ENTERIC METHANE AND NITROGEN EMISSIONS IN BEEF CATTLE GRAZING A TANNIN-CONTAINING LEGUME RELATIVE TO FEEDLOT AND TRADITIONAL PASTURE-BASED PRODUCTION SYSTEMS

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Range Science

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UTAH STATE UNIVERSITY
Logan, Utah

2019

ABSTRACT

Enteric Methane and Nitrogen Emissions in Beef Cattle Grazing a Tannin-containing Legume Relative to Feedlot and Traditional Pasture-based Production Systems

by

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The livestock sector produces 35.4% of all anthropogenic greenhouse gas emissions, mainly due to methane produced by enteric fermentation and manure storage. Beef cattle are ruminants typically finished on grain-based rations, which yield lower methane emissions than grass-based diets. Some “non-traditional” legumes contain beneficial chemicals like condensed tannins, which in addition to their high nutritional quality enhance the efficiency of nutrient use in ruminants relative to grasses and other legumes. I assessed animal performance, methane emissions, and concentration of nitrogen in urine and blood urea nitrogen in cattle grazing a tannin-containing legume (birdsfoot trefoil; BFT; Lotus corniculatus L.), relative to a legume without tannins (cicer milkvetch; CMV; Astragalus cicer), a grass (meadow brome; MB; Bromus riparius), or a feedlot ration (total mixed ration; TMR) with high contents of roughage (50%).

Cows grazing BFT showed greater weight gains than cows grazing CMV or MB (P=0.0006), but similar to cows fed the TMR (P=0.5790). Methane emissions per unit of intake from cows grazing BFT were lower than emissions from animals consuming the TMR (P=0.0740), when the fecal output/digestibility methodology was used to assess dry matter intake at pasture. This suggests a positive effect of condensed tannins or nutrients in BFT on methane abatement. Methane emissions were comparable among animals grazing CMV (P=0.1180), MB (P=0.6763) or fed the TMR. Blood urea nitrogen concentrations were similar in cows grazing legumes (P=0.1202), but greater than in animals grazing MB or consuming the TMR (P=<.0001). Urinary nitrogen concentrations were similar among all diets (P=0.5266). These results indicate grazing BFT is a viable alternative to high-roughage feedlot rations for maintaining beef production with similar or potentially lower levels of methane emissions.

Finally, I estimated the agricultural areas under irrigation in the state of Utah using remote sensing techniques. The estimated amount of agricultural land under irrigation in the state of Utah was 412,250 ha. These results suggest that legume-based grazing systems are a viable option for the state. In conclusion, this research project provides novel and valuable information for the future implementation of an alternative and more sustainable beef production system based on cows grazing tannin-containing legumes.

(132 pages)

PUBLIC ABSTRACT

Enteric Methane And Nitrogen Emissions In Beef Cattle Grazing A Tannin-containing Legume Relative To Feedlot And Traditional Pasture-based Production Systems

Raúl David Guevara Ballesteros

Beef cattle production is highly criticized because of the high use of land and water resources, and by the pollution (e.g., the gas methane in a cow's breath and nitrogen in urine) produced by cows fed in feedlots. In contrast to feedlots diets and grasses, some plants (e.g., legumes) contain bioactive compounds (condensed tannins) that reduce pollution and enhance animal nutrition. In my research, I observed that cows grazing a tannin-containing legume (birdsfoot trefoil; BFT) had methane emissions similar to cows fed a feedlot ration with comparable weight gains. Cows in the BFT treatment gained more weight than cows grazing grass (meadow brome) or a legume without tannins (cicer milkvetch). Additionally, I estimated the potential areas in the state of Utah than can sustain birdsfoot trefoil production, with 412,250 ha distributed mostly in the Box elder, Cache, Millard and Sanpete counties. Thus, feeding tannin-containing legumes to cows is a viable alternative to feedlot rations, with greater levels of productivity than other pasture-based systems, which can lead to a more sustainable production of beef.

ACKNOWLEDGMENTS

I want to express my gratitude to all the people that support my research project and also to the ones that help me and coincide with me during my experience living at Utah State University. Mainly, to my main advisor, Juan Villalba, who gave me the opportunity to reach a master degree and to have the chance to learn from him how to be an outstanding researcher. As well, I would like to show my appreciation to my committee members, Jennifer MacAdam, and Erick Thaker, who provided me with indispensable tools, resources, and knowledge for the proper performance of my grad program. I want to thank my mentors and friends: Nicolas DiLorenzo, Martin Moreno and all the people who worked with me in IFAS-NFREC of the University of Florida, for being an inspiration and encouraged me to pursue a research pathway.

My special thanks to the persons directly involved in the execution of the experiments, particularly, Alexis Copper, Justin Taylor and Brenan Britner for being the key support during long days of work, even in tough circumstances. As well, to other students that participate during the development of the study. I would like to praise Sebastian Lagrange and Andrea Bolletta, to stay attentive to me and being a guideline during this research experience.

I want to appreciate and dedicate this effort to my family, Patricia Ballesteros, Raúl Guevara, and Santiago Guevara for their preys and their confidence in the success of this challenge in my life. I´m deeply thankful with my friends in Colombia, mostly, Cindy Peña and Ginna Lorena Quevedo, for being the lighthouse of my mind in the complicated situations that I had over here. My expressed love and affection to my

friends and family around the world for cheering me and share with me their joy through this journey.

Raúl David Guevara Ballesteros

For my family and friends around the world

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Cattle production and global greenhouse gases landscape

Beef production systems have been criticized around the world in the context of food security and environmental issues as the demand for beef as a protein source is increasing worldwide (Smith et al., 2018). The Food and Agriculture Organization (FAO) stated that one-third of the global cropland area is dedicated to produce animal feed (FAO, 2006). Additionally, the livestock sector is responsible for 14.5% of anthropogenic global greenhouse gas (GHG) emissions (FAO, 2019). Methane is the main GHG released by the cattle during enteric fermentation and manure management. Methane released by enteric fermentation can reach 86 million tons per year, whereas, nitrous oxide, a more potent GHG than Methane (CH_4) is volatilized to the atmosphere by applications of fertilizer or by animals' urine (FAO, 2006). Moreover, frequent use of grains and concentrate feeds increase animal performance at the expense of competition with other sectors that use grains to feed the human population or with new uses such as production of ethanol (Holechek, 2009). United Nations Food and Agriculture Organization - FAO studies (FAO, 2013) pointed out that cattle produce 4.6 $GtCO_2eq\ yr^{-1}$, 2.1 $GtCO_2eq\ yr^{-1}$ comes from dairy cattle, and 2.5 $GtCO_2eq\ yr^{-1}$ from beef production. Total antropogenic emissions were around 35.8 $GtCO_2eq$ for that year (Janssens-Maenhout et al., 2017). However, with the overall trend towards high animal productivity levels, it is unlikely that the importance of enteric fermentation will increase further (FAO, 2006), although an increased population entails greater levels of animal products with at least proportional increments in GHG emissions.The scientific community has been proposing several options to mitigate GHGs emissions and reduce cattle environmental impacts. Alternatives such as changes in feed management, improvements in animal yields and, reduction in the consumption of animal products have been proposed as possible solutions. Feed additives have been tested for a long time with the aim of increasing the units of animal products per unit of GHG emitted. A wide spectrum of additives has been tested through time with differing levels of success. Additives such as essential oils (Machmuller and Kreuzer, 1999; Jordan et al., 2006); Ionophores like monensin (Sauer et al., 1998; McGinn et al., 2004; Van Vugt et al., 2005; Woodward et al., 2006; Odongo et al., 2007); yeasts (McGinn et al., 2004; Newbold et al., 2006), organic compounds (Kim et al., 2019), and enzymatic additives (Beauchemin et al., 2003), among others, are some examples of the approaches reported in the literature. The main constraints for the application of feed additives involve the public's negative perception of the approach and adaptation of the rumen microbiota, which reduces the long- term effects of some of these compounds. Immunization against rumen methanogens represent alternative options for the abatement of enteric methane emission by ruminants (Subharat et al., 2015)

Improvements in forage digestibility reduces energy used to produce methane and diverts that energy for the formation of other functional compounds such as the volatile fatty acid propionate. Such improvements in the efficiency of energy use is due to a decline in the digestion of plant fiber and an increment in the supply of non-fibrous carbohydrates to the diet. Consecuently, the proportion of propionic acid as a byproduct of ruminal fermentation is increased. Additionally, Plant Secondary Compounds (PSC) such as tannins and saponins, can reduce emissions by constraining the activity of methanogenic microorganisms (Hess et al., 2003; Pinares-Patino et al., 2003; Woodward et al., 2004; Carulla et al., 2005). Meanwhile, protein availability can be increased because PSC like condensed and hydrolizable tannins reduce protein degradation in the rumen, allowing for more dietary protein to be absorbed in the intestines (Muller-Harvey, 2006: Waghorn, 2008). Use of PSC can have a positive impact in developing countries with high levels of GHG emissions due to low productivity levels across large areas (Herrero et al., 2016) and the fact that many tannin-containing plants are present in these landscapes (Carulla et al., 2005; Ben Salem et al., 2005; Rubanza et al., 2007).

The reduction in methane emissions by the utilization of dense energy sources in the diet such as rations with grains relies in the fact that those feeds have the minimum content of fiber required for an animal to ruminate properly and keep ruminal pH values within normal levels. Rations with low fiber contents reduce the proportion of acetate to propionate in the rumen, and greater concentrations of propionate reduce the amounts of carbon available for the production of methane (Whitelaw et al., 1984; Daniels et al., 1984). Raising livestock based on corn-based diets is viable if relatively under cheap grain price persist. Such conditions are not likely to be maintained in the long-term because raising grains is highly dependent on fossil fuels. Thus, operations that strive to minimize production costs tied to oil as the main energy source are more likely to be sustainable in the time (Holechek, 2009).

A reduction in the demand for products from animal origin is a controversial mitigation alternative, given that this approach would significantly influence the economy of almost 1/3 of the world labor force (Garibaldi et al., 2017). Animal production in the US involves more than 1.6 millions of Americans and exports of animal products reach \$31.8

billion yearly, that equivalent to 22% of the income from all agricultural exports (White and Hall, 2017). The main reasons that support the proposal for a reduction in livestock products involve efficiency. Livestock production efficiency is lower than crop production, one third of the world cereal production is diverted to animal feeding and even if animal protein just represents 15% of the total energy for human diet, 80% of agricultural land is devoted to animal production (Herrero et al., 2016). Contrarily, studies show that human diets with moderated protein levels have positive benefits on lifespan and reduce the population growth rate (Simpson et al., 2017). Low calorie diets have positive benefits in humans health, such as reductions in the frequency of heart diseases, overweight in youth population, and motricity difficulties (Brehm et al., 2003; Story et al., 2008).

Adequate food production can be achieved with less agricultural land, allowing reforestation and promoting reductions in GHG emission by 4.3 GtCO$_2$e/year. Nevertheless, agricultural land used for cattle feeding is unsuitable for reforestation in many cases (Holechek, 2009). Reduction of livestock products demand, theoretically, is a powerful mitigation option (Popp et al., 2010; Bajzelj et al., 2014; Westhoek et al., 2014). Although is remarkable that removing animal production from US agriculture would cause a food supply incapable to cover the US population’s nutritional requirements given the diversity and density of essential nutrients provided by meat and milk products which cannot be found in other food sources (White & Hall, 2017), and diminution of the population growth rate can be achieve by decreasing human’s birth rate instead of constraining protein intake. In addition, eliminating animal agriculture would decrease total US emission by just 2.6% (White & Hall, 2017). Thus, mitigation strategies to reduce GHG is a more viable option that reducing livestock numbers.

Beauchemin and McGinn, (2008), stated different criteria in order to establish the applicability of different GHG mitigation strategies: 1) documented effectiveness in emission reduction, 2) profitable, or at least neutral revenue for the producer, and 3) feasible implementation on the farm. The second criteria is the most difficult to accomplish due to several factors that involve the economic sustainability of the production enterprise. It is unlikely that livestock producers adopt friendly ecologic measures if the positive economic impact is not perceived to be real. Development of positive incentives that encourage producers to apply mitigation practices is a key to reduce emissions without negative impacts on production levels (Herrero et al., 2016).

Condensed tannins to increase feeding efficiency and reduce methane and nitrogen emissions from the enteric fermentation

Condensed tannins are a heterogeneous family of highly-reactive, carbon-based plant secondary compounds of high molecular weight that bind to proteins with great affinity, forming tannin-protein precipitates (Hagerman and Butler, 1980). Plant condensed tannins have positive and negative impacts in the physiology of ruminants. The quality of the impact depends on the concentration of the PSC in the plant's tissues and on the biological activity of the PSC given by its chemical composition (Schofield et al., 2001). Condensed tannins must be present in the foliage in order to have real benefits for ruminants because there those would increase their contact with the ruminal microorganisms related with the proteolysis and the methanogenesis (Waghorn, 2008).

Tannins reduce the incidence of bloat in ruminants and protect dietary proteins in the rumen from microbial digestion, which reduces the formation of ammonia (Jones et al., 1976). This occurs because condensed tannins to form insoluble complexes with leaf and salivary proteins of pH values that range between 3.5-7.0 (Bunglavan and Dutta, 2013). However, complexes may dissociate when exposed to the neutral intestine's pH (Waghorn, 2008), a result of the blend of feed with pancreatic and biliary secretions (Jones et al., 1976; Getachew et al., 2000; Barry et al., 2001). However, when crude protein is below an animal 's requirement and fiber concentration is high, some condensed tannins may produce deleterious effects on ruminants, such as the reductions in fiber degradation due to the antibiotic actions of these compounds on fibrolytic bacteria, which limits voluntary intake and energy absorption (Waghorn, 2008).

It has been reported that low concentrations of condensed tannins in forages (20-45 grCT/kg Dry matter) (Min et al., 2003) decrease forage protein degradation in the rumen. A possible explanation to this phenomenon is that condensed tannins form reversible bindings on ruminal proteolytic populations, reducing their metabolic activity as result (Waghorn, 2008). Tannin-containing forages of several species have been tested (e.g. *Lotus corniculatus, Hedysarum coronarium, Onobrychis viciifolia,* among others.) showing positive effects on ruminant's physiology and performance, increasing milk production, wool growth, ovulation rate and, lambing percentage (Waghorn, 2008). Additional benefits include reductions in the incidence of bloat and in the extent of parasitic loads (Jones et al., 1976; Min et al., 2003). Those positive effects have been explained by increases in the absorption of essential amino acids from the small intestine, triggered by a greater protection of high-quality dietary protein in the rumen which is then digested and absorbed in the small intestine (Min et al., 2003; Waghorn, 2008) rather than by improvements in voluntary feed intake.

Researchers have also observed reductions in enteric methane production in response to condensed tannin intake (g/kg dry matter intake) with a wide range of results (Carulla, 1994; Woodward et al 2004; Waghorn & McNabb, 2003; Carulla et al., 2005; Lagrange et al, 2017; 2018). However, the mechanisms by which tannins affect methane production is not well defined. Proposed explanations involve the idea that condensed tannins indirectly affect the production of hydrogen ions or that through their antibiotic effects influence the rumen microflora involved in methane production. Jones et al. (1994) stated that condensed tannins could bind to the cell coat of some microbes in the rumen reducing their activity. Tan et al. (2011) observed that condensed tannins affect methanogens and other microbes that methanogens depend on to survive.

Condensed tannins containing legumes have the potential to increase sustainable ruminant production due to the beneficial effects of condensed tannins on protein use, animal health, and methane emissions with no deleterious effects on voluntary intake (MacAdam & Villalba, 2015). Thus, condensed tannins could increase the efficiency of forage use by grazing ruminants since less energy is diverted to methane production and more nitrogen is retained in the animal's tissues (Carulla et al., 2005). However, it is important to consider that excess exposure to condensed tannins, can induce tolerance mechanisms in the host which may also persist in future generations (Waghorn, 2008).

Birdsfoot trefoil (Lotus corniculatus) as alternative forage for environmentally friendly beef production systems

The ultimate goal for beef production is to develop a sustainable system that takes advantage of the unique abilities of ruminants to utilize plant fiber in an efficient manner (Villalba et al., 2019), minimizing environmental impacts while providing a high-quality product to consumers. The use of alternative perennial forages in agriculture can help achieve this goal. For instance, legumes increase the growth potential and the finishing rate of cattle (Kopp, 2003; Speijers et al., 2004; Fraser et al., 2004). In addition, and in contrast to cereal grains, perennial legumes fix their own nitrogen and are productive for multiple years after establishment without additional cultivation or planting (MacAdam & Villalba, 2015). Legume forages are digested more rapidly than grasses by ruminants, so intake and production are greater than for forage grasses (Van Soest, 1994).

Birdsfoot trefoil (BFT) is a tannin-containing legume originated in the Mediterranean region (Steiner et al., 2001), and performs well in grass-legume associations with forages like meadow brome, tall fescue and orchardgrass (MacAdam and Griggs, 2013). Birdsfoot trefoil has a higher concentration of non-fibrous carbohydrates than alfalfa and as a result it provides a protein to carbohydrates ratio that better matches the animal's nutritional requirements (Brummer et al., 2016). This legume can thrive in the same climatic conditions where alfalfa grows, but with higher tolerance to harsh soil conditions such as phosphorous deficiencies, low pH and, poorly drained soils. Additionally, BFT maintains its nutritional quality for longer periods compared with many other legumes and it persists by natural reseeding (Brummer et al., 2016). Moreover, BFT can tolerate more defoliation than alfalfa (Smith and Nelson, 1967). Condensed tannins from BFT consist primarily of procyanidins and these phenolic compounds tend to increase their concentration in mature forage (Gutek et al., 1974; Mueller-Harvey, 2006). Tannins in BFT are present in low concentration and do not constrain animal intake (Ramirez-Restrepo et al., 2015) or amino acids absorption in the small intestine (Waghorn et al. 1987;

Waghorn, 2008) On the contrary, absorption of essential amino acids is increased by 60% (Waghorn et al. 1987) under BFT diets.

Cicer Milkvetch and beef production

Cicer Milkvetch (*Astragalus cicer*) is a perennial legume native to Europe (Stroh et al., 1973) adapted to several soil types in the United States as it tolerates moderate acidity and alkalinity levels (Townsend, 1993). This legume is highly attractive to ruminants because its stems are hollow and succulent, allowing for high rates of forage intake. Due to its decumbent and rhizomatous growth habit, cicer milkvetch is suitable for grass-legume pasture mixtures (McGraw & Marten 1986; Loeppky et al., 1996), which increases nitrogen fixation, dry matter and protein content, complementing the grass crop (Acharya et al., 2006). Cicer milkvetch is a winter tolerant forage, and for that reason the legume maintains its nutritional quality under snow conditions (Acharya et al., 2006).

Cicer milkvetch is an outstanding option for ruminant nutrition because it does not cause bloat under high grazing pressures and it does not accumulate selenium like other milkvetch plants (Johnston et al., 1971; Johnston et al., 1975; Majak et al., 1995). The nutritional and digestible composition of cicer milkvetch is similar to alfalfa; cicer milkvetch's leaf to steam ratio is 74:26; it is rich in crude protein (~23%) and low in fiber content (NDF, ~35%) (Acharya et al., 2006). Irrigated pastures of cicer milkvetch can produce 570 kg of beef per hectare in one season (Russell et al., 1982).

Cicer milkvetch is ideally suited for cattle grazing, although some studies report anti-nutritional factors that affect animal performance. Studies done by Marten et al., (1987; 1990) described negative effects (i.e. photosesitization) in cattle and sheep grazing cicer milkvetch, and Weimer et al. (1993; 1998) observed inhibition of cellulose digestion in cicer milkvetch during *in vitro* fermentation studies. Nevertheless, these outcomes were specific to the conditions where those studies took place (Acharya et al., 2006).

Cicer milkvetch was selected for this study because this legume shares some of the attributes (e.g. productivity, plant structure, non-bloating) observed in birdsfoot trefoil, although it does not contain condensed tannins, thus making this forage a good control for exploring the potential effects of condensed tannins from BFT on grazing studies.

Meadow brome and beef production

Meadow brome (*Bromus riparius*) is a perennial cool-season grass introduced to North America in 1957, native to Southeast Europe and Western Asia (Knowles, 1990). This grass is characterized by a rapid regrowth of tillers after defoliation or grazing (Jensen et al., 2001). For instance, under Northern Utah management conditions (six harvest and multiple irrigation levels), this grass may produce dry matter yields 28% greater than smooth bromegrass and with fewer rhizomes (Jensen et al., 2001). Meadow brome showed good performances under rotational grazing conditions conducted in the mountain west region of the U.S. (Wiedmeier et al., 2004).

Meadow brome is commonly used as pasture, hay and haylage and because of a dense root network, it reduces soil erosion. When this grass grows under irrigation, it can reach 60-180 cm in height with high levels of production per unit area of land (> 11 tons of DM/ha in one year of production), but without being invasive. Plant growth starts early in the spring and due to its deep roots and tiller base, meadow brome is capable of strong summer development and regrowth after grazing or haying (Ogle et al., 2006). Calves may gain on average > 1 kg/day when they graze pure stands of meadow brome under the conditions of northern Utah (Wiedmeier et al., 2004).

Meadow brome was selected for this study as a typical forage used under grass-finishing systems due to its productivity and nutritional quality, with greater NDF digestibility than orchardgrass, tall fescue or perennial ryegrass (MacAdam et al., 2006).

Feedlot systems

The conventional feedlot system is characterized by high-grain inputs for a period of three to four months, leading to finishing product that takes a total of 14-16 months from birth to slaughter (Smith and Johnson, 2014). Feedlots aim to add muscle and fat to tissues, improving the final quality of the product. Intramuscular fat (marbling) gives consumers the taste and texture they desire from beef (USDA, 2011). A feed conversion ratio of 6:1 is common in modern feedlots (Reuter, 2009; Shike, 2013). Feedlot rations are generally 70-90% of grain and protein concentrates (USDA-ERS, 2018).

Although the majority of feedlots in the U.S. have a small capacity (less than 1,000 head), they are a minor share of the market. On the other hand, feedlots with more than 1,000 head capacity are less than 5% of total feedlots, but they provide 80-90% of the fed-cattle to the market. The beef production industry is likely to shift to a smaller number of animals and very specialized feedlots that are vertically integrated with the cow-calf phase and meat processing, sectors that produce high quality fed beef (USDA-ERS, 2018).

In the U.S., the majority of consumers prefer domestic feedlot-finished beef over alternatives meats like grass-finished beef. However, the demand for alternative beef is growing due to particular niches with concerns regarding human health, environmental impact and animal welfare (Banker, 2016; Felix, 2018). These consumer niches are willing to pay more for beef products that satisfy their ethical values (Umberger et al., 2002; Sitz, et al., 2005).

Grass-fed beef production

Approximately 12-14 months after cattle start grazing, animals finish their production cycle in either a feedlot (high grain diet) with a production cycle that takes between 14-16 months from birth to slaughter, or in pastures, representing the grass-finishing system with a production length between 18-24 months from birth to slaughter. In the U.S. it is difficult to complete grass-fed production cycles year-round because of constrains in temperature and/or precipitation that limit the growing season. Most of the grass-fed beef in the U.S. is imported from Australia and New Zealand because the forage supply in these regions is more or less constant across seasons (Nebrazka Corn Board, 2019). Cattle under grass-finishing systems are typically slaughtered at 18-24 months of age (Shattuck, 2013).

In order to maintain beef quality under reasonable production times, the goal for average daily gains under grass-fed systems ranges between 0.9 to 1 Kg per day, which requires pastures with 14-18% of CP, >20% of DM and >20% of water-soluble carbohydrates (Rutherford, 2009; Felix, 2018). In addition, forage availability should not restrict forage intake such that cattle have the opportunity to maximize their forage intake to reach the aforementioned production goals.

It has been claimed that grass-finishing systems provide several benefits to the environment and society, such as increases in carbon sequestration, improvements in animal welfare and product quality (Pirog, 2004; Banker, 2016; Felix, 2018), with concomitant increments in revenues (Umberger et al., 2002; Sitz, et al., 2005; Gwin, 2009). Grass-finished beef also presents greater proportions of omega-3 (antioxidant) fatty acids, and lower proportions of omega-6 (pro-inflammatory) fatty acids and cholesterol (Pirog, 2004; Daley et al., 2004; Banker, 2016). A particular benefit for grass-fed beef is that it can be produced locally, significantly reducing the "food miles" traveled for each steak, which in turn diminishes the total use of fossil fuels and the level of emissions produced for each item. Finally, farmers marketing grass-fed beef have more control over the price, making this production a very attractive option for small producers (Rutherford, 2009; Gwin, 2009).

Systems comparison

Gwin (2009) enlisted three factors that constrain the grass-fed operations from reaching productive magnitudes similar to feedlot productions. The first constraint is animal genetics. Angus or Herford breeds (Gwin, 2009; Fears, 2017) have big frames with high nutritional requirements, which are difficult to fulfill under pasture conditions, with lower nutrient density than feedlot rations. Consequently, cattle in grass-fed systems take longer to finish than cattle in feedlots (Shattuck, 2013; MacAdam & Villalba, 2015) with concomitant reductions in the quality of the product. Improvements on this matter can be achieved by means of using cows of smaller frames that are more adapted to grazing conditions (Riggs, 2007; Rutherford, 2009). Breeds like Red Devon, Murray Gray, and British White appear to be more suited to grass-finishing conditions because they mature faster and have a lighter finishing weight than large-framed continental animals, although small frame breeds tend to be rare and more expensive (Gwin, 2009; Rutherford, 2009). Large continental animals are more suited to grain-finishing conditions (Felix, 2018).

Another factor that impacts the grass-fed system is the supply of nutrients at a constant rate. Grasses in the U.S. have seasonal trends that directly affect the time of finishing because dietary changes that reduce forage availability and quality impact animal performance (Schwartzkopf-Genswein et al., 2003; Gwin, 2009). For that reason, pastures require irrigation to support grazing pressure during the growing season. Capper (2012) estimated $1,957,224 \times 10^6$ L of water per 1.0×10^9 kg beef in a grass-fed system. The reason of the differences in water use between feedlot production and grazing systems relies in the feed conversion efficiency (Mekonnen & Hoekstra, 2012). Thus, more feed implies more water to produce the feed. However, water use is higher in the production of grains relative to the forage and roughages production. Even though, this fact does not compensate the inefficient feed conversion rate of the grazing systems (Mekonnen & Hoekstra, 2012). In addition, managerial actions need to be applied during the finishing process in order to maximize yield and nutritional quality. Rotational grazing is a good strategy to increase the efficiency of pasture use but often requires adjustments in response to changing environmental conditions, thus demanding time, energy, facilities and observation to get the best results (Walton et al., 1981; Cros et al., 2001; Gwin, 2009). Even though, these inputs are even more high in a feedlot operation.

The final key factor that impacts grass-fed systems is the land required to finish the production cycle. Farmers would require more land to finish a certain goal of kilograms of meat in a grass-finishing production system (9.868×10^3 ha of land per 1.0×10^9 kg beef), approximately 80% more land than in a feedlot (Capper, 2012). However, these estimations do not take into consideration the amount of land required to produce forage and crops needed to feed the animals in a feedlot. Nevertheless, grass-fed production systems typically need two sets of pastures: one for cows and calves and another to finish heifers and steers (Pimentel et al., 1980; Gwin, 2009).

Basis of remote sensing and its applicability in agriculture

The feasibility of the proposed tannin-containing legume grazing system can be assess by high coverage and economic techniques such as remote sensing. This technique allows the user to evaluate the environmental conditions of certain area through the analysis and interpretation of electromagnetic reflectance data from the earth surface. This methodology is idea for research of locations with difficult access and size that cannot be easily evaluated by ground-based methodologies (Bateman, 2017).

Remote sensing is a technique that allows for the analysis and interpretation of reflectance data from the earth's surface. Such data are obtained from satellite imagery or sensors above ground level (Pinter et al., 2003; Rogan & Chen, 2004). Remote sensing applications in agriculture are based on a theory that relates morphological characteristics of a certain crop to their optical properties (Pinter et al., 2003).

The reflectance light from plants is the consequence of physiological conditions such as plant type, water content and other intrinsic factors (i.e. vegetable pigments) (Zhang & Kovacs, 2012; Xue & Su, 2017). Healthy green plants display low reflectance to visible light (400 to 700nm) due to strong absorbance capacity from photosynthetic processes and plant pigments (i.e., chlorophyll *a* and *b*, and carotenoids) (Pinter et al., 2003). The remote sensing applications are based on the following light spectra: (i) the ultraviolet region (10 to 380 nm); (ii) the visible light spectra (620 to 750 nm); and (iii) the near and mid-infrared (850-1700nm) (Cruden et al.; 2012; Abdul Rahim et al, 2016). Inversely, near-infrared reflectance (NIR) is high because there is little absorbance by subcellular particles (Pinter et al., 2003).

Environmental conditions and/or plant senescence directly affect chlorophyll concentration in leaf tissues, which increases the effect of plant secondary pigments such as, carotenes and xanthophyll. These compounds tend to increase visible light reflectance meanwhile decrease NIR reflectance is decreased (Curran et al., 1990; Pinter et al., 2003; Kira et al., 2015). The water content in plants can also be estimated using shortwave infrared (SWIR) band reflectance; vigorous plants have higher SWIR reflectance than dehydrated plants (Ustin et al., 2012; Hunt Jr. et al., 2016).

Agricultural production systems have the imperative need to optimize the system aforementioned technologies in order to achieve economic objectives while satisfying current environmental guidelines. In fact, producers have been increasing the use of these technologies that allow for an efficient accomplishment of different production expectations. Innovations such as genetic engineering to improve plant varieties, new chemical compounds to enrich fertilizers and crop supplements, along with sensing systems that allow more accurate information about environmental conditions are examples of new technologies that enhance the efficiency of agricultural production (Maggiori et al., 2017).

Remote sensing methodologies have the potential to provide information that would help to reduce production inputs and maximize profits, i.e., by obtaining better estimates about seeding and harvesting times, precipitation trends, and plant biomass production (Ramoelo et al., 2015; Maggiori et al., 2017). These reports are possible trough remote sensing that collects canopy reflectance data from crops.

Remote sensing to forecast yield and nutritional quality in crops

An application of remote sensing technique involves crop yield predictions and nutritional forecasting (Di Bella et al., 2004; Gitelson, 2016). Traditional methods to estimate crop yield or plant nutritional quality are considered inaccurate and invasive (Pinter et al., 2003), because samples do not guarantee a significant representation of all the plants in a plot, and samples are destroyed to analyze their nutritional content. For these reasons, remote sensing techniques represent a tool that aid in the provision of reliable information about the nutritional composition of crops across large scales without the need for multiple destructive chemical analyses.

The approach to measure biomass cover is by vegetation indices (VI) that allows to extract signals of plant abundance from complex canopy spectra (Khanal et al., 2017; Xue & Su, 2017). Such indices are often computed as differences, ratios, or linear combinations of reflected light in visible and NIR wavebands (Pinter et al., 2003).

Vegetation indices have served as the basis for many applications of remote sensing to crop management because they are well correlated with green biomass and leaf area index of crop canopies (Pinter et al., 2003; Xue & Su, 2017). One of the most used indices used to calculate vegetation canopy growth or vigor is the Normalized Difference Vegetation Index (NDVI) (Xue & Su, 2017). NDVI is based on the difference between the maximum absorption of radiation in the red band (as result of chlorophyll pigments) and the maximum reflectance in the NIR band (as result of leaf cellular structure) (Tucker, 1979). The soil spectrum is not detected in the difference between these bands, allowing the contrast between vegetation from the soil (Karnieli et al., 2010). This vegetation index can describe vegetation density, which can help producers to evaluate plant germination, growth and productivity (Precision Agriculture, 2018).

Hypothesis and objectives

I hypothesize that the nutritional composition and presence of bioactive compounds like condensed tannins in forages, will provide benefits to livestock production systems such as improvements in the efficiency of nutrient use and reductions in environmental impacts. Thus, I predicted that tannin-containing legumes like birdsfoot trefoil will reduce methane emissions in cattle relative to a grass-finishing system. I further predicted that such improvement in efficiency will lead to levels of productivity similar to feedlot-finished animals. Furthermore, dietary protein protected by condensed tannins in the rumen and released in the intestines will enhance nutrition promoting a shift in nitrogen excretion from urine to feces. In order to test my hypothesis, I determined animal performance, CH_4 production, blood urea nitrogen and urinary nitrogen excretion from cows fed a feedlot diet relative to cows grazing (1) meadow brome, a grass typically used in grass finishing systems, (2) a tannin-containing legume (birdsfoot trefoil), and (3) a legume of similar nutritional and structural characteristics to birdsfoot trefoil but without tannins (cicer milkvetch) (Chapter II).

In Chapter III, I explored the feasibility of producing legumes other than alfalfa (e.g. Birdsfoot trefoil) under irrigation by exploring the distribution and availability of agricultural areas under irrigation in the state of Utah. Thus, I applied GIS sensing techniques to produce a map that shows alfalfa stands currently under irrigation. This effort was made in order to guide future management efforts or to make predictions about land areas in Utah with potential to promote livestock finishing systems under irrigated legumes as an alternative or complement to hay production.

Expected benefits

Results from this research project provide information about the feasibility of implementing an alternative and more sustainable beef production system using legumes instead of traditional grain-based diets or traditional grasses during the finishing phase, given that legumes increase the growth potential and the finishing rate of cattle. Contrary to cereal grains, perennial legumes fix their own nitrogen and are productive for multiple years after establishment without additional cultivation or planting. Moreover, condensed tannins may increase the efficiency of energy and protein utilization in animals by reducing enteric methane emissions and increasing amino acid availability at the intestinal level. Switching to this alternative finishing system will allow for the use of grain in other systems and endeavors (i.e., human nutrition, ethanol production), reducing competition for land while reducing environmental impacts.

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CHAPTER II

A COMPARISON OF ENTERIC METHANE AND NITROGEN EMISSIONS IN FOUR BEEF FINISHING SYSTEMS: FEEDLOT, GRASS, TANNIN-CONTAINING AND NON-TANNIN-CONTAINING LEGUMES

ABSTRACT

The livestock sector produces 35.4% of all anthropogenic greenhouse gas (GHG) emissions, and beef cattle in the US is the largest contributor to this problem, mainly due to methane produced by enteric fermentation and manure storage. Beef cattle are ruminants typically finished on cereal grain-based rations fed in confinement, which yield lower methane emissions than grass-based diets. In contrast to both cereal grains and pasture grasses, perennial legumes fix their own nitrogen and are productive for multiple years after establishment. Some “non-traditional” legumes also contain beneficial chemicals like condensed tannins, which in addition to their high nutritional quality enhance the efficiency of energy and protein use in ruminants relative to grasses and other legumes like alfalfa. I assessed (i) animal performance, (ii) methane emissions (SF6 technique), and (iii) concentration of nitrogen in urine and blood urea nitrogen in cattle grazing a tannin-containing legume (birdsfoot trefoil; BFT; *Lotus corniculatus L.*), relative to cattle grazing a legume without tannins (cicer milkvetch; CMV; *Astragalus cicer*), a grass (meadow brome; MB; *Bromus riparius*), or to cattle fed a feedlot ration (total mixed ration; TMR) with high contents of roughage (50%).

Cows grazing BFT (1.9% condensed tannins) showed greater weight gains than cows grazing CMV or MB (P=0.0006), but similar to cows fed the TMR (P=0.5790).

Methane emissions per unit of intake (using the fecal output/digestibility method) from cows grazing BFT were lower than (when intake at pasture was assessed using the fecal output/digestibility method) emissions from animals consuming the TMR (P=0.074). This suggests a positive effect of condensed tannins or nutrients in BFT on methane abatement. Methane emissions were comparable among animals grazing CMV (P=0.1180), MB (P=0.6763) or fed the TMR. Blood urea nitrogen concentrations were similar in cows grazing BFT or CMV (P=0.1202), but greater than in animals grazing MB or consuming the TMR (P=<.0001). Urinary nitrogen concentrations were similar among the diet treatments (P=0.5266). These results suggest grazing BFT is a viable alternative to high-roughage feedlot rations for maintaining beef production with similar or potentially lower levels (i.e., methane emissions) of environmental impact.

INTRODUCTION

Beef cattle production systems are criticized for the use of large land areas, big water footprint and for the use of cereals as livestock feed (Thorpe, 2009; Herrero et al., 2016). Cattle production contributes to greenhouse gas emissions by releasing approximately 86 million tons of methane per year, representing 26.87% of the total global anthropogenic methane emissions per year, and 8 grams of nitrogen per kilogram of dry matter consumed to the atmosphere (FAO, 2006). Methane emissions are considered “leaks of energy” because energy used to form methane is diverted from the production of muscle fibers and therefore growth (Johnson & Johnson, 1995; Johnson et al., 2000). One option to mitigate enteric methane emissions is the utilization of high-quality feeds, i.e., grains in ruminant diets. The low fiber contents in grains, compounded with growth-enhancing technologies (e.g., addition of ionophores to concentrate rations), reduce the ruminal production of methane and boost animal performance (Whitelaw et al., 1984; Daniels et al., 1984). However, the use of grains to feed ruminants does not take full advantage of the unique ability of these animals to extract energy from roughages. Additionally, grains can be used to feed the human population or monogastric animals (Van Soest, 1994; Holechek, 2009; Eisler et al., 2014; White & Hall, 2017) while ruminants have no dietary requirement for concentrates.

In contrast to cereal grains and pasture grasses, perennial legumes fix their own nitrogen, and are productive for multiple years after establishment without additional cultivation or planting. Legume forages are digested more rapidly than grasses by ruminants, so intake and production are higher than for forage grasses. In addition, legumes like birdsfoot trefoil (*Lotus corniculatus*; BFT) and sainfoin (*Onobrychis viciifolia* Scop; SF) produce a class of plant secondary compounds, condensed tannins, which enhance the efficiency of energy and protein use in ruminants relative to other perennial legumes (Waghorn, 2008; Wang et al., 2015). Thus, forages with plant secondary compounds have the potential to reduce greenhouse gas emissions from ruminants (Hess et al., 2003; Pinares-Patino et al., 2003; Woodward et al., 2004; Carulla et al., 2005). For instance, the use of tannin-containing legumes has shown positive results regarding enteric methane abatement while maintaining high levels of animal performance (Carulla, 1994; Woodward et al., 2004; Waghorn & McNabb, 2003; Carulla et al., 2005; Lagrange et al, 2017, 2018).

In addition to the effects described above on enteric methane emissions, condensed tannins in some legumes like BFT have the ability to protect dietary protein from ruminal proteolysis (Waghorn et al., 1987), allowing for an efficient absorption of dietary amino acids at the intestinal level (Jones et al., 1976; Getachaw et al., 2000; Barry et al., 2001). Thus, condensed tannins can enhance the retention of nitrogen in ruminant animals and shift nitrogen excretions from urine to feces (Stewart et al., 2019). Fecal nitrogen is less volatile than urinary nitrogen, increasing the availability of this element for capture by the plant's roots (Somda et al., 1993; Hoste et al., 2006), instead of being volatilized as ammonia or nitrous oxide when excreted as urinary urea. Thus, I hypothesized that the nutritional composition and presence of condensed tannins in legumes would enhance the efficiency of nutrient use and consequently reduce environmental impacts in beef production systems. Thus, I predicted that tannin-containing legumes like birdsfoot trefoil would reduce methane emissions in cattle relative to a grass-finishing system. I further predicted that such improvement in efficiency would lead to levels of productivity similar to feedlot-finished animals. Finally, I predicted that dietary protein protected by condensed tannins in the rumen and released in the intestines would enhance nutrition, promoting reductions in urinary nitrogen and blood urea nitrogen. The objectives of this study were to determine animal performance, enteric methane production, blood urea nitrogen and urinary nitrogen excretion from heifers fed a feedlot diet relative to heifers grazing (1) meadow brome, a grass typically used in grass finishing systems, (2) a tannin-containing legume (birdsfoot trefoil), and (3) a legume of similar nutritional and structural characteristics to birdsfoot trefoil but without tannins (cicer milkvetch).

MATERIALS AND METHODS

I determined animal performance, intake, blood urea nitrogen (BUN), urinary nitrogen excretion, fecal output and enteric methane emissions in beef cows under two systems (i) Grazing: Animals grazed monocultures of birdsfoot trefoil (*Lotus corniculatus L.*), cicer milkvetch (*Astragalus cicer*) and meadow brome (*Bromus riparius*), and (ii) Confinement: Animals received a grain-based diet. The studies were performed in two agricultural facilities from Utah State University. The Intermountain Irrigated Pasture Project, located in Lewiston, UT (41°58′7″N 111°51′57″W) (Grazing experiment) and the Utah State University Animal Science Farm, located in Wellsville, UT (41°38′8″N 111°55′59″W) (Feedlot experiment).

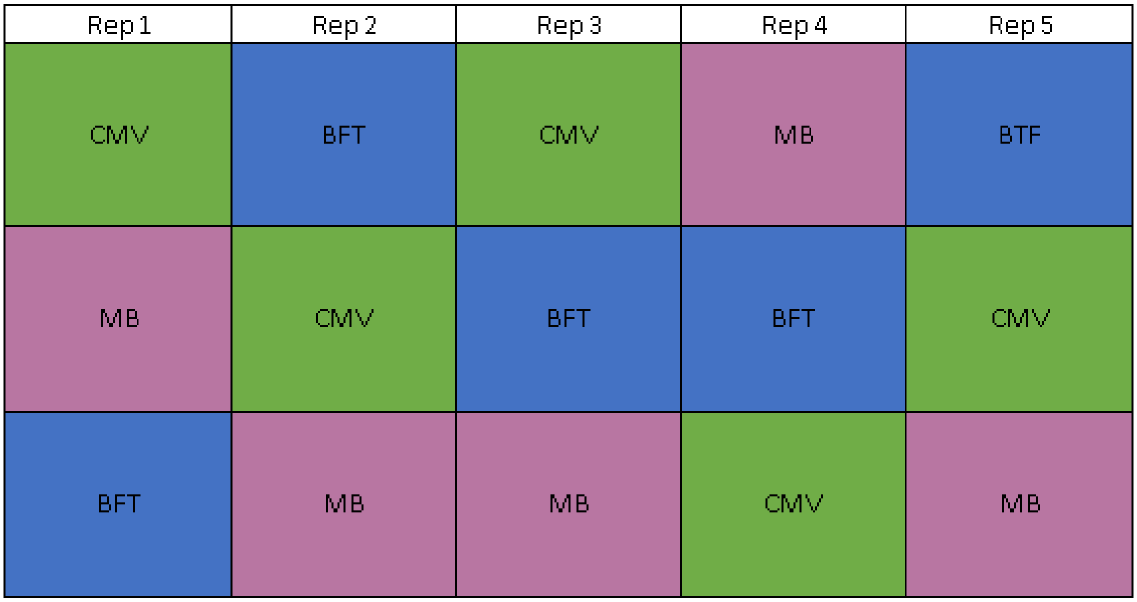
The grazing study occurred from May 19 until August 4 of 2017 during three consecutive periods. The feedlot study occurred during two years: from June 30 until August 8, 2017 (during 2 consecutive periods), and from May 21 to August 6, 2018 (during 3 consecutive periods). Both studies were conducted according to procedures approved by the Utah State University Institutional Animal Care and Use Committee (Approval # 2858).

Grazing experiment

Fifteen 2-year-old Angus heifers [541.09 kg BW ± 30 kg (Mean ± SD)] were randomly assigned to one of three treatment pastures: (1) Birdsfoot Trefoil, *Lotus corniculatus* (BFT), a tannin-containing legume (McAllister et al., 1994; Steiner et al., 2001; Mueller-Harvey, 2006; Waghorn, 2008; Wang et al., 2015); (2) Cicer Milkvetch, *Astragalus cicer* (CMV), a control non-bloating legume of similar nutritional and agricultural characteristics to BFT but without tannins (Stroh et al., 1973; Majak et al., 1995; Acharya et al., 2006; MacAdam & Villalba, 2015); and (3) Meadow Brome, *Bromus riparius* (MB), a grass well-adapted for high-altitude irrigated grass-fed systems (Knowles, 1990; Jensen et al., 2001; Jensen et al., 2006; MacAdam et al., 2006). The average nutritional quality of the pastures and nutritional quality across the three experimental periods is reported in Table 1. The use of the aforementioned forages in the study was based on their characteristics of adaptation, establishment and persistence under the environmental conditions present in the Intermountain West for irrigated pastures (MacAdam et al., 1997).

Each treatment had 5 spatial replications (experimental plot that represented the experimental unit of the design). Each replication was randomly divided into three paddocks (64 x 57m; 0.3648 ha), seeded with birdsfoot trefoil, cicer milkvetch and meadow brome (Figure 1). One heifer was assigned to graze in each paddock (N=5 animals/pasture). Heifers were allowed to graze in one-twelfth of the paddocks, and they were moved to a new section every 3.5 days. The perimeters of the experimental plots and paddocks were fenced using t-posts and electric fence.

Throughout the experiment, animals had free access to water and mineral salt blocks (mineral composition: 96% NaCl, 320 mg/kg Zn, 380 mg/kg Cu, 2,400 mg/kg Mn, 2,400 mg/kg Fe, 70 mg/kg I, and 40 mg/kg Co). Cows grazed their respective pastures for 77 days, from May 19 to August 4, 2017. There were 3 sampling periods: Period 1: From June 15 to June 23; Period 2: From July 6 to July 14; and Period 3: From July 27 to August 4. During in-between sampling periods (2 weeks), cows continued to graze at their respective paddocks moving to fresh pasture every 3.5 days, as described before.



Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
CMV	BFT	CMV	MB	BTF
MB	CMV	BFT	BFT	CMV
BFT	MB	MB	CMV	MB

Figure 1. Pasture plot plan: Birdsfoot Trefoil (BFT), Meadow Brome (MB), and Cicer Milkvetch (CMV) paddocks randomly distributed across five spatial replications.

Each sampling period represented four consecutive days of adaptation to daily doses of Chromic oxide (Cr_2O_3) and five days of sample collection (see below).

Pasture sampling

Forage samples were collected once per period, mimicking animals grazing behavior. Samples were placed in plastic bags and labeled, stored immediately on an ice cooler, and then transported to the laboratory and stored in a freezer at 20 °C. Frozen samples were sunsequently dried in a freeze drier (Free Zone 18 Liters, Labconco Corporation, Kansas City, MO) for approximately 8 days until constant weight. Dried pasture samples were ground to pass through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA) and then used for forage quality determinations (see below).

Pasture quality data were analyzed and presented as the average of the whole study and as the average of each sampling period.

Body weights

Cows were weighed at the beginning of the study (May 19 of 2017) and at the end of period 3 (August 4 of 2017). Average daily gains were determined for each of the treatments. Average daily gains (ADG) were calculated for each of the treatments by dividing the BW gain by the number of days that elapsed to accrue such gains. ADGs are presented as the average for the entire grazing study.

Fecal output

Each day during each sampling period, animals were gathered from their respective pastures at 0900 and they walked (approximately 300 m) to the handling facility, and then through the handling chute where they were sampled and dosed. Fecal output was estimated for each sampling period using Chromic oxide (Cr_2O_3) as an external indigestible fecal marker. The marker Cr_2O_3 was dosed once daily (15 g of Cr_2O_3/d) using gelatin capsules lubricated with mineral oil and delivered with a balling gun. This procedure started 4 days prior to sampling feces and then for 5 additional days, when fecal grab samples were collected daily from each animal (approximately 50g on a DM basis). Fecal grab samples were collected at 0930 daily and immediately deposited in a cooler with dry ice for transportation until final storage in freezer (-20°C) before analyses.

Fecal samples were composited per cow and for each period. The homogenized composited samples were frozen and dried by lyophilization for 5 to 7 days in a freeze dryer (Free Zone 18 Liters, Labconco Corporation, Kansas City, MO). At the end of the process, the dry sample weight was recorded, and then feces were ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). Fecal samples were analyzed for chromic oxide content (Kolver et al., 1998). Concentration of fecal Cr was used to calculate fecal output, which was then used to estimate pasture DMI as described by Kolver et al. (1998).

$$Fecal\ output(g) = \frac{Daily\ Chromic\ Oxide\ dose\ (g)}{Chromic\ Oxide\ concentration\ in\ Feces(\frac{g}{g\ feces})}$$

Fecal output estimations are presented as the mean of the grazing study and as the mean of each sampling period during the grazing study (Table 2 and 4).

Intake determinations

Estimation using fecal output and diet digestibility. In order to estimate intake in grazing animals, I used fecal output values determined through the use of an external marker (Cr_2O_3) as described above, and digestibility of the forage dry matter estimated through NIRS analyses (NIRS, AOAC,1990; see below). Intake was calculated by the following equation.

$$Intake\ at\ Pasture = \frac{Fecal\ output}{(1 - Digestibility\ of\ Dry\ Matter\ )}$$

Estimation using the rising plate meter. A rising plate meter (RPM) calibrated for each pasture species was used to estimate the amount of forage DM availability from the pastures (MacAdam and Hunt, 2015). At least 30 RPM measurements were averaged to estimate pre- and post-grazing biomass, and DM calibration samples were collected from each replication of each pasture each week. Calibration samples were cut, oven-dried

(60°C) to constant weight, and then weighed to determine the DM content of each forage. The DM of RPM calibration samples was regressed on the corresponding RPM readings to develop a linear regression equation for each one of the forage species (birdsfoot trefoil, cicer milkvetch, and meadow brome). Pre- and post- grazing pasture DM readings were predicted from these equations. DM disappearance from each paddock was calculated as the difference between pre- and post-grazing estimates of pasture DM.

Pasture dry matter intakes by both estimation methods (biomass disappearance using the RPM and fecal output-digestibility relationship) were expressed as DMI/day, DMI/kg LBW (BW^0.75), and as DMI as % of BW. DMI was analyzed and presented as the average of the whole grazing study and as the average of each sampling period in the grazing study (Tables 2 & 3).

Estimations based on the NRC model. Intake by individual animals on each treatment was estimated with the Beef Cattle Nutrient Requirements Model (NRC, 2018 software version 1.0.37), using the individual ADG of each animal and the total digestible nutrients (TDN) of each diet, which estimated the dry matter intake required by each animal to achieve the observed ADG.

<u>Methane determinations</u>

Enteric methane emissions by grazing animals were measured using the sulfur hexafluoride (SF_6) tracer gas technique described by Johnson et al., (1994; 2007). The quantity of methane produced on a daily basis was determined by sampling air close to the nostrils of cows that had received a permeation tube deposited into their rumens.

Fourteen days before the first methane collection period started, cows were trained daily to the methane capture equipment (canister and halter), and 37 days before Period 1 started, each animal received an SF_6 slow-release permeation tube delivered with a balling gun. The permeation tubes emitted on average 5.24 ± 0.546 kg (Mean ± SD) of SF_6 marker per day.

During each sampling day, every cow was fitted with a halter and an evacuated canister to gather exhaled air for a 24-h period. Every morning and for five consecutive days, canisters valves connected to air collecting capillary tubes were closed, canisters were replaced, and valves were opened for a new collection period. Control samples of air were collected from canisters placed in areas surrounding the experimental plots to measure background atmospheric concentrations of methane; which were used to correct values obtained from the air samples collected from the animals (Williams et al., 2011). Replaced canisters were taken back to the laboratory to measure final captured pressure and then they were pressurized again with nitrogen. A subsample was acquired from the pressurized canisters and placed in evacuated vials for temporary storage until methane and SF_6 determinations (see below; chemical analyses).

Methane emissions were analyzed and presented as the average of the whole study and as the average of each sampling period, moreover these emissions were compared with methane emissions from the feedlot study.

Nitrogen in blood and urine

Blood and urine samples were collected at the end of each sampling period for each animal (3 periods, 15 animlas/period). Blood samples were taken from the coccygeal vein of the tail using 10 ml vacuum tubes with no additives (Kolver & Muller, 1998). The blood was allowed to clot, serum was separated by centrifugation (2000 rpm for 20 minutes) and extracted using a disposable pipette. The serum sample was placed in two 1mL tubes and frozen at -20°C until urea analysis (see below).

During the last day of each sampling period, urine (25 mL/cow) was collected through vulva massage. Urine volume was measured using a calibrated cylinder and then 3.125 ml of HCl 6N were added to reduce pH (<1) in order to avoid nitrogen volatilization. Samples were stored at -20°C until nitrogen analysis (Bargo et al., 2002).

BUN and urinary nitrogen were analyzed and presented as the average of the whole grazing study and as the average of each sampling period. These parameters were compared between the grazing and feedlot studies.

Chemical analyses

Forage quality

Forage nutritive value was determined from composited samples from the hand-plucked forage samples for each collection period, forming one sample per experimental unit of the design and per species (5 spatial replication x 3 species = 15 total samples per period). Ground forage samples were analyzed for Crude Protein (CP), Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), Lignin, Non-fibrous carbohydrates (NFC) and digestibilities of the NDF (DNDF) and dry matter (DMD) (NIRS, AOAC,1990) at the Utah State University Analytical Laboratories (USUAL). Forage samples were also analyzed for ther condensed tannin contend (Grabber et al., 2013).

Methane analyses

Gas samples extracted from the canisters were analyzed for methane and sulfur hexafluoride by gas chromatography (Chavez et al., 2006). Enteric methane was expressed as the total number of grams produced per day and grams per kg of dry matter intake.

Nitrogen analyses

Urine samples were analyzed for urinary nitrogen contents (Leco Corporation FP-528 Protein/Nitrogen Determinator) at the USDA-ARS Poisonous Plant Research Lab. Urinary nitrogen was expressed in g/L. Blood serum samples were analyzed for urea nitrogen (BUN) (Siemens Urea Nitrogen Flex Reagent, Siemens Healthcare Diagnostics, Newar, Deleware) at the Utah Veterinary Diagnostic Laboratory (UVDL). BUN was expressed in mg/dL.

Feedlot experiment

Five 2-year-old Angus cows [2017 BW, 526.83 kg ± 18.71 kg; 2018 BW, 563.44 kg ± 83.61 kg (Mean ± SD)] were randomly assigned to individual adjacent pens (measuring 10 x 5 m) inside a covered barn to receive a TMR ration (25% of Alfalfa hay, 25% Corn silage and 50% Chopped barley). Enteric methane emissions, BUN, Urinary nitrogen, and animal performance were measured using the same methodologies described for the grazing experiment. The nutritional value of the TMR ration (composited for all sampling periods) is presented in Table 1.

The experiment was performed during two consecutive years. During 2017, cows received the TMR ration for 42 days from June 30 to August 8 (Period 1: July 3 to July 10; Period 2: July 27 to August 6). During 2018, animals received the TMR ration for 78 days, from May 21 to August 6 (Period 1: June 1 to June 9; Period 2: June 28 to July 7; Period 3: July 26 to August 4). Different cows were used in 2017 and 2018, but periods and days were repeated measures on the same cow during each year.

The ration was offered each day at 0900 and the amounts offered were 27 kg/ animal in both years. Refused feed was collected at 0850 on the following day and weighed; fresh feed was offered immediately upon refusal collection. During the 2017 study, the feed was offered in two feeders/pen (13.5 kg feed/feeder; feeder volume = 79 L) placed inside each pen. During the 2018 study, the feed was offered in one larger feeder (27 kg feed/feeder; feeder volume = 378 L) also placed inside each pen. Each animal was given *ad libitum* access to water and trace mineral salt blocks (mineral composition: 96% NaCl, 320 mg/kg Zn, 380 mg/kg Cu, 2,400 mg/kg Mn, 2,400 mg/kg Fe, 70 mg/kg I, and 40 mg/kg Co) in both years. Additionally, animals were dosed daily with 15 grams of Cr_2O_3 to estimate fecal output as described before.

During both the 2017 and 2018 studies, enteric methane emissions were determined as described for the grazing study and fecal samples were collected during the last five days of each collection period. During 2018, urine and blood samples were collected during the last day of each sampling period as described for the grazing study.

Body weights

Cows were weighed at the beginning (2017: June 28; 2018: May 21) and the end (2017 and 2018: August 6) of each of the studies. Average daily gains were calculated for each of the treatments by dividing the BW gain by the number of days that elapsed to accrue such gains. ADG was presented as an average of the two years of the feedlot study.

Fecal output

Animals were assigned to individual pens on June 26 of 2017 and May 31 of 2018. Cows received a daily dose of Cr_2O_3 in gelatin capsules (15g/day), following the same protocol for marker delivery and fecal collection described for the grazing study.

For each sampling period, fecal samples were composited by cow and frozen as described before. Composited samples were dried by lyophilization and ground with the same protocol described above. Chromic oxide contents from feces and the amounts of Cr_2O_3 delivered daily were used to calculate fecal output as described by Kolver et al. (1998).

Fecal output estimations are presented as the mean of the feedlot study and as the mean in each sampling period of this study.

Intake determinations

Feed intake was measured from day 5 to 9 of each collection period. The feed was placed every morning in individual feeders inside each pen. Feed offered was weighed at 0900 and refused feed was measured at 0850 on the following day. The difference between feed offered and refused was recorded as feed intake. Additionally, food intake was estimated as described before using the mathematical relationship between fecal output and digestibility of the dry matter.

Feed intake by both estimation methods (Gravimetrically and fecal output/digestibility), were expressed as DMI/day, DMI/kg LBW (BW^0.75), and as DMI as % of BW.

Feed intake is presented as the mean of the feedlot study and as the mean in each sampling period of this study.

Intake was also estimated with the Beef Cattle Nutrient Requirements Model (NRC, 2018 software version 1.0.37), using the individuals ADG of each animal and the total digestible nutrients (TDN) of each diet.

Methane determinations

Cows had an adaptation period followed by a collection period as described for the animals in the grazing study. During each sampling day, cows were gathered from their individual pens to pass through a chute, where canisters were replaced as described for the grazing study.

Methane emissions are presented as the average of the two years of the feedlot study and as the mean emissions in each sampling period.

Nitrogen in blood and urine

Blood and urine samples were collected just in the second year of the feedlot study (2018) using the same sampling protocol described for the grazing study. BUN and urinary nitrogen results are presented as an average of the study and as the average for each sampling period during 2018.

Chemical analyses

Representative samples from feed collected in each period of the study during 2017 and 2018 were freeze dried as described before and sent to Cumberland Valley Analytical Services Inc. (Hangerstown, MD). All frezze-dried samples were analyzed for dry matter content (AOAC, 1990; method 967.03), neutral detergent fiber (NDF) (Van Soest et al., 1991), acid detergent fiber (ADF) (AOAC, 1990; method 973.18), ash (AOAC, 1990; method 942.05), total nitrogen (AOAC, 1990; method 990.03), ether extract (EE) (AOAC, 1990; method 920.39), lignin (Goering & VanSoest, 1970) and digestibility of DM and NDF (Tilley and Terry, 1963). Non-fibrous carbohydrates (NFC) were calculated from the equation:

NFC%=100%-[CP%+(NDF%-NDFICP%)+EE%+Ash%], where CP was estimated as N% x 6.25, and NDFICP (the NDF insoluble curde protein) was estimated as NDF x 0.93 (Undersander and Moore, 2002). Total digestible nutrients (TDN) were calculated from CP and fiber concentration based on equations by Weiss et al., (1992).

Rations were also analyzed for their condensed tannin contents (Grabber et al., 2013).

Methane in exlaed air, urinary N concentration, BUN were analyzed as described for the grazing study.

Statistical analyses

I analyzed pasture quality (CP, ADF, NDF, Lignin, TDN and DDM), DMI at pasture and in confinement, methane emissions, fecal output, blood urea nitrogen and urinary nitrogen excretions. Response variables for the grazing and feedlot experiment were analyzed as a split-plot design with repeated measures. In both experiments, cows (random factor) were the whole plot units with treatment (pasture species; ration) as a fixed factor and day, period and year (feedlot experiment) as the repeated measures. The variance-covariance structure used was the one that yielded the lowest Bayesian information criterion. Plots were blocked by design and there was no evidence of a block effect and thus the block term was omitted from the statistical models. There was not interaction effect between treatment and rep.

For the grazing study, treatment effects were analyzed across the three sampling periods of the experiment. Results are presented as the average of the three sampling periods, and as the mean of each sampling period.

For the feedlot experiment, heifers (random effect factor) were the whole plot units in the design with year (2017 or 2018) day and period as the repeated measures on each heifer. Results are presented as the average of the five sampling periods of the feedlot study and by each sampling period during each year.

Variables gathered across the grazing and feedlot experiment using the same methodology [dry matter intake (estimated by Fecal output/Digestibility), methane emissions, ADG, BUN, concentration of nitrogen in urinary excretions and fecal output] were compared using the overall means for each study (Grazing: mean of 3 sampling periods; Feedlot: mean of 5 sampling periods).

All analyses were computed using PROC GLIMMIX in SAS/STAT 14.1 in the SAS System for Windows Version 9.4 (SAS Inst., Inc. Cary, NC). Normal distribution of the error residuals and homogeneity of variance were graphically assessed. Data were transformed when needed with logarithm for methane emission per kg of DMI, DMI/kg LBW, DMI as %BW, and fecal output. Pairwise mean comparisons were adjusted for experiment-wise Type I error using the Tukey method.

RESULTS

Nutritional composition of the pastures and ration

The average nutritional composition of the different diets in the grazing study and the ration used in feedlot study are reported in Table 1. As expected, dry matter content was greater for the TMR (P=<.0001) followed by MB, whereas the legumes (BFT and CMV) showed the lowest values for this parameter. In contrast, crude protein concentration was greater for the legumes diets, and in particular for CMV (P=<.0001). The TMR and MB had similar crude protein contents (P=0.6473). Meadow brome had the highest concentrations of fiber [ADF (P=<.0001) and NDF (P= <.0001)] and lignin compared with the legumes diets and the TMR diet (P=0.0001). Non-fibrous carbohydrates concentration was similar between the TMR and the CMV diet (P=0.1324), both legumes diets were comparable among them (P=0.4178) regarding NFC, and MB was the diet with the lowest concentration of NFC (P=<.0001). Condensed tannins concentration was greater in BFT than in the other diets used in this study (P=<.0001), with low and similar (P=0.8935) concentration for MB and CMV (BFT>CMV=MB). The digestibility of the NDF at 48 hours was significantly different among the diets (P=<.0001), TMR had the higher digestibility of the NDF at 48 hours (TMR>MB>BFT>CMV). Digestibility of the dry matter was CMV=TMR>BFT>MB (P=<.0001; Table 1). The level of TDN was the greatest for CMV, BFT and TMR were similar among them (P=0.1224), and MB showed the lowest values (P=<.0001).

Forage quality during the grazing experiment remained fairly stable across periods for BFT and CMV. On the other hand, MB showed a significant reduction in CP content and increase in fiber concentration during Period 2, which led to reductions in DM digestibility and TDN contents for this grass (Figure 2). Lignin concentration was greater for the grass than the legumes in Period 1 and lignin concentration of the three forages did not differ in Periods 2 and 3 (Figure 2).

Table 1. Average nutritional quality during the grazing[1] and feedlot[2] studies. Treatments: TMR (feedlot diet), BFT (Birdsfoot trefoil), CMV (Cicer Milkvetch) and MB (Meadow Brome). Means in a row with different letters ($^{a\text{-}d}$) are significantly different at the α = 0.10 after Tukey adjustment. SEM: Standard error of the mean. ND: Not determined.

Item	**[1]Grazing study**						**[2]Feedlot study**	
	BFT		**CMV**		**MB**		**TMR**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Dry matter, %	94.84 ^{bc}	0.096	94.64 ^{c}	0.096	95.03 ^{b}	0.096	95.03 ^{a}	0.167
Crude protein, %	20.70 ^{b}	0.717	25.39 ^{a}	0.717	13.39 ^{c}	0.717	13.12 ^{c}	1.241
Acid detergent fiber, %	23.86 ^{b}	0.843	21.23 ^{b}	0.843	35.13 ^{a}	0.843	22.51 ^{b}	1.540
Neutral detergent fiber, %	33.74 ^{b}	1.571	25.46 ^{c}	1.571	57.41 ^{a}	1.571	34.66 ^{b}	2.721
Lignin, %	4.97 ^{ab}	0.162	4.56 ^{bc}	0.162	5.41 ^{a}	0.162	3.85 ^{c}	0.297
Non-fibrous carbohydrates, %	38.93 ^{b}	0.944	41.01 ^{ab}	0.944	23.84 ^{c}	0.944	45.40 ^{a}	1.723
Tannins, %	1.91 ^{a}	0.053	0.18 ^{b}	0.091	0.12 ^{b}	0.091	0.06 ^{b}	0.096
Digestibility of NDF, %	22.38 ^{c}	0.598	19.98 ^{d}	0.598	35.69 ^{b}	0.598	48.16 ^{a}	1.093
Digestibility of dry matter, %	86.40 ^{b}	0.972	91.60 ^{a}	0.972	77.06 ^{c}	0.972	87.51 ^{ab}	1.775
Total digestible nutrients, %	74.57 ^{ab}	1.069	77.53 ^{a}	1.069	61.99 ^{c}	1.069	69.74 ^{b}	1.852

[1] Grazing study data were collected on year 2017, (Period 1: June 15 to June 23; Period 2: July 6 to July 14; Period 3: July 27- August4).

[2] Feedlot study data were collected on years 2017 (Period 1: July 3 to July 10; Period 2: July 27 to August 6) and 2018 (Period 1: June 1 to June 9; Period 2: June 28 to July 7; Period 3: July 26 to August 4).

Grazing experiment

Pasture biomass production

Table 2 shows results for standing biomass averaged across periods and within each period. Averaged across periods, CMV produced the greatest amounts of biomass followed by MB and then by the BFT pastures (P=<0.0001). When looking at biomass in different periods, CMV>MB>BFT for Period 1 (P=0.0002), whereas there was less BFT biomass than CMV and MB during the second grazing period (P=0.0002; MB=CMV>BFT). During the third period of the grazing study, there were no differences in standing biomass among the pastures (P=0.3463).

Table 2. Average pasture biomass availability in kg/ha. Treatments: BFT (Birdsfoot trefoil), CMV (Cicer Milkvetch) and MB (Meadow Brome). Means in a row with different letters ($^{a\text{-}c}$) are significantly different at the α = 0.10 after Tukey adjustment. SEM: Standard error of the mean.

Term	**[1]Pasture**			**SEM**
	BFT	**CMV**	**MB**	
Period 1	5160 ^{c}	8825 ^{a}	7328 ^{b}	288.82
Period 2	4699 ^{b}	7246 ^{a}	7277 ^{a}	288.82
Period 3	5378	5836	5523	288.82
Overall	5079 ^{c}	7302 ^{a}	6709 ^{b}	168.14

[1] Grazing study data was conducted on year 2017, (Period 1: June 15 to June 23; Period 2: July 6 to July 14; Period 3: July 27- August4).

Body weights

Average daily gains were greater for cows under the BFT treatment, followed by cows grazing the MB and then the CMV pastures (BFT> MB > CMV; P=0.0006; Table 3).

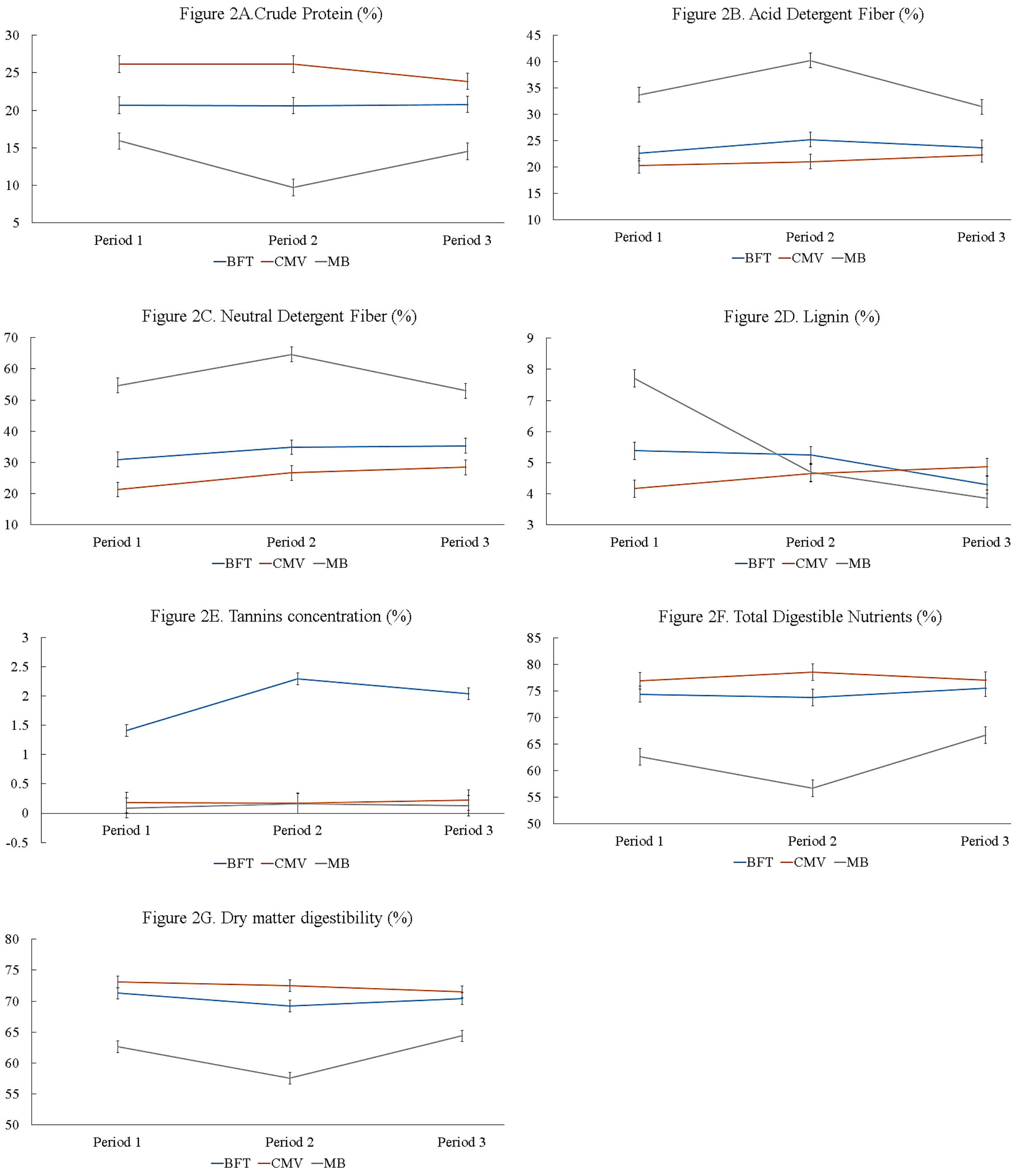


Figure 2A.Crude Protein (%)

Figure 2B. Acid Detergent Fiber (%)

Figure 2C. Neutral Detergent Fiber (%)

Figure 2D. Lignin (%)

Figure 2E. Tannins concentration (%)

Figure 2F. Total Digestible Nutrients (%)

Figure 2G. Dry matter digestibility (%)

Figure 2. Nutritional quality of three treatment pastures during the grazing study across three sampling periods. Treatment pastures: BFT (Birdsfoot trefoil), CMV (Cicer Milkvetch) and MB (Meadow Brome). Data were collected in 2017. Period 1: June 15 to June 23; Period 2: July 6 to July 14; Period 3: July 27- August 4.

Fecal output

Averaged across periods, fecal output did not differ among cows grazing the three experimental pastures (P=0.1658; Table 3). Likewise, fecal output did not differ among grazing treatments across the first two periods of the study (Period 1, P=0.5762; Period 2, P= 0.4094). During the third period, heifers grazing BFT showed a greater level of fecal output (P=0.0157; Table 5).

Intake determinations

Intake in the grazing study is repoted by the fecal output and digestibility relationship because by using this methodology values were closer to those estimated using the NRC model for predictions of intake based on ADG and TDN contend of the forages (National Research Council, 2018: Table 3). Further comparisons of dry mater intake and methane in relation with the dry mater intake for the grazing study were performed using the fecal output and digestibility relationship.

Intake values (expressed per kg of LBW and as % of BW) estimated through fecal output and dry matter digestibility showed that cows consumed similar amounts of BFT and CMV during the three sampling periods (Table 4). In contrast, cows grazed lower amounts of MB than CMV during Period 2 (DMI/kg LBW, P=0.0418; DMI%BW, P=0.0386) and lower amounts of MB than BFT (DMI/kg LBW, P=0.0239; DMI%BW, P=0.0270) during Period 3 (Table 4).

Table 3. Average dry matter intake, fecal output, methane emissions, average daily gains (ADG) and nitrogen in blood and urine by animals during the grazing study[1.] Treatments: BFT (Birdsfoot trefoil), CMV (Cicer Milkvetch) and MB (Meadow Brome). Means in a row with different letters ($^{a-c}$) are significantly different at the α = 0.10 after Tukey adjustment. SEM: Standard error of the mean. ND: Not determined.

	[1]Grazing study					
	BFT		**CMV**		**MB**	
Item	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Dry mater intake and fecal output						
DMI, kg/d [2]	14.84^{a}	1.063	13.14^{ab}	1.063	10.12^{b}	1.063
DMI, kg/d [3]	12.68	ND	10.11	ND	14.18	ND
DMI/kg LBW	0.13^{a}	0.010	0.12^{ab}	0.010	0.09^{b}	0.010
DMI%BW	2.77^{a}	0.238	2.57^{ab}	0.238	1.94^{b}	0.238
Fecal output, kg/d	4.37	0.285	3.61	0.285	3.87	0.285
Enteric methane emissions						
Methane per day, g/d	283.56	13.254	261.37	13.128	254.28	12.955
Methane/kg DMI	20.55	2.119	21.04	2.119	25.42	2.119
ADG, kg/d	0.70^{a}	0.079	0.18^{c}	0.079	0.46^{b}	0.079
Nitrogen in blood and urine						
BUN, mg/dL	17.80^{a}	0.748	20.06^{a}	0.748	8.40^{b}	0.748
Urinary Nitrogen, g/L	4.55	0.536	4.14	0.536	2.87	0.536

[1] Grazing study data were collected on year 2017 (Period 1: June 15 to June 23; Period 2: July 6 to July 14; Period 3: July 27- August4).

[2] Dry mater intake estimated by the fecal output and digestibility method.

[3] Dry mater intake estimated by th NRC (2018) model.

Table 4. Dry matter intake and fecal output per period during the grazing study[1]. Dry matter intake estimated through the relationship between fecal output and the digestibility of the dry matter of the diet. Means in a column with different letters ($^{a-c}$) are significantly different at the α = 0.10 after Tukey adjustment. SEM: Standard error of the mean.

[1]**Grazing study: Dry matter intake and fecal output per period**						
Item	**Period 1**		**Period 2**		**Period 3**	
DMI, kg/d						
BFT	13.54	1.190	13.02 ^{a}	0.914	17.94 ^{a}	1.737
CMV	12.78	1.190	13.60 ^{a}	0.914	13.04 ^{ab}	1.737
MB	10.24	1.190	9.91 ^{b}	0.914	10.21 ^{b}	1.737
DMI/kg LBW						
BFT	0.12	0.012	0.11 ^{ab}	0.008	0.15 ^{a}	0.016
CMV	0.12	0.012	0.12 ^{a}	0.008	0.12 ^{ab}	0.016
MB	0.09	0.012	0.09 ^{b}	0.008	0.09 ^{b}	0.016
DMI%BW						
BFT	2.68	0.268	2.44 ^{ab}	0.178	3.21 ^{a}	0.357
CMV	2.54	0.268	2.65 ^{a}	0.178	2.53 ^{ab}	0.357
MB	2.03	0.268	1.89 ^{b}	0.178	1.88 ^{b}	0.357
Fecal output, kg/d						
BFT	3.87	0.332	3.94	0.236	5.30 ^{a}	0.491
CMV	3.42	0.332	3.73	0.236	3.68 ^{b}	0.491
MB	3.82	0.332	4.2	0.236	3.60 ^{b}	0.491

[1] Data were collected in 2017. Period 1: June 15 to June 23; Period 2: July 6 to July 14; Period 3: July 27- August 4.

Methane emissions

Averaged across periods, cows grazing CMV, BFT and MB pastures showed similar average levels of methane emissions in g/day (P=0.3719; Table 3), and emissions per day were greater during Period 2 (P=<.0001; Table 5).

Average methane emissions in g/kg DMI, revealed no differences among grazing treatments (P=0.2943; Table 3). Likewise, when looking at methane emissions/kg DMI within each period, no differences were observed among grazing treatments for Periods 1 (P=0.7033) and 2 (P=0.3975; Table 5). During the third sampling period, cows grazing

Table 5. Methane emissions per day, and as function of the intake, per period. Means in a column with different letters ($^{a-c}$) are significantly different at the α = 0.10 after Tukey adjustment. SEM: Standard error of the mean.

[1]Grazing study: Methane emissions per period						
Item	**Period 1**		**Period 2**		**Period 3**	
Methane, g/d	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
BFT	254.44	16.292	332.21	22.559	266.88	18.828
CMV	232.79	15.774	305.03	22.672	246.10.	18.518
MB	225.11	15.733	284.41	21.926	253.19	18.518
Methane/kg DMI						
BFT	18.92	2.832	26.68	2.829	10.20 ^{b}	4.366
CMV	20.41	2.832	23.30	2.829	14.65 ^{ab}	4.366
MB	22.10.	2.832	29.03	2.829	25.17 ^{a}	4.366

[1] Data were collected in 2017. Period 1: June 15 to June 23; Period 2: July 6 to July 14; Period 3: July 27- August 4.

BFT had lower emissions than cows grazing MB (P=0.0675), and cows grazing CMV showed intermediate values that did not differ from BFT (P= 0.4361) or MB (P=0.4056; Table 5).

Nitrogen in blood and urine

Legume diets promoted on average, greater levels of blood urea nitrogen (BUN) than the grass diet (P=<.0001; Table 6). The same trend was observed for Periods 1 and 2 (P=0.0006 and P=<.0001, respectively), and during Period 3 BUN values were CMV>BFT>MB (P=<.0001; Table 6).

Urinary nitrogen concentration did not differ among cows grazing BFT or CMV pastures (P=0.7081), and cows grazing MB had the lowest urinary nitrogen excretions (P=0.0249; Table 6). The same trend was observed for Periods 1 and 2 (P=0.0001) and for Period 3, cows grazing BFT had greater urinary nitrogen concentrations than cows grazing

Table 6. Blood urea N (BUN) and urinary N (g/L) in cows that grazed three treatment pastures: BFT (Birdsfoot trefoil), CMV (Cicer Milkvetch) and MB (Meadow Brome). Means within a column with different superscripts ($^{a\text{-}c}$) are significantly different at the $\alpha = 0.10$ after Tukey adjustment. SEM: Standard error of the mean.

[1]**Grazing study: Nitrogen metabolism**						
Item	**Period 1**		**Period 2**		**Period 3**	
BUN, mg/dL	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
BFT	19.00 ^{a}	1.622	17.40 ^{a}	0.989	17.00 ^{b}	0.886
CMV	19.40 ^{a}	1.622	19.80 ^{a}	0.989	21.00 ^{a}	0.886
MB	9.80 ^{b}	1.622	6.80 ^{b}	0.989	8.60 ^{c}	0.886
Urinary Nitrogen, g/L						
BFT	2.88	0.451	2.64 ^{a}	0.153	3.01 ^{a}	0.353
CMV	2.32	0.451	3.08 ^{a}	0.153	2.36 ^{ab}	0.353
MB	2.45	0.451	1.39 ^{b}	0.153	1.54 ^{b}	0.353

[1] Data were collected in 2017. Period 1: June 15 to June 23; Period 2: July 6 to July 14; Period 3: July 27- August 4.

MB (P=0.0447), and cows grazing CMV showed intermediate values that did not differ from BFT or MB (Table 6).

Feedlot study

Body weights

Average daily gains for the feedlot study were in the range of 0.8 kg/d and gains did not differ between years (P=0.3375; Table 7). Overall, feedlot cows had similar ADG to animals grazing BFT (P=0.5790) and greater than animals grazing MB or CMV (P=0.001).

Fecal output

Fecal output was different between years (P=0.0211), with the greatest fecal output values for the year 2017 (Table 7). During this year, fecal output was greater for the second than for the first period of the study (P=0.0119; Table 8), whereas fecal output did not differ among periods for the year 2018 (P= 0.1932; Table 9).

Heifers in the feedlot study showed greater fecal output values than cows in the grazing study (P=0.0219; Table 7).

Intake determinations

Intake values for the feedlot study are only reported by the gravimetric methodology as these values are closer to those obtained through the NRC model using ADG and TDN of the ration. Estimate of methane emissions per unit of intake also used these intake values.

Intake estimations of heifers on the feedlot study showed that intake values from 2018 were greater than in 2017 (DMI/d, P=0.0001; DMI/LBW, P=<.0001; DMI%BW, P=<.0001; Table 7).

Intakes expressed in kg/LBW or as % of BW were greater for Period 2 than for Period 1 during 2017 (kg/LBW, P=0.0004; %BW, P=0.0005; Table 8). In contrast, no differences in DMI were detected among periods during 2018 (kg/d, P=0.8104; kg/LBW, P=0.2703; %BW, P=0.1167; Table 9).

Average feedlot intake estimates (DMI/d; DMI/kg LBW; DMI%BW) revealed no differences in TMR intakes among periods during 2018.

Table 7. Average dry matter intake, fecal output, methane emissions, average daily gains (ADG) and nitrogen in blood and urine in the feedlot study[1]. Means in a row with different letters ($^{a-c}$) are significantly different at the α = 0.10 after Tukey adjustment. SEM: Standard error of the mean. ND: Not determined.

[1]Feedlot study						
Item	**Overall**		**2017**		**2018**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Dry matter intake and fecal output						
DMI, kg/d [2]	10.87	0.362	7.97 ^{b}	0.463	11.61 ^{a}	0.387
DMI, kg/d [3]	13.59	ND	12.90	ND	14.14	ND
DMI/LBW	0.08	0.003	0.07 ^{b}	0.004	0.09 ^{a}	0.003
DMI%BW	1.78	0.087	1.46 ^{b}	0.098	1.99 ^{a}	0.092
Fecal output, kg/d	6.68	0.438	7.34 ^{a}	2.053	3.22 ^{b}	1.222
Enteric methane emissions						
Methane per day, g/d	224.69	12.464	253.09 ^{a}	10.345	212.65 ^{b}	9.124
Methane/kg DMI	28.87	1.966	32.52	3.624	26.43	3.451
ADG, kg/d	0.81	0.069	0.73	0.097	0.88	0.097
Nitrogen in blood and urine						
BUN, mg/dL	7.06	0.748	ND	ND	7.06	0.748
Urinary Nitrogen, g/L	3.92	0.536	ND	ND	3.92	0.536

[1] Feedlot study data were collected in years 2017 (Period 1: July 3 to July 10; Period 2: July 27 to August 6) and 2018 (Period 1: June 1 to June 9; Period 2: June 28 to July 7; Period 3: July 26 to August 4).

[2] Dry mater intake estimated by the gravimetric method.

[3] Dry mater intake estimated by th NRC (2018) model.

Table 8. Dry matter intake estimations by the gravimetric method, fecal output, methane emissions and nitrogen in blood and urine in the feedlot study during 2 Periods[1] for 2017. Means in a row with different letters ($^{a\text{-}c}$) are significantly different at the $\alpha = 0.10$ after Tukey adjustment. SEM: Standard error of the mean.

Feedlot Study 2017				
Item	**Period 1**		**Period 2**	
	Mean	**SEM**	**Mean**	**SEM**
Dry matter intake and fecal output				
DMI, kg/d	6.86 ^{b}	0.578	9.09 ^{a}	0.578
DMI/kg LBW	0.06 ^{b}	0.004	0.08 ^{a}	0.004
DMI%BW	1.30 ^{b}	0.099	1.62 ^{a}	0.099
Fecal output, kg/d	5.82 ^{b}	1.704	14.29 ^{a}	1.704
Enteric methane emissions				
Methane per day, g/d	212.78 ^{b}	14.842	289.28 ^{a}	15.453
Methane/kg DMI	31.69	4.136	33.3642	4.136

[1] Data collected during 2 Periods: Period 1: July 3 to July 10; Period 2: July 27 to August 6.

Table 9. Dry matter intake estimations by the gravimetric method, fecal output, methane emissions and nitrogen in blood and urine in the feedlot study during 3 Periods[1] for 2018. Means in a row with different letters ($^{a\text{-}c}$) are significantly different at the $\alpha = 0.10$ after Tukey adjustment. SEM: Standard error of the mean.

[1]Feedlot Study: 2018						
Item	**Period 1**		**Period 2**		**Period 3**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Dry mater intake and fecal output						
DMI, kg/d	11.50	0.582	11.84	0.582	11.48	0.582
DMI/kg LBW	0.10	0.003	0.09	0.003	0.09	0.003
DMI%BW	2.09	0.103	1.99	0.103	1.87	0.103
Fecal output, kg/d	3.86	0.954	5.64	0.954	3.77	0.954
Enteric methane emissions						
Methane per day, g/d	194.06	15.774	217.76	22.294	231.05	19.371
Methane/kg DMI	24.03	4.136	26.53	4.136	28.73	4.136
Nitrogen in blood and urine						
BUN, mg/dL	7.40	1.090	7.60	1.090	6.20	1.090
Urinary Nitrogen, g/L	4.51	0.709	4.15	0.709	3.10	0.709

[1] Data collected during 3 Periods: Period 1: June 1 to June 9; Period 2: June 28 to July 7; Period 3: July 26 to August 4.

Methane emissions

Feedlot methane emissions (in g/d) for 2017 were greater than emissions for the 2018 study (P=0.0041; Table 7). Methane emissions expressed per unit of intake were similar between years when DMI was estimated through the gravimetric method (P=0.2903; Table 7).

During 2017, methane emissions (in g/d) were greater in Period 2 than in Period 1 (P=0.0021; Table 8). However, this parameter did not differ among periods for the study in 2018 (P=0.1439; Table 9).

When methane was expressed per unit of intake, there were no differences for this parameter among sampling periods for both years of the study (2017: P=0.6046; 2018: P=0.5945; Table 8 and 9).

Averaged across years and periods, methane emissions (in g/d) for the feedlot study were similar to the MB (P=0.3771) and CMV (P=0.2201) grazing treatments, but lower (P=0.0285) than the BFT treatment.

When methane emissions were expressed per unit of intake, emissions from the feedlot study were greater for the BFT treatment (P=0.0740), whereas they did not differ from the animals under the MB (P=0.6763) or CMV (P=0.1180) pasture treatments.

Nitrogen in blood and urine

There were no significant differences among the periods for BUN (P=0.2449) or nitrogen concentration in the urine excretions (P=0.3933).

BUN concentrations in feedlot animals were similar to values observed in cows grazing MB (P=0.5530), but lower than in cows grazing the legume pastures (BFT and

CMV) (P=<.0001). Urinary nitrogen concentrations were similar between cows in the feedlot and the grazing study (P=0.2036).

DISCUSSION

I hypothesized that legumes like BFT with bioactive compounds like condensed tannins would benefit livestock production systems by enhancing animal performance and reducing carbon and nitrogen emissions from enteric fermentation and urine volatilization to the environment relative to conventional grass-fed systems. Thus, I predicted that methane and nitrogen emissions and animal performance would be similar between cattle that graze tannin-containing legumes and cattle consuming a feedlot diet, but tannin-containing legumes will promote lower emissions and greater weight gains than animals consuming grass. Consistent with these predictions, cows grazing BFT showed greater ADG than animals grazing MB (grass) and similar gains to animals fed a TMR with high roughage contents. In addition, methane emissions from cows grazing BFT per unit of intake were similar (RPM method) or lower (fecal output/digestibility method) than emissions from animals consuming the feedlot ration in confinement. Nonetheless, cows grazing MB showed methane emissions that were similar to those observed in cows grazing BFT. Animals grazing BFT and CMV showed greater blood urea nitrogen concentrations than cows grazing MB or consuming a TMR explained by the greater crude protein contents of the legumes. Finally, nitrogen excretions in urine were similar for all the diets suggesting greater N retention in cattle grazing legume pastures.

Body weights

Average daily gains for grazing animals were in generally low (0.46 and 0.70 kg/d for MB and BFT treatments, respectively), and in particular for CMV (0.18 kg/d). For instance, Pitcher (2015) observed ADG values of 0.63 kg/d in Angus steers grazing CMV, and MacAdam et al., (2011) reported ADG values between 1.42 to 1.64 kg/day in steers grazing CMV. One explanation for the lower ADG values found in the grazing experiment is the growth stage of the animals (2 years old) with lower rates of gain than younger individuals. It is known that the growth rate in ruminants is inhibited after puberty (Owens et al., 1993). Additionally, another explanation for the low weight gains in animals grazing CMV is the presence of anti-nutritional components in this forage that impede fiber digestion through inhibition of cellulose fermentation, as reported by Weimer et al., (1993). There is also evidence that fiber components from CMV might be less digestible than components from other forages (Kephart et al., 1990), and Marten et al., (1987) observed lower ADG in heifers grazing CMV than in heifers grazing alfalfa, BFT or sainfoin. The particularity of Marten's results is that animals grazing CMV presented photosensitization and low intakes, suggesting low palatability of the forage (Marten et al., 1987). Therefore, it is possible to conclude that the low ADG observed in animals grazing CMV in this study was a consequence of the potentially negative influence of anti-nutritional factors and low levels of fiber digestibility in this forage (Weimer et al., 1993).

Under typical feedlot conditions, ADG for beef cattle ranges from 0.79 to 2.43 Kg/d (Reinhardt et al. 2012). Thus, the ADG for TMR-fed animals reported in the current study are at the low-end of this range, which is reasonable when considering that cows were in the postpubertal deceleration phase of their growth rate (i.e., animals were 2 years old), did not receive growth promoters and the roughage contents of the TMR were high (50%). An important outcome of this study is that animals grazing BFT showed ADG values similar to those observed by animals in confinement consuming a TMR. This outcome is explained by the high nutritional quality of BFT (Table 1) with greater concentrations of CP and similar concentration of fiber relative to the feedlot ration. Consequently, BFT had a greater TDN content than the TMR. Moreover, tannins from BFT are responsible for an increase in the efficiency of use of dietary protein in the intestines (Waghorn et al. 1987; Min et al., 2003; Waghorn, 2008; MacAdam and Villalba., 2015; Brummer et al., 2016; Lagrange et al., 2017). The magnitude of ADG observed in this study for cows grazing BFT is consistent with previous studies (Pitcher, 2015; Lagrange et al., 2017; 2018). Cows grazing MB showed lower ADG than animals grazing BFT (Table 3), although gains for the MB treatment was lower than values reported by Pitcher (2015) (0.88 Kg/d). An explanation for this pattern may entail the greater contents of fiber and lower concentration of protein in the pastures grazed during the present study (Table 1) than on those reported by Pitcher (2015). The greater ADG observed for animals grazing BFT than for animals grazing MB could also be explained by the greater intakes (fecal output/digestibility method) displayed by cows grazing BFT, a legume of greater nutritional quality than MB.

Intake determinations

Dry matter intake is related to the concentration of the main nutritional components of forages: non-structural carbohydrates, nitrogen (protein) and fiber (Forbes, 2007). During the grazing study, two methods to assess dry matter intake in grazing animals were used: The rising plate meter (RPM) (Scrivner et al., 1986) and a mathematical relationship between fecal output (estimated with chromic oxide as an external marker) and digestibility of the dry matter of the forage (estimated through NIRS analysis) (Holden et al., 1994).

Results from the RPM method showed that animals grazing CMV consumed the greatest amounts of DM (Table 3). However, dry matter intake estimations for CMV, BFT, and MB treatments using this method appear to be overestimated,, and in particular for CMV, relative to intakes predicted by the NRC model (National Research Council, 2018), using the nutritional quality of the forages and observed ADG of cows as inputs (Table 10). Thus, and as it was mentioned in previous sections, intake estimations for the grazing study were reported by the FO/D relationship because such estimations were closer to the predicted intake values obtained with the NRC model. Likewise, for the feedlot study, intake estamtions by the gravimetric method were reported due to estimations by the FO/D method were highly different than the intake values from the NRC model (Table 10).

Table 10. Observed (gravimetric, rising plate meter-RPM and fecal output/digestibility method-FO/D) and predicted [using the NRC (2018) model] dry matter intakes in kg/d for cows in the grazing and feedlot studies. ND: Not determined.

DMI, kg/d	**BFT**	**CMV**	**MB**	**TMR**
Observed (Grav)	ND	ND	ND	10.87
Observed (RPM)	17.73	25.83	16.55	ND
Observed (FO/D)	14.84	13.14	10.12	31.89
Predicted	12.68	10.11	14.18	13.59

The technique to compute intake based on estimations of the fecal output and forage digestibility (FO/D method) resulted in lower intake values that those obtained with the RPM method, but still above the predicted values for BFT and CMV, and below predicted values for MB using the NRC (2018) model (Table 10).

Smith & Reid (1955) reported that chromic oxide excretions in feces vary during the day and for that reason two grab fecal samples per day is recommended (at 0600 and 1600 hours) to generate accurate calculations of fecal output. However, we collected one sample per day in order to reduce alterations in the grazing behavior of cows that had to walk to the handling facility located 300 m from the pastures. Lardner et al., (2015) calculated MB intake in beef cows at pasture using Chromic sesquioxide as an external marker (with two fecal samplings per day, 0700 and 1700), obtaining values of 0.14 kg DM/kgLBW in steers, these results are similar to those obtained in the present grazing study with the RPM method (0.14 kg DM/kg LBW), but lower than those obtained with the FO/D method (0.09 kg DM/kg LBW).

For feedlot animals, intake determinations by the FO/D method were significantly greater than those values obtained gravimetrically or predicted by the NRC model (Table 10). Ration intake in the feedlot study was assessed gravimetrically by the difference between the amounts of feed offered and refused. Bevans et al., (2005), and Beauchemin & McGinn (2005) report greater intake values by mature animals consuming a TMR (0.10 kg DM/ kg LBW in both studies) than overall intake values observed in the current study (0.08 kg DM/kg LBW), although intakes were in the same range for the second year (2018) of the study (0.10, 0.09 and 0.09 kg DM/kg LBW for Periods 1, 2 and 3, respectively). Lower intake values during 2017 may be explained by a smaller volume of the feeder that in conjunction with the methane collection equipment likely constrained access to the forage. Intake estimations by the gravimetric methodology showed that intake values in 2018 were greater than in 2017.

Intakes by grazing animals under this technique (10.12 to 14.84 kg/d range) were closer to values obtained using the NRC (2018) model (12.68 to 14.18 kg/d; Table 10) than those estimations from the RPM method (16.55 to 25.82 kg/d range). Intakes estimated with the FO/D technique were similar for cows grazing legumes (BFT and CMV), and cows grazing BFT had greater intakes than cows grazing MB.

Methane emissions

Enteric methane production is directly related to the amounts of plant fiber digested by the animal (Johnson & Johnson, 1995; Johnson et al., 2000). Therefore, animals grazing forages low in fiber produce lower emissions than animals consuming grass or more fibrous feeds (Beauchemin et al., 2005), and daily methane emissions from cows consuming concentrate diets (e.g., low fiber content) should be lower than emissions from cows grazing forages (e.g., grass) with greater concentrations of fiber (Johnson & Johnson, 1995; Beauchemin & McGinn, 2005; Beauchemin et al., 2008). Methane emissions are negatively correlated with intake levels because passage rate increases with increments in feed intake. Consequently, the residence time of feed in the rumen declines which decreases the time needed for microbial fermentation and as a consequence the rate of methane production in the rumen is reduced (Jiao et al., 2014).

Heifers grazing the different pasture treatments and heifers in the feedlot study emitted similar amounts of methane in g/d, although animals in the feedlot study emitted lower absolute amounts (in g/d) than cows in the BFT treatment. Given the aforementioned relationship between intake and methane production, when methane emissions were expressed per unit of intake (estimated through the FO/D method for the grazing study), no differences among grazing treatments were observed, and emissions by cows grazing BFT were lower than emissions per unit of intake in the feedlot study (feedlot intake was estimated through the gravimetric method). Prior studies reported methane emissions from animals grazing BFT pastures that were similar to values obtained in the current study (Waghorn & McNabb, 2003; Woodward et al., 2004; Lagrange et al., 2017). There is also evidence that condensed tannins in forages reduce methane emissions likely due to an antibiotic effect on methanogenic bacteria or to a reduction in the availability of hydrogen ions from the rumen microflora involved in methane production (Jones et al., 1994; Woodward et al., 2004; Tan et al., 2011.). Nevertheless, concentrations of tannins in BFT for the present study were low (< 20g CT/kg DM), but still much greater than the concentrations observed in CMV or MB. Alternatively, the high-nutritional quality of BFT with lower fiber contents and greater concentration of CP than grass may contribute to explain methane emissions in this study. Collectively, these results support my hypothesis that condensed tannins in legumes may improve livestock production systems by reducing carbon emissions from enteric fermentation.

I proposed that the comparison in methane emissions per unit of intake within and between studies using the FO/D (grazing study) and gravimetric method (feedlot study) is the most accurate approach among all intake estimates explored in this study. This is because intake values obtained through these methods were closer to estimates obtained through the NRC model (2018) and the gravimetric method yields accurate measurements of food intake in confinement.

Contrary to what was expected due to the high fiber contents in MB, cows grazing this forage produced low amounts of CH_4 per unit of intake, which were comparable to values recorded for cows grazing BFT. Ruminants display selectivity at pasture (Theron & Booysen, 1966; Andrew, 1986; Provenza, 1995; Sheremetev et al., 2017) and thus this result can be explained by cows selecting a diet with lower fiber and greater CP contents (i.e. by selecting young grass tissues) than values reported in the chemical analyses (Table 1) for meadow brome, even when hand-plucked samples were collected in a way that tried to mimic the animal's selection.

Nitrogen in blood and urine

Blood urea nitrogen and urinary nitrogen are the consequences of digestion and absorption of ammonia excesses that pass from the rumen to the bloodstream (Lobley & Milano, 1997). Indeed, urea concentration in blood has been used as an indicator of the level of degradable protein in the rumen, with high correlations with the levels of urinary nitrogen excretions (Kebreab et al., 2004). During this study, cows grazing legumes showed high levels of BUN due to the high concentration of CP in these forages (Table 1). We used a control legume (CMV) of similar architecture and nutritional quality to BFT, but without tannins, to explore the influence of condensed tannins on nitrogen metabolism and methane emissions in grazing animals.

Lagrange et al., (2017) observed BUN values of 13.8 mg/dL in steers grazing BFT pastures with 24% of protein in the forage. Meanwhile, Stewart et al., (2019) reported BUN values for cows consuming BFT, CMV, and MB hays in the range of 8.0, 16.0, and 8.0 mg/dL, respectively.

Despite the aforementioned effects of tannins on ruminal proteolysis, legume diets (BFT and CMV) in this study yielded similar levels of BUN and urinary nitrogen concentrations in grazing animals, which were greater than for animals grazing MB or consuming the feedlot ration. The lower concentration of BUN in cows grazing MB or consuming the TMR is explained by the low concentrations of CP in the grass and the ration relative to BFT and CMV (Table 1). The similar concentrations of BUN and urinary nitrogen in tannin- (BFT) and non-tannin (CMV) containing legumes, particularly when considering that the concentration of CP was lower in BFT than in CMV, gives no indication that tannins in BFT reduced BUN or urinary nitrogen concentration in the present study. It is likely that the low concentration of condensed tannins in BFT (Table 1) explains this outcome. Although the optimal concentration of condensed tannins to reduce ruminal proteolysis is not universally established (Wen et al., 2003), Aerts et al., (1999) and Min et al., (2003) suggested that concentrations between 20 to 45g CT/kg DM have positive effects, forming reversible bindings to reduce proteolytic populations in the rumen, which reduce the degradation of dietary protein and production of ammonia. However, this threshold can vary depending of the molecular structure of the CT, due to strong bonds of tannins with the protein may reduce dissociation of the complex and posterior release of the protein in the intestines. Birdsfoot trefoil in my study presented tannin concentration bellow this optimal threshold (<20g CT/kg DM), additionally, CT present in the BFT in this study may not released the protein in the intestines because complexes tannin-protein were precipitated in the rumen slowing down the digestion of the protein (Waghorn, 2008). Thus, contributing to explain the lack of differences in BUN and urinary nitrogen concentrations in cows grazing tannin (BFT) and non-tannin (CMV) containing legumes. Waghorn (2008) concluded that concentrations of 30g CT/kg DM in BFT are beneficial for ruminant production, whereas concentrations over 50 g CT/kg DM have negative impacts on forage intake, although such concentrations may contribute to control gastrointestinal parasite populations in ruminants. Alternatively, the type of condensed tannins presented in BFT (procyanidin-rich tannin type) may also be involved in the lack of differences for BUN and urinary nitrogen concentration between tannin- and non-tannin containing legumes (Mangan, 1988; Hatew et al., 2016).

No differences were observed in urinary nitrogen concentration between cows consuming the TMR and cows grazing tannin-containing legumes, despite the fact that the concentration of CP was greater in the legumes than in the TMR. Thus, the excess of dietary protein in the legume species likely retained by the grazing animal due to greater synchrony in the availability of non-fibrous carbohydrates and nitrogen (Table 1). Finally, as the total urine output in pasture was not measured, it is likely that cows in the legume treatment excreted more urine as a consequence of more physical activity that increases the frequency of urination (Hirata et al., 2011), or because more urine is excreted as a consequence of consuming a diet with greater concentration of nitrogen (Sannes et al., 2002; Broderick, 2003; Wattiaux & Karg, 2004). For instance, Stewart et al., (2019) reported urine excretion values of 17.72, 20.82, and 6.44 L/d for heifers fed with hay of BFT, CMV and MB, respectively, being BFT and CMV forages with higher protein concentration than the MB forage in that study. Thus, greater urine volumes can account for similar urinary nitrogen concentrations despite the fact that diets are different in CP contents. On the other hand, the lower concentration of urinary nitrogen in cows grazing MB can be explained by the lower concentration of crude protein in this grass (Table 1). Based on the fiber contend of the forages (NRC, 2000), it is possible to assume that water intake could been higher in the animals that graze the grass diet relative to the animals that graze the legume diets due to a lower concentration of fiber in such forages.

Previous research has shown positive effects of BFT at reducing urinary nitrogen excretions. For instance, Woodward et al., (2009) recorded a 15% reduction in the urinary nitrogen excretions in dairy cows by the inclusion of 45% of BFT in the diet. Eriksson et al., (2012) fed BFT silage (low concentration of tannins) to dairy cows, obtaining urinary nitrogen values (2.98 g/L) 13-14 % greater than expected based on the predicted benefits of condensed tannins at reducing nitrogen excretions. Hymes-Fecht et al., (2013) observed lower levels of urinary nitrogen excretions (6.65 g/L) in dairy cows eating BFT silage than animals fed alfalfa silage (7.19 g/L) or red clover (7.48 g/L). Nevertheless, these values are greater than those observed in the present study for cows grazing BFT, although there are differences in nitrogen excretions between beef and dairy cows (Xiccato et al., 2005). Former studies by Stewart et al., (2019)) report urinary nitrogen concentrations of 3.72, 4.95 and 4.89 g/L when feeding beef cows BFT, CMV and MB hays, respectively. These last values are comparable to the urinary nitrogen concentration reported in the current study. Considering that the concentration of N in the urine are similar, it is posible to expect that the urinary volumes are similar as well, and then given the conetrations in this study the volumes might be as result, that animals fed with BFT would excreate 20.51 L/d, CMV animals 17.59 L/d and MB heifers 3.63 L/d.

CONCLUSIONS

In summary, cows grazing a tannin-containing legume (BFT) showed greater weight gains than cows grazing a non-tannin containing legume (CMV) or a grass (MB), but similar to cows fed a feedlot ration with high contents of roughage. Methane emissions were comparable among animals grazing BFT, CMV and MB, or consuming the high-roughage ration, although in some instances (e.g., using an external marker and forage digestibility to estimate intake), emissions per unit of intake were lower in cows grazing BFT than in cows consuming the ration. This suggests a positive effect of condensed tannins or nutrients in BFT on methane abatement. Blood urea nitrogen and urinary nitrogen concentrations were similar in cows grazing tannin- (BFT) or non-tannin (CMV) containing legumes, suggesting that tannins in BFT did not reduce ruminal proteolysis or shifted the site of nitrogen excretion from urine to feces. Overall, my findings suggest grazing BFT is a viable alternative to high-roughage feedlot rations for maintaining beef production with similar or potentially lower levels (i.e., methane emissions) of environmental impact.

The development of standard methodologies to measure intake under grazing and confinement conditions is important to generate more accurate comparisons between these two feeding systems. The use of external markers like Cr_2O_3 (this study) to determine fecal output and estimates of digestibility is one of the most common and accurate approaches to measure intake at pasture. Nevertheless, the frequency of fecal sample collection (i.e., at least 2 collections per day) required to increase precision in the estimation of intake, constrains its applicability since animals are disturbed in their grazing patterns every time a sample needs to be taken. Emerging techniques where herbage intake is estimated through concurrent measurements of chewing behavior and sounds appear promising. In a recent study, energy flux density of chewing sounds have successfully predicted intake in grazing dairy cows offered experimental swards (Galli et al., 2018). The same technique could be used to determine intake in confinement. Further validation of this technique is underway to assess forage intake in noisy, natural environments and over prolonged periods of time.

Future projects should focus on the improvement of the agronomic characteristics of birdsfoot trefoil and other tannin-containing legumes, such that biomass production and regrowth rates are enhanced across the growing season, leading to high and sustained rates of body weight gains under grazing. This endeavor will contribute to create an alternative feeding system that in terms of production is comparable to the feedlot system, with the added benefits of reduced greenhouse gas emissions and enhanced rates of carbon and nitrogen sequestration. Foraging chains, where forage species with different phenology are grazed in a sequence that matches the forages' peaks of production and quality (Pordomingo, 2007), the use of tannin-containing legumes, shrubs or trees to extend the grazing season and enhance nutrition (Revell et al., 2013), or the use of legumes in general to fix atmospheric nitrogen in grass pastures are all approaches that could be integrated into new animal management systems that contribute to achieve the goal of enhanced and sustained animal productivity with lower environmental impacts.

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CHAPTER III

ESTIMATION OF IRRIGATED AGRICULTURAL AREA IN THE STATE OF UTAH

ABSTRACT

The ultimate goal of beef production is the development of a sustainable system that takes advantage of the unique ability of ruminants to utilize plant fiber in an efficient manner, minimizing environmental impacts while providing a high-quality product to consumers. The use of alternative legumes (e.g. birdsfoot trefoil) as a mixed crop-livestock farming system could help ranchers achieve this goal in the Intermountain West. For this reason, an estimation of the available land in the state of Utah for growing legumes such as birdsfoot trefoil would inform future management efforts about the feasibility of raising and finishing cattle under this alternative beef production system.

Remote sensing is a technique that allows for the capture of electromagnetic (EM) energy reflected from the earth's surface and to analyze and interpret that reflectivity as surface land cover or land use features. The reflectance of EM energy by plant communities is modulated by leaf tissue and pigments that regulate absorption or reflection of light. The measurement of plant community reflectance utilizing different parts of the EM spectrum provides information that can help improve land management programs focused on vegetation production and biomass conservation. For the agricultural sector, estimation of crop status, soil quality, or plant development are important variables used in the decision-making process that directly affect food supply and market prices. Thus, knowledge of proper soil condition in agricultural areas is a key factor used by managers to increase the likelihood of success in agricultural operations.

An estimation of the areas occupied by irrigated agriculture in the state of Utah was performed using remote sensing imagery collected by the Moderate Resolution Imaging Spectroradiometer (MODIS). The Normalized Difference Vegetation Index (NDVI) (Rouse et al., 1973) is a commonly used and accepted vegetation index for vegetation studies where values of the index are positively correlated with photosynthetically active biomass. Irrigated agriculture in Utah is generally characterized as areas with a high NDVI, and in relatively flat landscapes within low-lying valleys. The identification of agricultural land was performed using a Random forest (decision tree) using NDVI layers from 18 years of MODIS imagery, a digital elevation model (ASTER data), and a training data set of 1,050 random points. The estimated amount of agricultural land in the state of Utah was 412,250 ha, and the major regions of irrigated land were observed in the northern part of the state (Box Elder and Cache counties); and in the central region of the state in Millard and Sanpete counties. Thus, legume-based grazing systems are a viable option for the state, particularly when considering the implementation of mixed crop-livestock farming systems where growers could diversify their operations by producing both hay and beef.

INTRODUCTION

As described in Chapter II, an alternative finishing system based on tannin-containing legumes would take advantage of the capacity of the ruminants to extract energy and nutrients from forages in an efficient manner, minimizing environmental impacts while providing a high-quality product to consumers.

One of the steps to evaluate the feasibility of a tannin-containing legumes finishing system is the quantification of potential areas that can be dedicated to sustain these type of forages (i.e., birdsfoot trefoil). Thus, techniques capable of providing information about these areas and their location are important to accomplish such an endeavor. Remote sensing is a technique that allows for the analysis and interpretation of reflectance data from the earth's surface (Pinter et al., 2003; Rogan & Chen, 2004). There are several remote sensing-based data sources that can provide information about surface resources (i.e., drones, aircraft and satellites) that is otherwise would be difficult to obtain using traditional ground-based methodologies (Hunt et al., 2003).

Remote sensing (RS) of vegetation is mainly performed by recording the electromagnetic (EM) reflectance information from vegetation canopies using passive sensors (Rogan & Chen, 2004). A passive remote sensing instrument is a device that records EM energy originating from the sun, or other independent energy source (NASA, 2017). Light reflectance characteristics vary between plant types, water content within the plant tissues and other intrinsic factors (i.e., plant health, pigments, secondary compounds). Reflectance is determined by chemical and morphological characteristics on the surface of plant organs or leaves (Zhang & Kovacs, 2012; Xue & Su, 2017), although, the quality of a captured image depends on the sensitivity of the sensor to detect reflected energy (Rogan & Chen, 2004).

Remote sensing of vegetation is typically based on the following light spectra: (i) the ultraviolet region (UV), which ranges from 10 to 380 nm; (ii) the visible spectra, composed of the blue (450 to 495 nm), green (495 to 570 nm), and red (620 to 750 nm) wavelength regions; and (iii) the near and mid-infrared band (850 to 1700 nm) (Cruden et al., 2012; Abdul Rahim et al., 2016; Xue & Su, 2017).

The primary pigments in plant leaves are chlorophyll *a* and *b*, and carotenoids. These pigments are responsible for the absorption and reflectance of visible light used in photosynthetic process. Leaf morphology (cellular structure) also affects the amount of reflectivity of near-infrared light, and the comparison of visible light (blue and red) absorption by chlorophyll of near-infrared by leaf tissue can provide an insight into the physiological status of plant communities (Blackburn & Steele, 1999). Changes in the reflectivity features due to the health status of different plants are depicted in Figure 3.

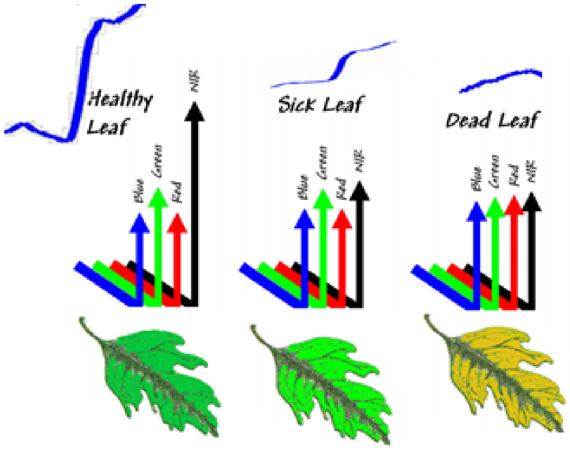

Figure 3. Relationship between leaf health with visible light spectra reflectance (blue, green and red) and Near-Infrared reflectance (Dobrowski, 2012).

Physiological characteristics, such as leaf water content and transpiration, are detected by measuring the middle infrared energy incident to the leaf (Ustin et al., 2012; Hunt Jr. et al., 2016). Variations in the reflectance and absorption of EM energy of these different wavelengths are the result of the variation of the concentration of pigments in leaf tissues, which are related to the physiological function of the plant (Pinter et al., 2003). For instance, high concentrations of chlorophyll in leaves result in high photosynthetic rates, and thus an increased absorption of visible light. Increased amounts of chlorophyll for a specific plant is positively correlated to the amount of leaf tissue present. An increase in leaf tissue is positively correlated with an increase in the reflectance of NIR light. The differential absorption of visible light (particularly red) compared to the reflectivity of NIR light is indicative of the total amount of biomass (Dobrowski, 2012).

The relationship between light reflectivity/absorption by photosynthetically active biomass can therefore be estimated through the use of vegetation indices (VI) that mathematically combine the differential reflectance/absorption of various wavelengths of EM energy due to changes in the photosynthetically active material (Khanal et al., 2017; Xue & Su, 2017). Such indices are often computed as differences, ratios, or linear combinations of reflected light in visible and NIR wavebands (Pinter et al., 2003; Angerer et al., 2016). Variation in these indices can be attributed to changes in characteristics related to plant growth and vigor, such as water, pigments, sugar, protein, aromatics and carbohydrate content, among others (Meera Gandhi et al., 2015). In addition, these indices can be used to estimate stomata dynamics related to plant transpiration or plant water stress (Tucker, 1980; Xue & Su, 2017). This information can be used to make decisions related to the management of a crop, such as irrigation levels or shading manipulations.

One common application of remote sensing is the measurement of characteristics from a vegetation community (Di Bella et al., 2004; Meera Gandhi et al., 2015; Angerer et al., 2016; Gitelson, 2016; Khanal et al., 2017; Xue & Su, 2017). Thus, remote sensing has been widely used to assess agricultural activities and will continue to be more commonly used given the current trends of human population growth and the need to optimize the use of natural resources (Crist et al., 2017). Remote sensing has an important role in precision agriculture by increasing data available to make decisions, providing information about the dynamics of the environment such as biomass status and abundance, or data about drought and water stress in agroecosystems (Ramoelo et al., 2014; Maggiori et al., 2017; Precision Agriculture, 2018).

Different applications of remote sensing techniques in agriculture depend on the reflectivity peaks along the range of visible and near/mid-infrared regions of light spectra (Di Bella, et al., 2004; Gitelson, 2016). For example, plant emissions and transpiration levels can be portrayed using plant emissivity as measured in the thermal infrared (Soer, 1980; McCarty, 2011).

Thermal remote sensing is used in agriculture to detect environmental changes that can produce water or temperature stress in a crop (Ustin et al., 2012; Hunt Jr. et al., 2016). Some uses of thermal remote sensing in agriculture are: (i) soil moisture detection, which monitors humidity in the soil, improving timing of irrigation; (ii) land soil temperature, a characteristic that has a strong relationship with soil texture, that is an indicator of the quality of the soil (structure, porosity, hydraulic properties, and nutrient retention ability); (iii) plant disease detection by changes in temperature of the plant community, due to physiological responses in the plant caused by pathogens; (iv) drainage mapping, the presence of water in the soil and humidity in the environment directly affect plant thermal radiation, which in turn allows water stress to observed in crops; and (v) crop maturity and crop yield mapping (Serrano et al., 2000; Buitrago et al., 2016; Khanal et al., 2017; Yen Mee et al., 2017).

Remote sensing techniques have been used as tool to forecast biomass yield (Serrano et al., 2000; Shanahan et al., 2001; Khanal et al., 2017; Xue & Su, 2017; Noland et al., 2018) and forage quality (Kalu & Fick, 1981; Owens et al., 1995; Starks et al., 2016).

This information can complement traditional visual estimations utilized to decide the harvesting of the crop. Remote sensing analysis can be particularly advantageous where research is economically and spatially restricted (Bateman, 2017).

The objective of this study was to use remote sensing in order to estimate the agricultural areas under irrigation in the state of Utah. Results from this study will provide estimates on the agricultural areas that can be suitable for "non-traditional" forage legumes that grow under irrigation such as birdsfoot trefoil (*Lotus corniculatus L)* or sainfoin (*Onobrychis coronarium)* to be used in beef-finishing systems as an alternative/complement to hay production such that plant growers can diversify their operations. Thus, this information will be of value for novel local or regional programs aimed at developing mixed crop-livestock farming systems based on the inclusion of tannin-containing legumes, and to estimate the capacity for local perennial legume-based pasture finishing.

STUDY AREA

The state of Utah is in the center of the Intermountain West region, between the longitudes from 109° West to 114°West and the latitudes 37° North to 42° North latitude. The approximate size of the state is 219,807 square kilometers and it has 29 counties, with San Juan, Tooele, Millard, and Box Elder (McGinty & McGinty, 2009) being the largest ones, which are distributed in three major eco-regions: Rocky Mountains, Basin and Range, and Colorado Plateau (Figure 4). Utah is ranked as the 11th largest state in the U.S (UACD-UDA-NRCS, 2005). The state altitude ranges from 663 meters at Beaver Dam Wash in the southwestern corner of the state to 4,123 meters above sea level at the summit of King's Peak in the Uinta Mountains (Netstate.com, 2016).

Three-quarters of the land in Utah is not permanently inhabited by humans, because the landscape is too dry, too low in vegetation cover or it is owned by the federal government. Utah is the second driest state US (UACD-UDA-NRCS, 2005). However, these lands provide several ecological services such as habitat for plants and animals, water source for irrigation, minerals and fossil fuels, and numerous recreational activities related to the natural resources (West, 2009).

Agriculture in Utah is dominated by livestock production, animal products, and crops that provide feed to the livestock industry (Banner, 2009; Ward & Salisbury, 2016). Livestock grazing has been an important economic activity in Utah since colonization by Europeans (West, 2009; Forrest, 2016). Utah is the second producer of mink pelts in the U.S., third-largest in apricots and tart cherries, sixth in sheep and sweet cherries, seventh in onions, and ninth in pears and farm-raised trout. Barley production in Utah ranks eleventh and alfalfa hay production ranks thirteenth (UACD-UDA-NRCS, 2005).

The acreage utilized for production of grains and forages for livestock production has increased by almost 16% since 1940. Introduction of irrigation technology has contributed to this growth, but 82% of the irrigated land in Utah is considered to have low to marginal crop production potential (Banner, 2009), and is more suitable for forage production or grazing than for crop production.

Beef and dairy operations are abundant in the state, there are 830,000 head of cattle being raised by more than 8,000 ranchers (Utah Beef Council, 2018). Cropland irrigation is necessary for sustained plant growth, and 80% of the diverted water in Utah is for agricultural irrigation purposes (Allen, 2017). Dryland farming and grazing are also noted as major agricultural businesses. However, grazing on public land is decreasing due to reduction in the range quality, overgrazing; conflicts in public land use policies, lease availability and pricing (UACD-UDA-NRCS, 2005; Coppock et al., 2009).

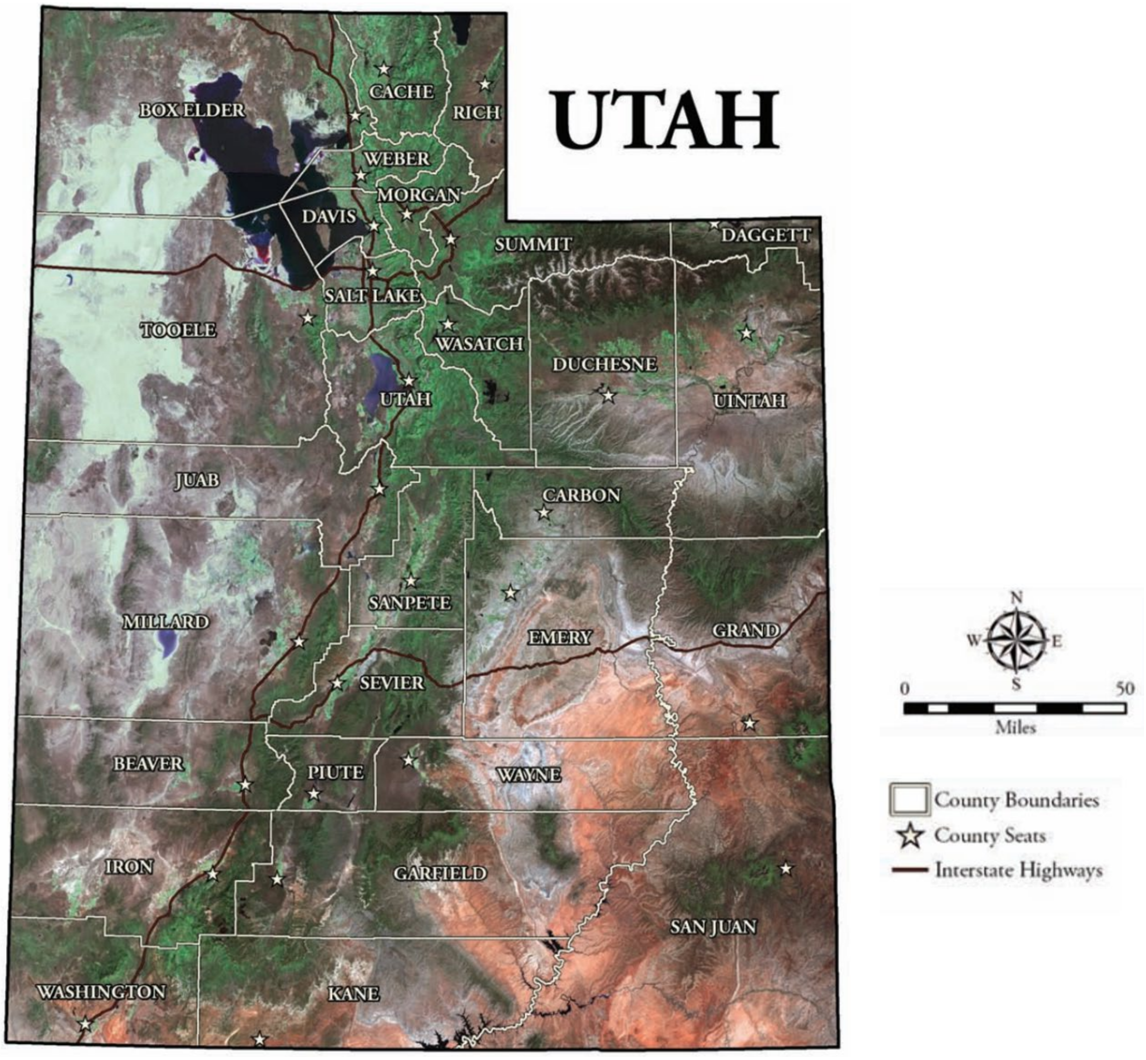

Figure 4. General geography of the state of Utah. Image provided by the remote sensing/GIS laboratory at Utah State University (Utah State University, 2009).

MATERIALS AND METHODS

The estimation of the agricultural areas in the state of Utah was performed by the development of a map with data from the MODIS sensor (Moderate Resolution Imaging Spectroradiometer), an instrument installed on two satellites: Terra (EOS AM-1) and Aqua (EOS PM-1). Terra orbits the earth from north to south across the equatorial line in the morning, while Aqua crosses the equatorial line from south to north in the afternoon (NASA, 2002). Both platforms, Terra and Aqua MODIS sensors acquire information of the Earth's surface every one to two days in 36 spectral bands. MODIS is a key tool sued to generate information related to the development of interactive Earth system models. Such information is important to predict global changes accurately enough to assist the decision-making process regarding the environment (NASA, 2002).

The MODIS sensor provides a high radiometric fidelity (12 bit) for each of the 36 spectral bands ranging in wavelength from 0.4 to 14.4 µm. Two bands collect reflectance values at a resolution of 250 m, five bands at 500 m and, the remaining 29 bands at 1 km (NASA, 2002).

The MODIS data product analyzed was MOD13Q1, because it provides vegetation index values for every pixel. The MOD13Q1 product consists of imagery from the Terra platform collected every day. Daily imagery is combined into 16-day intervals to generate cloud-free composites. For each 16-day interval, an algorithm selects the highest quality pixel from the images collected during that 16-day period. The quality criteria used consists of low cloud cover, low view angle and the highest NDVI value (Didan, 2015).

MODIS data are organized by a tile system that covers the Earth. The extent of the state of Utah intersects tiles H08V05, H09V05, and H09V04. To map irrigated agriculture across the state, I used data collected between day 97 (April 7, spring) and day 273 (September 30, end of the summer) from 2000 through 2018. The data were reduced to a yearly NDVI value by identifying the maximum value of NDVI for each of the 19 years.

Yearly NDVI, data were filtered by topographic conditions, to distinguish between cropland and forest or other vegetated areas in the mountains. The topography data was obtained from the Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER). ASTER is an imaging instrument aboard Terra, the flagship satellite of NASA's Earth Observing System (EOS) (NASA-JPL, 2012). ASTER captures high-resolution images of the Earth surface in 14 different wavelengths of the electromagnetic spectrum (from visible to thermal infrared light). Scientists use ASTER data to create detailed maps of land surface temperature, emissivity, reflectance, and along-orbit track stereo capability is used to generate elevation data (NASA, 2019). Additionally, a training data set was produced through selection of a representative sample of each land cover class, with a total of 1,050 sample sites distributed in across of the state. These training sites were classified by visual appreciation of the user of high-resolution imagery.

A supervised classification utilizing the Random forest decision tree algorithm (Breiman, 2001) and a training data set of 1,050 random points separated NDVI and topographic into four different land use/cover categories. The categories were: (i) Irrigated Agricultural lands, such as croplands or pastures, (ii) Forest areas, (iii) non-agricultural land like pastures in the mountains, urbanized areas, shrublands or vegetation without irrigation, and (iv) bare ground areas, lands with low or no vegetation cover.

In general, irrigated agriculture (crops and grazing pastures under irrigation) was characterized by high NDVI values across the 19 years and occurring in flat areas. Forest areas are represented by pixels with high NDVI, but occurring on higher elevation, steeper landscapes. Non-agriculture had variable NDVI and occurred in higher elevations or urban areas where NDVI was high and lowland flat areas where NDVI was low. These were considered non-irrigated pastures or shrublands. Bare ground areas consistently have the lowest NDVI values and occur in any topographic landscape. Area in hectares for each type was estimated by multiplying the area of one pixel by the total number of pixels assigned to a specific class and dividing the product by 10.000 (number of meters in one hectare).

$$Land\ Cover = [(Cell\ Size^2 \times Class\ (count)]/10{,}000$$

RESULTS

Land cover classification revealed that the state of Utah has 412,250 ha dedicated to agricultural activities under irrigation. Based on the current assessment, the majority of the hectares in the state of Utah are dedicated to non-agricultural activities (urbanized areas, shrublands, short vegetation areas with no irrigation) (Table 11, Figure 5). Accuracy of the map is 72.2% and the agreement index is 54.22%(Table 12).

Table 11. Pixel count and hectares estimated by remote sensing by each land cover use for the classification.

Land Cover	ha
Irrigated Ag	412,250
Forests	7,388,819
Non-Ag	11,481,350
Bare Ground	2,867,906

Table 12. Confusion matrix of the land cover classification for the state of Utah.

ClassValue	IrrigatedAg	Forests	Non-Ag	BareGround	Total	U_Accuracy
IrrigatedAg	52	0	8	0	60	86.66%
Forests	4	134	66	10	214	62.61%
Non-Ag	1	16	248	50	315	78.73%
BareGround	0	0	32	79	111	71.17%
Total	57	150	354	139	700	-
P_Accuracy	91.22%	89.33%	70.05%	56.83%	-	73.28%

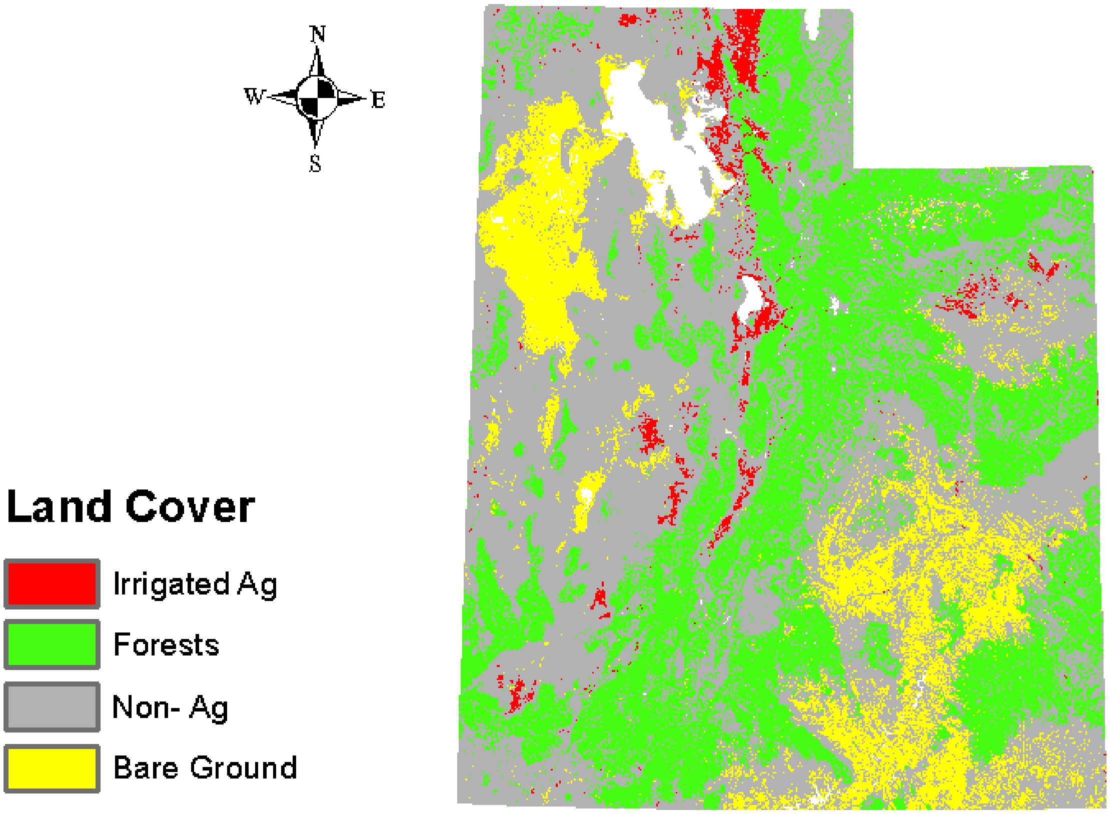

Figure 5. State of Utah, classified by land cover use.

DISCUSSION

The USDA-NASS reported 444,028.77 ha under irrigation in Utah in the agricultural census of 2017 performed through a survey (USDA-NASS, 2019), this estimate is similar to the value obtained in the present study using remote sensing. The difference between the values obtained in this study and those reported by USDA-NASS can be explained by several factors such as the size of the study area, size of the training data or sensor utilized in the present study. These factors have shown to affect the accuracy of the land cover classification (Table 12).

Geurts et al., 2006; Millard & Richardson, 2015; Ma et al., 2017; Tang et al., 2018). Moreover, a constraint in the classification methodology is the inability to know what is the spectral overlapping among certain classes (Bateman, 2017), and consequently areas with similar spectral sign can be misclassified. Thus, the main misclassification occurred when Forest and Bare Ground pixels were classified as Non-Ag, because of the wide variety of areas that were defined as non-agricultural (pastures in the mountains, urbanized areas, shrublands or vegetation without irrigation). Additionally, small agricultural areas under irrigation (<6.25 ha) were undetected by the classification because a pixel would be classified under a certain category depending of the dominating land cover type in the pixel (Bateman, 2017; Ma et al., 2017). Finally, it is important to consider that different measurement methodologies can produce different results and different error rates. Therefore, USDA-NASS agricultural census is self reported by agricultural owners or managers (USDA-NASS, 2019). Consequently, differences between the remote sensing results and the agricultural census are possible.

Figure 5 shows that agricultural areas under irrigation are concentrated in the northern part of the state, in Box Elder and Cache counties; and in the central region of the state in Millard and Sanpete counties. The USDA-NASS' census of agriculture registered these counties as the ones with the most irrigated agricultural areas in the state (USDA-NASS, 2019).

Overall accuracy of the MODIS generated map was assessed with the individual accuracies of each land cover type. The producer and user accuracies provide a method to evaluate the confusion between different land cover types (Janssen & van der Wel, 1994; Carfagna & Gallego, 2005; Brown et al., 2013). User's accuracy is the likelihood that a classified pixel actually represents that category on the ground. Meanwhile, producer's accuracy indicates the probability that a classified pixel in the same category, in fact belongs to that class (Story & Congalton, 1986; Brown et al., 2013). Carfagna & Gallego (2005) reported producer's accuracy values ranging from 40 to 80% and user's accuracy between 50 to 90% for crops such as wheat, barley, corn, sunflower, among others. Thus, the accuracy of the current classification is reasonable. Moreover, and as it was mentioned previously, the agricultural census reported values similar to those obtained through remote sensing in the present study.

Finally, the assessment of the accuracy assumes that the reference data (training data set) are totally correct and in many cases this is not completely true (François Mas et al., 2003), as the creation of the reference data set is through the examination of aerial imagery. Generation of the reference data was performed by assigning a land cover type to a sample pixel, but there were cases that assignation of the land cover type was not completely clear. For these reasons it is highly recommended to create a reference data set based on physical sampling. However, because of the size of the study area (the state of Utah), such practice was not feasible. Collectively, I suggest that the greatest error rate in this study was generated by the general nature and spectral overlap of the categories utilized in the classification.

The Utah Association of Conservation Districts (UACD), Utah Department of Agriculture (UDAF), and the Natural Resources Conservation Service (NRCS) reported a total of 979,803.83 ha dedicated to agricultural activities in the state of Utah (UACD-UDA-NRCS, 2005), this value is twice the area obtained in this remote sensing assessment. However, the UACD-UDA-NRCS report did not differentiate between irrigated or farmable areas with rangeland or shrublands used for some agricultural activities such as animal grazing. Thus, the irrigated agricultural areas have a big potential to increase if irrigation is implemented in those areas without irrigation.

CONCLUSIONS

The main goal of the current remote sensing assessment was to estimate the irrigated land in the state of Utah in order to gain knowledge about the potential areas that can sustain alternative grazing crops such as tannin-containing legumes. The final output showed the number of hectares and the regions with the greatest concentration of irrigated land in the state. These results were compared with statistical records from the USDA-NASS to corroborate the accuracy of the values obtained. This study shows that the state of Utah has 412,250 ha that could sustain tannin-containing legume crops (e.g. birdsfoot trefoil) for grazing during the finishing phase of beef production. The major regions of irrigated land were observed in the northern part of the state, at the Box elder and Cache counties; and at the central region of the state, particularly in Millard and Sanpete counties. This effort suggests that legume-based grazing systems are a viable option for producers in the state, particularly when considering the implementation of mixed crop-livestock farming systems where growers could diversify their operations by producing both hay and beef. Finally, feeding tannin-containing legumes to cows during the finishing phase is a viable alternative to feedlot rations, with greater levels of productivity than other pasture-based systems, which can lead to a more sustainable production of beef.

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CHAPTER IV

SUMMARY

Livestock production systems are being criticized for the negative environmental impacts of the greenhouse gases (e.g., methane and nitrous oxide) they generate during the process of raising and finishing animals, and thus alternatives to further reduce environmental footprints of beef cattle production represent a significant aspect of sustainable farming. This Thesis shows that the use of non-traditional forages such as tannin-containing legumes (e.g., birdsfoot trefoil; *Lotus corniculatus L.*) have the potential to optimize the utilization and cycling of nutrients inside the animal, contributing to a reduction in environmental impacts during the process of beef production.

An evaluation of (i) animal performance (DM intake and ADG), (ii) methane emissions, and (iii) concentration of nitrogen in urine and blood urea nitrogen was performed in beef cattle grazing three different pastures or a feedlot ration with high (50%) content of roughage (total mixed ration). The pastures used during grazing studies were: i) a tannin-containing legume diet (birdsfoot trefoil; BFT), ii) a non-tanniferous legume (cicer milkvetch; CMV), and iii) a typical grass diet (meadow brome; MB).

Cows grazing BFT showed greater weight gains than cows grazing CMV or MB, but similar to cows fed the TMR. Methane emissions per unit of intake from cows grazing BFT were similar to (when intake at pasture was assessed using the rising plate meter method), or lower than (when intake at pasture was assessed using the fecal output/digestibility method) emissions from animals consuming the TMR. This suggests a positive effect of condensed tannins or nutrients in BFT on methane abatement. Methane emissions were comparable among animals grazing CMV, MB or fed the TMR. Blood urea nitrogen concentrations were similar in cows grazing BFT or CMV, but greater than in animals grazing MB or consuming the TMR. Urinary nitrogen concentrations were similar among the diet treatments. These results suggest grazing BFT is a viable option to reduce environmental impacts by livestock production systems while maintaining levels of productivity, relative to traditional feeding systems such as feedlots, or increasing productivity relative to grass-feeding systems.

In order to explore the viability of legume-based feeding systems, a remote sensing estimation of the irrigated land in the state of Utah was also implemented in this Thesis. This effort was undertaken with the aim of informing future management and research projects about the amount of land in the state that can potentially be used to feed and finish cattle under legume-based grazing systems. The estimated amount of agricultural land under irrigation in the state was 412.250 ha, and the major regions of irrigated land were observed in the northern part of the state, such as Box Elder and Cache counties, and at the central region of the state in Millard and Sanpete counties. Thus, these findings integrated with the positive effects of birdsfoot trefoil on grazing cattle suggest that alternative legume-based grazing systems are a viable option for producers in the state, particularly when considering the implementation of mixed crop-livestock farming systems, where growers could diversify their operations by producing both hay and beef.

Future research should focus on alternative tannin-containing forages, in addition to birdsfoot trefoil, (e.g., sulla [*Hedysarum coronarioum* L.], crown vetch [*Coronilla varia* L.], small burnet [*Sanguisorba minor*]), and on forages that contain other secondary compounds (e.g., sesquiterpene lactones in chicory [*Cichorium intybus*]) with potential to reduce environmental impacts and enhance livestock productivity. In addition, the creation of a “tanniferous index” may be useful for classifying tannin-containing plants into categories that reflect their tannin concentration in the leaf tissues. This index may help producers select species that better fir their management and production goals. For instance, forages with higher concentration of condensed tannins might be of preference when concentrations of protein at pasture are high, such as in legume-based dairy production systems or in grazing systems where gastrointestinal parasitism is a concern. On the other hand, lower concentrations of tannins may be more advisable for systems where beef cows graze on forages with lower contents of crude protein.

Combinations of a diversity of forages in pasturelands may lead to benefits that are larger than when animals graze single species due to positive associative effects. Moreover, grazing diverse systems that match biomass production peaks among forage species of different phenologies with the dry matter requirement of the animals may contribute to enhanced intake on pasture and thus productivity through the year. Thus, future research efforts may need to focus on the influence of plant secondary compounds and forage diversity at attenuating environmental impacts while improving beef production.

Finally, research may need to concentrate efforts at improving biomass yield, regrowth, and persistence traits in non-traditional forages containing secondary compounds, increasing their competitiveness and adaptability, as it occurs with more traditional species (e.g., Alfalfa [*Medicago sativa*] or Ryegrass (*Lolium spp*]). These improved varieties would enhance the likelihood of adoption by producers interested in finishing animals at pasture. Thus, animal scientists need to work hand-by-hand with plant breeders such that several of these “non-traditional” forage species become more adapted to different ecoregions with concomitant enhancements in productivity and persistence, with optimal concentrations of nutrients and plant secondary compounds, which will ultimately “breed” more sustainable beef production systems.