolism chambers. Three studies looked at the difference in measurable CH_4 from the two different techniques; Johnson et al. (1994) compared metabolism chambers and individual pens, Boadi et al. (2002) used respiration hoods and individual stalls and Grainger et al. (2007) looked at the SF_6 technique while cattle were kept in metabolism chambers. All three studies showed similar values using the SF_6 method in comparison to metabolism chamber (Pinares-Patiño et al., 2011), demonstrating the accuracy of the SF_6 technique.

The SF_6 method utilizes a brass permeation tube containing SF_6 gas with a known release rate that is placed in the reticulorumen. The release rates of the gas from the permeation tubes are assessed gravimetrically for six weeks prior to insertion into the rumen. A halter containing a collection system comprised of a filtered intake tube, capillary tubing and an evacuated PVC collection canister are fitted to the animal, and the intake tube is placed near the mouth and nose of the animal. The evacuated canister has a negative pressure, which draws air continuously through the filter. By varying the length and/or diameter of the capillary tubing and the size of the canister, the duration of the sampling time period can be adjusted. After the sample has been collected for a known time, the canister is removed and is pressurized with nitrogen gas. The collected gas is sampled and assayed using a gas chromatograph, which determines the concentrations of CH_4 and SF_6. The emission rate of the permeation tube and the ratio of SF_6 to CH_4 in the collection canister are used to calculate the enteric emission rate of CH_4 from the animal (Johnson et al., 2007).

OBJECTIVE AND HYPOTHESIS

This study quantified the effects of different pasture diets on enteric CH_4 emissions for cows in a beef production operation. The null hypothesis of this study was that cattle grazing on BFT, meadow brome (MB; *Bromus riparius*), and CMV will not differ in enteric CH_4 emissions.

MATERIALS AND METHODS

Experimental Design

This study was carried out on 15 acres at the USU Intermountain Irrigated Pasture Project Farm in Lewiston, Utah with three different pasture treatments replicated five times. The study was designed as a randomized complete block with three treatments, which were BFT, MB and CMV. Meadow brome is a bunchgrass that grows well under rotational stocking in the northern Mountain West. Meadow brome was chosen as the grass in this study because MB has higher NDF digestibility than orchardgrass, tall fescue or perennial ryegrass (MacAdam et al., 2006) and the cv. “Cache” was selected because it is high-yielding compared with older MB cv (Jensen et al., 2004).

Pasture Establishment

In the spring of 2012, soil tests were conducted and deficiencies of phosphorus and potassium were addressed with fertilizer applied prior to planting. Each pasture was broadcast seeded with CMV, BFT or MB at 20, 34, and 37 kg/ha (18, 30, 33 lbs./acre); cultivars were ‘Monarch’ cicer milkvetch, ‘Langille’ birdsfoot trefoil, and ‘Cache’ meadow brome. These high seeding rates were used to reduce the competition with weed species that is common in monoculture perennial pasture establishment. Legumes were inoculated with the proper *Rhizobium* bacterium species before planting to supply nitrogen, and grass pastures received 150 kg nitrogen ha^{-1} y^{-1} fertilizer as 30-0-0 on 2 June, 14 July and 2 September 2014. All pastures were sprinkler irrigated using hand lines every two weeks throughout the summer into early fall.

Grazing and Feeding

Experiments and procedures involving livestock were carried out following review and approval by the USU Institutional Animal Care and Use Committee. In 2014, 15 beef cows, 4 to 5 years old with a mean body weight of 616 kg +/- 8 kg (SEM) were sorted into three groups of 5 cattle such that each group had a similar total body weight and similar weight distribution. One cow from each group was randomly assigned to each of the five replications of a given treatment. Each cow rotationally grazed the same 0.9-acre pasture for the period from 16 June to 22 August. Each cow was dewormed with albendazole (Valbazen broad spectrum dewormer, Pfizer Animal Health, Exton, PA 19341, USA) at 4 mL/100 lb of body weight before going on pastures, and provided with permethrin ear tags (GardStar Plus, Y-Tex Corp., Cody WY, 82414 USA) to minimize horn flies.

Rotational stocking was used because it maintains a high level of pasture quality and each paddock is grazed to a similar degree even though forage regrowth changes with season. Each cow was moved to a fresh paddock within her pasture every 3.5 days; fresh water and trace-mineralized salt blocks (Morton iOFIXT T-M) were provided at all times. All the cows used were in the later stages of gestation, and calved between mid-August and early September. All cows were to have been sampled for enteric methane emissions before mid-August, but some calves were born prior to the end of the experiment, so cows in one replicatin were sampled in two different weeks. Cows were used in this study because they are the major source of enteric methane emissions in beef production systems (Beauchemin et al., 2011).

Plant Production, Intake, Nutritive Value and Chemical Composition

Available herbage dry matter in the next paddock to be grazed, and remaining dry matter in the paddock being vacated were non-destructively sampled using a Farmworks (Feilding, NZ) rising plate meter (RPM) calibrated for each forage species. Forage dry matter disappearance (intake) was approximated as the difference between pre- and post-grazing dry matter. To sample, pre- and post-grazing paddocks were walked in a "lazy

W" pattern to accumulate a total of 30 rising plate meter readings, which were averaged for that paddock. Care was taken to bring the rising plate meter down perpendicularly to the ground for the most accurate and repeatable readings. Once average readings were recorded, a single forage stand with a RPM reading as close to the average as possible was identified for a calibration sample. A three-sided quadrat with an inside diameter identical to the area of the RPM plate was slipped around the forage under the RPM, and all forage within the quadrat was removed to a 1-cm height and placed into pre-labeled paper bags. These bags were placed in coolers, transported to a drying oven and dried to constant weight. This process was repeated for each pre and post grazing paddock each week for the duration of the study. Forage nutritive value and tannin samples were also taken weekly in pre-grazing paddocks. These samples were collected by walking a transect across the paddock, sampling available forage every few steps. Tannin samples were taken similarly but included only the primary pasture treatment species (BFT, CMV or MB). Sufficient samples were taken to half-fill a gallon sized zip-closure plastic bag, which was immediately sandwiched between blocks of dry ice. Frozen samples were stored at -20 °C, freeze dried, ground to pass a 1 mm screen and analyzed for crude protein, neutral and acid detergent fiber, acid detergent lignin and other forage nutritive value components with calibrated near-infrared spectroscopy (NIRS). Forage nutritive value samples were also assayed for fatty acid concentrations. Forage nutritive value, dry matter intake and digestibility data were used to compute the nutritional value of forage consumed relative to the energy requirements for beef cattle maintenance and gestation.

Ruminant Methane Emissions

In collaboration with Dr. Kristen Johnson at Washington State University and Dr. Karen Beauchemin at Agriculture and Agri-Food Canada, Lethbridge, enteric CH_4 was measured using the SF_6 technique (Johnson et al., 1994, 2007). A permeation tube with a known release rate of SF_6 was placed in the reticulorumen of each cow to serve as an internal standard. Exhaled gas was collected in an evacuated canister using a collection system attached to a halter placed on the animal's head and neck with an inlet near the nose and mouth of each animal. A sample was collected from the three cows in one replication of the pasture study for four successive days each week. Each cow and pasture constituted an experimental unit.

Permeation Tubes

Permeation tubes were comprised of 3.2-cm-long, 1.1-cm outside diameter brass rod machined with a 25.4-mm-deep by 4.77-mm-dia inner cavity and outside threads at one end. Machined tubes were fitted with a stainless steel frit, a Teflon® filter, a #10 nylon washer and a 6.35-mm-deep brass Swagelok® nut. Before assembly, each nut was stamped for identification with a unique letter-number combination. Assembled, empty permeation tubes were weighed, then disassembled for filling. Permeation tube bodies were submerged in liquid nitrogen (LN_2) to chill, then excess LN_2 was drained, and tube bodies were placed in a Styrofoam® insulating block for filling, which was carried out in a fume hood to minimize contamination of laboratory air with SF_6 gas. A 60-mL syringe fitted with an 18-gauge needle less than 25 mm in length was filled from a canister of pure SF_6 gas. The needle was placed inside the permeation tube, allowing the needle coupling to assist in trapping SF_6 inside the tube as the gas was slowly discharged into the permeation tube. The SF_6 gas condenses to a solid in the frozen permeation tube. Approximately 600 mg of SF_6 is required to adequately fill these permeation tubes, and one 60-mL syringe delivered slightly more than 300 mg SF_6. As each permeation tube body was filled with SF_6, the permeation tube was reassembled, sealing the SF_6 gas inside. Permeation tubes were rinsed under warm water to remove condensation, dried, weighed, and placed in glass jars submerged in a 39 °C (rumen temperature) water bath for seven weeks. Each jar was continuously evacuated with pressurized air through inlet and outlet ports in the lid. Outlet port tubing was vented into a fume hood. Permeation tubes were weighed weekly to calculate an hourly SF_6 release rate through the Teflon® filter, which stabilized after 1-2 weeks.

Collection Canisters

Canisters for enteric methane sample collection were constructed from 10.16-cm (4-inch) schedule-40 PVC pipe rated for 220 psi or higher. For a canister with a volume of 2.38 L, pipe was cut it into 27.94-cm (11-inch) lengths. A 10.16-cm (4-inch) slip cap was joined to one end with Oatey PVC cement and primer (#30244). After the cap was set, a 1.74625-cm (11/16-inch) hole was drilled and tapped, and a Swagelock ball valve with threads coated with Teflon® tape was threaded into place. Drill and tap shavings were removed and a cap was joined to the open end as previously described. After the joints were set, canisters were pressurized to 30 psi and submerged in soapy water to check for leaks. Canisters were evacuated to -0.150 psi with a diaphragm vacuum pump (Vacuubrand Model MZ2NT) and joints were allowed to set for 48 hrs. Canisters were again pressurized and checked for leaks, and any that lost more than 0.500 psi were discarded. Canisters were prepared to be hung from each side of halters with two sections of three 2.54-cm (1-inch) long, 0.3429-cm (0.135-inch) thick links of welded steel chain affixed to the ends of the canisters with 45.72-cm (18-inch) cable ties through a 3.175-cm (1.25-inch) diameter steel o-ring that was attached to the chain through a 0.3175-cm (0.125-inch) chain quick link. Chains were attached to halters with a 0.635-cm (0.25-inch) snap link.

Halter Capillary Tube Collection System

The vacuum created in evacuated canisters was used to sample air exhaled from each cow's nose and mouth through capillary tubing attached to a halter, and halters were fitted to specific cows. At the gas collection end of the capillary tubing, an inline particulate filter was attached to 0.3175-cm (1/8-inch) diameter PTFE tubing which in turn was attached to a 5.08-cm (2-inch) length of Tygon® tubing, which rested on a leather pad across the nose of the cow. The other end of the inline filter was attached to a 0.159-cm (1/16-inch) to 0.317-cm (1/8-inch) reduction union to which was attached to 0.159-cm (1/16-inch) capillary tubing. The length of the capillary tubing could be varied to vary the rate of gas sampling, and a length of about 49.53-cm (19.5-inches) allowed for a 24-hr sampling period, filling canisters evacuated to 1 atm to roughly 0.5 atm. A 0.317-cm (1/8-inch) to 0.159-cm (1/16-inch) reduction union was attached to the other end of the capillary tubing, to which a length of 0.317-cm (1/8-inch) PTFE tubing was attached, which in turn was attached to a male quick-connect. The length of the PTFE tubing varied depending on the build of the cow, but in general, a 17.78-20.32-cm (7- to 8 inch) piece was a good length.

Regular horse halters provided good support for the collection system and had attachment points for the collection canister. Halters with a chin strap allowed sufficient adjustment for cattle with different head and muzzle sizes. A piece of leather 0.317-cm (1/8-inch) thick by 10.16-cm x 10.16-cm (4- x 4-inches) was stitched to the halter on the nose piece and 0.635-cm (¼-inch) diameter holes were punched through the leather flap. The filter of the collection system was attached to the leather nose flap with two 10.16-cm (4-inch) long zip ties so that roughly 2.54-cm (1-inch) of the tygon tubing protruded from the end. The filter was adjusted after the halter was placed on the animal. A position just above the nose was best and reduced the chance of feed debris or water entering the filter when the cow was foraging or drinking. From the nose piece, the capillary tubing ran along the jaw strap to the junction of the throat/head strap, and was wrapped with electrical tape to this point, leaving only the reducing union and a 17.78-cm (7-inch) piece of PTFE tubing with a male quick connect hanging free. This was needed to attach the capillary tubing to the collection canisters.

Testing Canisters

To test the halter capillary tube collection system with canisters, all canisters and collection systems were labeled with unique numbers and canisters were draw down to 0.150 psi or lower. After recording canister and halter I.D. numbers and noting the time, a canister was connected to the capillary tubing on a halter. After 24 hrs, the canisters were disconnected from the collection system and connected to a pressure indicator. The desired level of vacuum remaining in the canister after the sampling period was ½ atm, but this can vary greatly depending on the elevation at which the sampling is done. If the remaining vacuum was less than ¼ atm, the capillary tubing was either plugged with debris or the tubing had become kinked. If the remaining vacuum was 2/3 atmosphere or higher, the fittings were either not tight or there was a broken connection. With use, the broken connections most often occurred between the capillary tubing and the collection canister.

Daily Sampling

Each week one cow from each of the three treatments was subjected to four days of enteric methane sampling. The needed number of canisters plus spares were evacuated to less than 0.100 psi early in the morning, and initial pressures were noted. Halters and evacuated canisters were taken to the field, and the cows to be sampled were moved to the working area. Cows were held in a hydraulic squeeze chute while a halter with a collection system was placed on her head. The leather pad holding the filter and intake tubing was placed directly over her nostrils. The head strap was cinched down and the chin strap was left loose enough to fit three fingers between it and the cow to prevent chafing and the development of sores. The canister was then clipped to the halter using the snap links, the halter capillary collection system was attached to the canister via the quick-connect valve, and the ball valve was turned on. The canister and halter were identified along with the cow, and the start time for collection was recorded. Cows were returned to their pastures, and the next morning, the canister on the cow was turned off when the cow was secured in the squeeze chute. The time sampling ended was recorded and the canister was disconnected from the collection system. This process was repeated through Friday morning when halters were also removed from cows. A control canister was installed in one pasture of each treatment in a different replication on each day that samples were collected from cows. For this purpose, a capillary collection system identical to those attached to halters was attached to evacuated canisters, but the nosepiece was attached to the top of a step-in post.

Canisters containing samples from the previous 24 hrs were transported back to the lab for analysis. In the lab, each canister was hooked up to a pressure indicator and the pressure was recorded. Then the canister was pressurized to 1.2 atm with pure N_2 gas and the final pressure was recorded. Samples were drawn by connecting a male quick release valve fitted with a septum to the female quick release connection. This allowed the ball valve to be opened without any loss of sample. Four-mL glass sample vials fitted with a septum were evacuated through a 20-gauge needle connected with Tygon® tubing to a vacuum pump. To sample canister gas, a 20-gauge needle attached to a 20 mL syringe was inserted into the septum connected to the sample canister, allowing a sample to be drawn by hand. A 15- to 18-mL sample was drawn into the syringe, and the needle was pulled out of the septum while slowly depressing the plunger. This prevented air from the surrounding atmosphere from contaminating the sample. The needle was inserted into the vial and the remaining sample was forced into the vial. There was some back pressure on the plunger, and some sample was lost through the septum of the vial as the needle was retracted. Vials were labeled and shipped to Dr. Karen Beauchemin's lab at Agriculture and Agri-Food Canada for CH_4 analysis.

Statistical Analysis

Differences in dry matter yield and allowance were analyzed using a randomized block design in which replicates of the experiment were the blocking units and species within each block were the fixed factor. Week was treated as a covariate. The correlation of grazed paddock within grazing unit was modeled through R-side covariance within each unit. For post-grazing dry matter, forage allowance and utilization, pre-grazing dry matter was also included in the model as a covariate. Enteric methane emission data were analyzed using species and week as fixed factors and animal as blocking unit. All analyses were conducted using PROC GLIMMIX of SAS/STAT 13.2 (SAS Institute, Cary, NC).

RESULTS

Mean pasture DM production (pre-grazing DM) was less for MB than for BFT and CMV (Table 3.1), and post-grazing dry matter was greater for BFT than for CMV and MB (Table 3.1). Week-to-week variation was considerable, and is illustrated in Fig. 3.1. Forage allowance of all forages in this study was much less than for steers (see Chapter 2), because this study used large pregnant cows. Forage utilization of MB was not significantly different from BFT and CMV (Table 3.1; Fig. 3.2) although the utilization of BFT was significantly lower than CMV. The forage allowance (pre-grazing DM as a function of cow live weight; Table 3.2) was higher for MB, suggesting that while MB pre-grazing DM availability was less than for other species, it was not low enough to be limiting. Initial enteric methane data were collected after a four-week adjustment period, and data for each date (week) in Fig. 3.2 are the mean of four measurements per week in most cases. Methane emissions of BFT and CMV were not significantly different (Fig. 3.2), but were higher for MB than for either BFT or CMV (Table 3.3).

DISCUSSION

The contribution of beef enteric methane emissions should be viewed in the larger context of greenhouse gas (GHG) emissions and life cycle assessment (LCA) of the production system. An LCA is a birth to death analysis that quantifies all GHG inputs and outputs, and allows differences in environmental impacts to be calculated. Using a LCA, the methane emissions of our cows on all pasture treatments can be compared to published values for cows on pasture (e.g., Beauchemin et al., 2011).

Forage Production

Wen et al. (2002) reported BFT forage production of 4266 DM kg/ha, similar to BFT production in this study, with a mean of 4206 DM kg/ha for the five BFT pastures. Smoliak and Hanna (1975) reported production of CMV in sub-irrigated pastures to be 4295 DM kg/ha, which is similar to the production of 4595 DM kg/ha on these irrigated pastures. Kopp et al. (2003) reported MB production of 3950 DM kg/ha when fertilized with 95.75 kg/ha of N fertilizer. Production in this study was 4617 kg/ha, possibly because a higher rate (168 kg/ha) of N fertilizer was applied. Legumes produce their own nitrogen through a symbiotic association with rhizobia bacteria that infect the roots, forming nodules that are the site of nitrogen fixation. These nodules eventually break down and decay, providing N to the plants (Heichel et al., 1985). The small differences in DM production on the BFT, CMV and MB pastures compared to prior research could be attributed to differences in rainfall timing, irrigation intervals and amounts and mean monthly temperature differences.

Methane Production

The quantity of enteric CH_4 produced in g per cow per day was lower when cattle grazed on BFT and CMV compared with cows on MB. This difference in methane production on grass and legume pastures is similar to that reported by Woodward et al. (2004) and Williams et al. (2011b). McCaughey et al. (1999) reported methane emissions of 411 g per 24 h on MB, which is nearly the same as the mean of 355 g per 24h produced on MB pastures in this study. Digestibility of the legumes BFT and CMV is higher than that of the grass, MB. The higher concentration of crude fiber in MB will increase the proportion of acetate to propionate, increasing the production of CH_4 in the rumen and eventually expelled by the animal (Hook et al., 2010; Mountfort et al., 1982). Although the differences in enteric methane production between the legumes and grass used in this study are striking, irrigated pastures are only available for 3-4 months of the year. However, year-round sources of high-quality forages should be investigated to develop more sustainable ruminant production systems.

Because BFT tannins bind excess plant proteins in the rumen, resulting in higher protein digestion in the abomasum and better absorption in the small intestine than would occur with non-tannin-containing forages, it might be expected that more efficient forage utilization and lower CH_4 emissions would occur on BFT than on the non-tannin legume CMV. In an in vitro study comparing CMV and BFT with alfalfa (ALF), CMV and BFT produced significantly less CH_4 per time period than ALF, and CMV produced less CH_4 per unit of digested fiber than either ALF or BFT (Williams et al., 2011a). There was a similar trend in the present study for methane emissions to be lower from CMV than from BFT. Because the intake of cattle grazing legumes is higher than that of cattle grazing grasses, we did anticipate lower CH_4 emissions per unit of forage intake on both CMV and BFT than on MB. Meadow brome has higher NDF digestibility than other grasses (MacAdam et al., 2006), which may reduce enteric methane emissions below that of other, less digestible grasses even under irrigation.

CONCLUSION

The use of pasture DM was proportional to the pre-grazing DM, and the forage allowance was less than 1% for these late gestation cows, for forage utilization was only about 40%. Mean CH_4 emission was significantly less for the two legumes, BFT and CMV, than for the grass, MB.

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Table 3.1 Mean season-long pasture pre- and post-grazing dry matter, and forage disappearance.

	Pasture Dry Matter		
Grazing period treatment	Pre-grazed DM	Post-grazed DM	DM utilization[†]
		kg/ha	
BFT	4089[‡] a	2479 a	36 b
CMV	3781 a	2031 b	43 a
MB	2741 b	2038 b	39 b

[†] Estimates of forage DM utilization are the difference between pre- and post-grazing DM divided by pre-grazing DM.

[‡] Values in columns followed by different letters are statistically different at $P \leq 0.05$.

Table 3.2 Forage DM allowance[†] of pasture treatments.

Grazing period treatment	Forage Allowance
	% of BW/d[†]
BFT	0.74
CMV	0.92
MB	0.99

[†] Values are means of pre-graze minus post-graze forage DM divided by cow live body weight (BW) and days per grazing unit * 100.

Table 3.3 Enteric Methane Emissions from Beef Cows, 2014

Grazing period treatment	Enteric Methane	
	Methane emission rate, g/d	
BFT	167[†]	b
CMV	159	b
MB	355	a

[†] Values in columns followed by different letters are statistically different at $P \leq 0.10$.

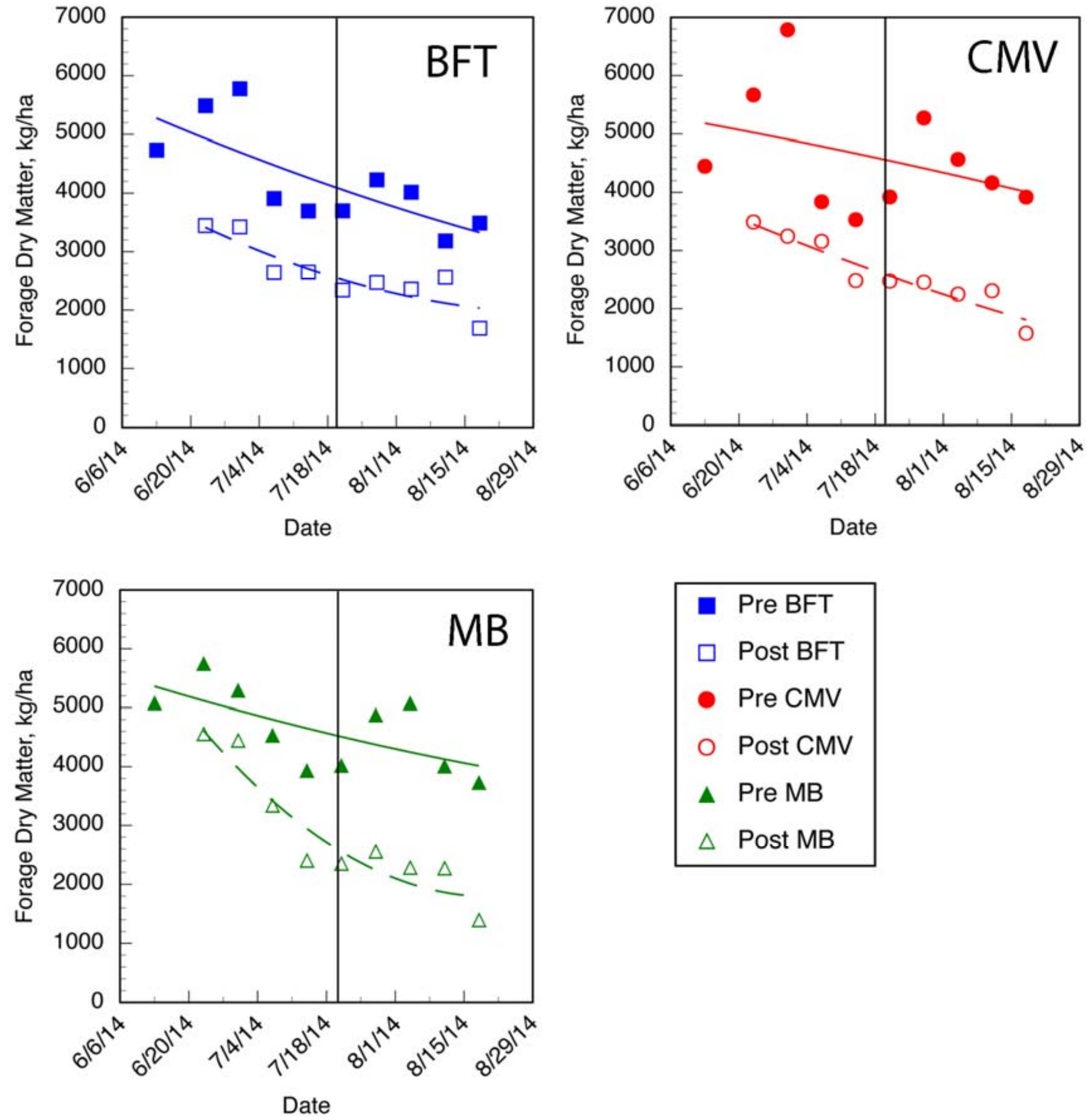

Figure 3.1. Pre- and post-grazing dry matter production on birdsfoot trefoil (BFT, cicer milkvetch (CMV) and meadow bromegrass (MB) pastures. The vertical line indicated the first week of enteric methane data collection.

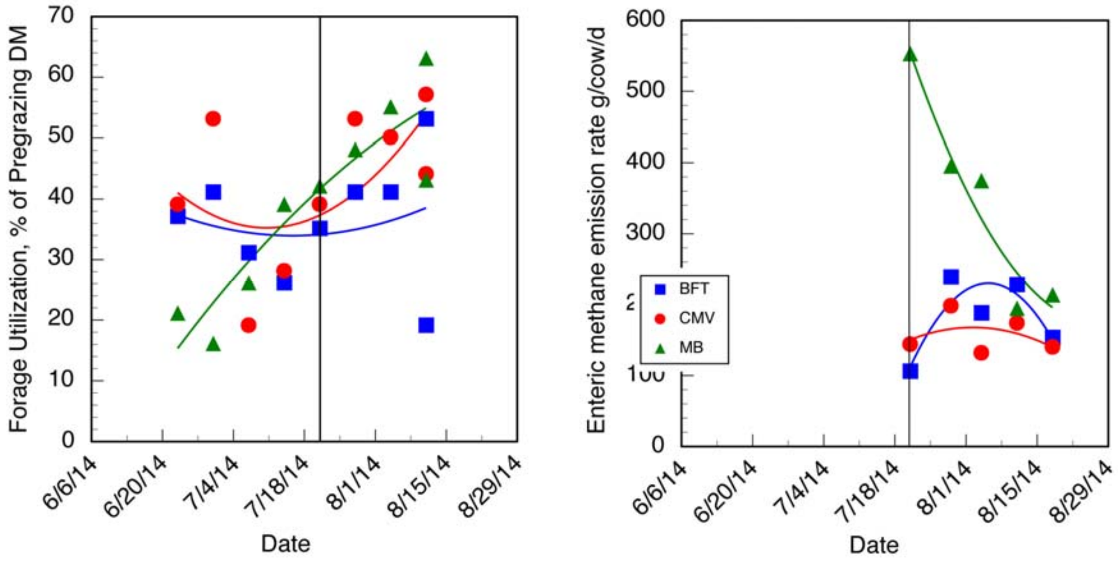

Figure 3.2. Forage disappearance (left) was calculated as a proportion of the pre-grazing pasture dry matter and data are included for the entire grazing season. Enteric methane emissions (right) were collected from the 6th to the 12th week of grazing, and data are the weekly means of daily data.

CHAPTER 4

SUMMARY AND CONCLUSION

We examined differences in ADG and blood plasma fatty acid composition of steers rotationally grazing high-quality perennial pasture forage species, BFT, CMV and MB, and compared them with steers on a typical feedlot finishing diet. The dry matter production for each forage was evaluated during grazing, along with forge utilization, the concentration of condensed tannins in these forage legumes, and its response to changing temperatures and water availability during the grazing season. A greenhouse gas byproduct of ruminant production, methane, was also investigated in mother cows grazing these same forage species using the SF_6 technique.

Average daily liveweight gain was 1.31 kg d^{-1} for feedlot-fed cattle, which was significantly higher than the ADG of any pasture treatment. Cattle grazing CMV gained an average of 0.69 kg d^{-1}. This gain was significantly lower than that of cattle grazing BFT, which ranged from 0.83 kg d^{-1} for Oberhaunstadter to 0.94 kg d^{-1} for Norcen. The ratio of omega-6 to omega-3 fatty acids in blood plasma is predictive of the fatty acid composition of intramuscular fat, and was higher in feedlot cattle than for any pasture-fed cattle. Omega-6 fatty acids averaged 42 g per 100 g fat, and were 50% higher than those of BFT-fed cattle and twice that of cattle grazing grass pastures. The omega-3 fatty acids of feedlot-fed cattle averaged 6.4 g per 100 g fat, and were as little as one-quarter that of pasture-finished cattle.

Forage dry matter increased from year to year as perennial forages continued to develop. Cattle numbers were adjusted to accommodate changes in cattle live weights at the beginning of the season. By assessing pre- and post-grazing dry matter, intake was estimated, and pastures were managed to maintain high intakes. Condensed tannins were not present in either MB or CMV and the concentrations of tannins were generally higher in Oberhaunstadter than in Norcen BFT. The quantity of CH_4 produced by cows on BFT and CMV pastures were less than half that produced on MB. Digestaibilty of BFT and CMV is higher than that on MB because the crude fiber in the cell walls of MB requires more time for digestion by rumen microbes. This leads to an increased ratio of acetate to propionate, which in turn leads to higher production of CH_4.

Gains of cattle grazing legumes, even when lower than for feedlot-fed cattle, were greater than for cattle grazing irrigated high-quality grasses. The SF_6 technique produced enteric CH_4 production values for MB comparable to values previously reported in the literature, and values for BFT and CMV were significantly less. This suggests that grazing systems including legumes have enhanced productivity as well as lower environmental impacts.

Perennial non-bloating legume pastures are productive and persistent in the Mountain West. Legumes fix their own nitrogen, eliminating the need for chemical nitrogen fertilization synthesis and application to produce higher yields of meat and milk than most grass pastures, and result in lower greenhouse gas emissions than grass pastures. In particular, tannin-containing perennial legumes improve the sustainability of ruminant livestock production by reducing the activity of internal parasites and transferring nitrogen from liquid to solid fraction of waste. Tannin-containing legumes can be grazed without risk of bloat. These forages will play an important role in developing more environmentally and economically sustainable agricultural production systems.