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EFFECT OF TANNIN-CONTAINING LEGUME HAYS ON ENTERIC METHANE
EMISSIONS AND NITROGEN PARTITIONING IN BEEF CATTLE

by

Elizabeth K. Stewart

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

of

MASTER OF SCIENCE

in

Range Science

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2018

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ABSTRACT

Effect of Tannin-containing Legume Hays on Methane Emissions and Nitrogen
Partitioning in Beef Cattle

by

Elizabeth K. Stewart, Master of Science

Utah State University, 2018

Major Professor: Dr. Juan J. Villalba
Department: Wildland Resources

Beef consumption in the United States is expected to increase over the next decade putting increasing pressure on beef production systems to decrease their environmental impacts. The cow-calf phase counts for approximately 80% of the total beef production system greenhouse gas emissions and there is potential for tannin-containing hays to reduce these environmental impacts. The objective of my study was to determine if feeding tannin-containing hays to mother cows and heifers influences enteric methane and nitrogen emissions relative to feeding traditional legume and grass hays. Fifteen mature beef brood cows and nine yearling beef heifers were blocked by age and randomly assigned to three groups of five cows or three heifers and fed tannin-containing (birdsfoot trefoil-BFT, sainfoin-SAN, small burnet-SML), or non-tannin containing (alfalfa-ALF, cicer milkvetch-CMV, meadow bromegrass-MB) hays in four trials. Groups of cows were fed BFT, CMV or MB in Trial 1 and ALF, SAN or SML in Trial 3. Groups of heifers were fed ALF, MB or SAN in Trial 2 and BFT, CMV or SML in Trial

4. Each trial used a completely randomized block design with repeated measures during five consecutive days following a 14-d adjustment period. Nine cows and nine heifers were selected for total collection of enteric methane emissions, feces and urine while intake was measured for all animals. Methane emission from cows and heifers was lower for SML than for any other treatment, however digestibility was reduced for animals consuming this hay. Additionally, cows and heifers fed tannin-containing hays showed lower urine urea nitrogen and blood urea nitrogen levels than animals fed ALF or CMV. This resulted in a shift in nitrogen excretion from the urine to the feces. Furthermore, animals on either the BFT, SAN, or CMV treatments showed the greatest balances. These high efficiencies can be attributed to the presence of condensed tannins in the case of BFT and SAN and to the high rates of digestibility and structural carbohydrate digestion in CMV. These results suggest that tannin-containing legumes have the potential to reduce environmental impacts of beef cattle fed in confinement

(99 pages)

PUBLIC ABSTRACT

Effect of Tannin-containing Legume Hays on Methane Emissions and Nitrogen Partitioning in Beef Cattle

Elizabeth K. Stewart

Cattle are responsible for greenhouse gas emissions such as carbon dioxide, methane and nitrous oxide. In particular, the cow-calf phase of production accounts for approximately 80 percent of the total beef production system greenhouse gas emissions. Tannins are chemical compounds found in certain forages and they have the potential to help reduce these negative environmental impacts. Thus, given that the cow-calf phase often relies on feeding hay, feeding tannin-containing hays may represent a significant mitigation practice.

With my MS program, I sought to explore whether tannin-containing hays fed to mother cows and heifers influence methane and nitrogen emissions relative to feeding traditional legume and grass hays. I found that “non-traditional” hays such as cicer milkvetch and tannin-containing hays such as sainfoin, birdsfoot trefoil and small burnet can help mitigate greenhouse gas and nitrogen emissions produced from heifers and mature cows. Therefore, these hays could be used to feed cattle during the fall and winter to help create a more environmentally friendly cow-calf phase of beef production.

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Elizabeth Stewart

For my family

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INTRODUCTION

In the United States, the conventional beef production system consists of two to three phases. The first phase is cow-calf operations which consists of mature beef brood cows and their calves and lasts until the calves are weaned between six to nine months of age. The second phase of production is the stocker, or backgrounding, phase which is made up of weaned calves grazing pasture or fed stored forage and typically lasts 6 to 12 months (Gadberry et al., 2016). Not all calves, however, will participate in the stocker phase and may be sent directly to the third production phase after weaning. This phase is referred to as the feeder phase and lasts around four to six months, during which time the calves are fed a prepared diet that is high in concentrate to enhance growth and weight gain of the calves (Gadberry et al., 2016).

In the western United States, the cow-calf phase of beef production commonly takes place on rangelands where the diet of the animals consists mainly of medium to poor quality forage with little to no supplementation. Since there is not much supplemental or controlled feeding of cow-calf pairs, at least for parts of the year when there are suitable amounts of forage available on rangelands, there is little ability for producers to control for the quality of the diet that the grazers are consuming. This poses a problem because, since they produce copious quantities of greenhouse gases, beef cattle have a reputation for being bad for the environment and low-quality rangeland diets enhance the production of greenhouse gases such as methane (Van Soest, 1994), whereas if animals were to be fed higher quality forages, such as legumes, this effect could be

lessened due to an enhancement in the efficiency of forage utilization (Huang et al., 2011).

Despite being higher in lignin content, legumes are lower in neutral detergent fiber and can therefore be digested more quickly by ruminants than grass forages (Smith et al., 1972) which can lead to increased intake and therefore, increased production (Van Soest, 1994). Most legume species also have the ability to form symbiotic associations with soil bacteria (*Rhizobia* spp.) and fix their own nitrogen (de Faria et al., 1989). This ability means that the need for nitrogen fertilization for legumes is replaced by nitrogen fixation which, along with the fact that perennial species of legumes can survive and continue to be productive for multiple years, promotes lower expenses and greater profits for producers.

Sustainability of Beef Production

According to the USDA (2017b), there were 103 million cattle in the United States in July of 2017, an increase of approximately four percent since July of 2015. Previously, total numbers of cattle in the United States had shown a steady decline over the last 15 years (USDA, 2017b) which coincided with increasing beef prices (USDA, 2017c). Despite this recent trend, decreasing costs of production and increasing demand from international markets are expected to result in a rise in beef production in the United States at a rate of about one percent per year for the next 10 years (USDA, 2017a).

One of the challenges emerging from an increasing number of livestock animals involves the concomitant increase in environmental impacts. The production of beef cattle results in the production of three greenhouse gases; methane, nitrous oxide, and

carbon dioxide (Stackhouse-Lawson et al., 2012). Total emissions of carbon dioxide are usually higher than either methane or nitrous oxide, however, based on global warming potentials, the latter two gases present a bigger concern (Stackhouse-Lawson et al., 2012). The term global warming potential refers to how much energy a gas absorbs, and thus how much it can warm the earth, over the course of 100 years. To standardize the global warming potentials of the different greenhouse gases, an equivalence index was developed and is expressed as carbon dioxide equivalent units (CO₂ Eq) (IPCC, 2001a). Carbon dioxide is used as the reference gas for global warming potential, so it has a value of 1 kg of CO₂ Eq per kg of carbon dioxide whereas, methane has a value of 25 kg of CO₂ Eq per kg of methane and nitrous oxide has a value of 298 kg of CO₂ Eq per kg of nitrous oxide (IPCC, 2007).

The U. S. EPA (2017a) estimates that 522.3 Tg CO₂ Eq, or 7.9 percent, of the total anthropogenic greenhouse gas emissions in the United States in 2015 were the result of agricultural activities and of this, beef cattle contributed 118.1 Tg CO₂ Eq from enteric methane emissions and 7.7 Tg CO₂ Eq from nitrous oxide from manure management. Due to manure and nitrogen fertilization, areas that have been cultivated for the production of crops and forages represent the largest sources of nitrous oxide emissions and contributed 251.3 Tg CO₂ Eq, in 2015 (U. S. EPA, 2017a). Therefore, beef cattle also contribute to nitrous oxide emissions through the consumption of cultivated forages and grains. Furthermore, approximately 80 percent of the greenhouse gas emissions that result from beef production are the result of the cow-calf phase and this includes emissions from cattle themselves as well as emissions from the production of feed, and manufactured inputs such as fertilizer and herbicides (Beauchemin et al., 2010).

Nitrogen

Once ingested, protein either escapes the rumen intact and makes its way to the small intestine or it is broken down in the rumen by microbes with most of it being transformed into ammonia (Satter and Roffler, 1975). There are a couple of different fates for this ammonia including utilization by rumen microbes as a source of nitrogen or, if the amount of ammonia in the rumen exceeds the capacity for uptake by microbes, it can be absorbed through the rumen wall (Owens and Bergen, 1983). Once absorbed, the ammonia enters the hepatic portal vein and from there it is transported to the liver where it is converted to urea and a portion of this urea is recycled by the animal, while the majority of the remaining urea is excreted in the urine (Satter and Roffler, 1975). Additionally, if there is not a sufficient supply of fermentable carbohydrates in the rumen, dietary protein that is ingested by cattle can be utilized by the microbes in the rumen for the production of energy (Satter and Roffler, 1975). In this scenario, the growth of the rumen microbial population is much slower and the amount of ammonia that can be utilized is less than if there were synchronous sources of both protein and fermentable energy available to the microbes (Satter and Roffler, 1975).

The mode of excretion of ingested nitrogen (i.e. urine vs. feces) impacts how much nitrous oxide and ammonia enter the environment. Urea that has been excreted with the urine can rapidly be transformed into ammonia through interactions with fecal or soil urease (Whitehead, 1995). Under aerobic conditions, the ammonia then undergoes nitrification, where it is converted to nitrite and, subsequently, to nitrate with nitrous oxide being produced when the ammonia is oxidized to nitrite (Bremmer, 1997). Anaerobic denitrification is also a source of nitrous oxide where it is produced as an

intermediate in the reduction of nitrate or nitrite (Bremmer, 1997). Excreted urea from livestock along with high inputs of nitrogen fertilizer have led to pastures and croplands as the primary sources of nitrous oxide emissions (Brown et al., 2001; Stackhouse-Lawson et al., 2012). The nitrification and denitrification processes are both components of the normal nitrogen cycle in the soil that takes place after the deposition of nitrogen onto the soil via fertilization or urination and defecation by animals. However, these processes are also the primary pathways through which nitrous oxide is formed (Henault et al., 2012). Volatilization and subsequent deposition of ammonia can also indirectly result in the formation of nitrous oxide (IPCC, 2001b).

In contrast to urea in urine, organically bound nitrogen is the main form of nitrogen that is present in the feces (de Klein and Eckard, 2008). Since the mineralization of organic nitrogen to ammonium is slower than the hydrolysis of urea to ammonia, the nitrogen in feces is broken down at a slower rate than urea from the urine, so it is not immediately available to soil microbes for nitrification and denitrification (de Klein and Eckard, 2008).

In addition to being excreted in the urine and feces, some of the nitrogen is retained and recycled by cattle (Lapierre and Lobley, 2001). After being transformed into urea in the liver, some of the urea is excreted in the urine and the rest is recycled back into the rumen through the saliva or directly from the blood across the epithelium of the rumen (Reynolds and Kristensen, 2008). Once inside the rumen, microbial urease degrades the urea to ammonia which can then be utilized by the microbes for microbial protein synthesis (Reynolds and Kristensen, 2008). This recycling is especially important when the nitrogen in the diet is insufficient to meet the protein requirements of the

microbes in the rumen and can also enable cattle to remain productive on diets that contain nitrogen only present in non-protein nitrogen forms (Virtanen, 1966). Therefore, ways to increase nitrogen recycling and the transfer of recycled nitrogen into the rumen are important processes for ruminants. According to a review by Reynolds and Kristensen (2008), increasing carbohydrate fermentation increases urease activity in the rumen, thus enhancing the ability of urea to be transferred into the rumen by diffusion. Additionally, higher ruminal ammonia concentrations correspond to lower rates of transfer of ammonia into the rumen (Kennedy and Milligan, 1980).

The amounts of nitrous oxide and ammonia resulting from production can also depend heavily on the type and management of the operation (Monteny et al., 2006; Dijkstra et al., 2013). Feedlot diets typically have a high rumen degradable protein content with little of the protein actually being utilized by the animals (Gay, 2009) resulting in high nitrogen losses. In contrast, and when inputs from the entire production system (including fertilizer applications) are included in the life cycle assessment, these emissions are less when cattle are allowed to graze pasture instead of consuming a high concentrate diet during the finishing phase of beef production (Stackhouse-Lawson et al., 2012). Furthermore, when cattle are allowed to graze pastures containing legumes the nitrogen fertilizer is replaced by biologically fixed nitrogen (Stackhouse-Lawson et al., 2012).

High levels ammonia can have many negative impacts on the environment including subsequent nitrous oxide production (de Klein and Eckard, 2008), lowering soil pH, and eutrophication of water if nitrate produced from ammonia leaches through the soil and enters the water tables (Kebreab et al., 2010). The cow-calf phase is responsible

for 39 to 48 percent of the ammonia resulting from beef production (Stackhouse-Lawson et al., 2012). Furthermore, based on carbon dioxide equivalents, approximately 27 percent of the total greenhouse gas emissions resulting from beef production are due to nitrous oxide that comes from manure and soil (Beauchemin et al., 2010).

Methane

The production of methane is a normal part of ruminant digestion. Methanogenic archaea bacteria, or methanogens, are capable of producing methane as a byproduct during the fermentation process in the rumen and hindgut of ruminants (Hook et al., 2010). Hydrogen is produced during normal ruminal fermentation and methanogens use this element as a source of energy (Janssen, 2010), resulting in the formation of methane which plays the role of a sink for hydrogen ions (Beauchemin et al., 2008) by using carbon as an electron acceptor. Most of the methane is then evacuated from the body via the mouth and nostrils (Murray et al. 1976; Woodward et al., 2004). According to Anderson et al. (1987), the process of the production and expulsion of methane begins when the calf is about four weeks old which is also around the time when the reticulorumen begins to hold solids.

Methane production in the rumen is an important process because removing the hydrogen ions helps keep the partial pressure of hydrogen in the rumen low which, in turn, promotes hydrogenase activity and decreases lactate and alcohol production (Wolin et al., 1997). Furthermore, removing excess hydrogen allows ruminal microorganisms to oxidize substrates more completely during fermentation leading to greater production of energy (Stewart and Bryant, 1988; Raskin et al., 1997; Janssen, 2010).

Methane production in the rumen is promoted by diets that induce the production of the volatile fatty acids acetate and butyrate (i.e. from high fiber diets) (Wilkinson, 2012). Thus, high-fiber (i.e. neutral detergent fiber) diets promote high levels of enteric methane emissions (Van Soest, 1994; Jayanegara et al., 2009). Conversely, diets that increase the production of propionate, such as high-starch concentrate feedlot rations, typically reduce the amount of methane that is produced during enteric fermentation because methane and propionate are in competition with one another for the available hydrogen present in the rumen environment (Bhatta et al., 2009). Decreasing the particle size of the feed by pelleting and grinding can also decrease methane production (Blaxter, 1989) due to the increased passage rate of the feed (Johnson and Johnson, 1995). Generally, factors that increase the amount of feed intake will decrease the amount of methane produced per unit of dry matter intake (Puchala et al., 2005).

Cattle can produce roughly 250 – 500 L of methane per animal per day and there are many factors that play a part in determining the amount that any individual cow will produce, including amount of feed intake, dietary additives such as ionophores, and composition of the microflora in the rumen (Johnson and Johnson, 1995). This production of methane is an energy-costly process and results in gross energy losses of three to six percent (Johnson and Johnson, 1995; Blaxter and Clapperton, 1965) because methane emissions represent a source of hydrocarbons that have not been burned to yield their stored energy to the consumer (Blaxter and Clapperton, 1965). Therefore, reducing the amount of methane produced by 25 percent could result in an increase in gains of growing calves by 75 g/d (Nkrumah et al., 2006) and increased production by lactating

dairy cows of 1 L/d (Bruinenberg et al., 2002). Thus, a reduction in methane production involves an increase in feed efficiency (Huang et al., 2011).

Domestic ruminant species contribute approximately 86 Tg of methane each year to the atmosphere and of this, beef cattle are responsible for 62.8 percent of such amount (McMichael et al., 2007). Enteric fermentation from ruminants accounts for around 35 percent of the total methane emissions that result from anthropogenic activities and, again, beef cattle are responsible for the majority of this (U.S. EPA, 2017a). Furthermore, 63 percent of all greenhouse gas emissions resulting from beef cattle production consists of enteric methane and the cow-calf phase of beef production represents over 80 percent of the methane produced by beef cattle in terms of carbon dioxide equivalents (Beauchemin et al., 2010).

Condensed Tannins

All plants consist of both primary and secondary compounds. Primary compounds are those that are directly involved with growth, development and reproduction of the plant, whereas, plant secondary compounds are not involved with these processes (Crozier et al., 2006). Plant secondary compounds have a variety of functions including discouraging herbivory (Waghorn, 2008), protection against harmful bacteria, promotion of symbiosis with beneficial microbes, and transportation of metals within the plant (Demain and Fang, 2000). Out of all plant secondary compounds existing in nature, condensed tannins are of particular interest because they have been shown to exhibit properties that are beneficial to ruminant animals. Some of these properties include antimicrobial activity (Scalbert, 1991), reductions of internal parasite burdens, as well as

reductions to the risk of bloat by binding to proteins in the feed (Waghorn, 1990; Min et al., 2003).

Condensed tannins may also be able to reduce the negative impacts that cattle production has on the environment. It has been shown that condensed tannins have the potential to reduce the amounts of enteric methane as well as the amounts of urinary nitrogen produced by ruminants (Carulla et al., 2005; Maamouri et al., 2011; Tan et al., 2011; Williams et al., 2011a; Aguerre et al., 2015). The exact mechanism by which condensed tannins reduce methane production is unknown, however some different mechanisms have been proposed. According to Jones et al. (1994), condensed tannins can bind with the cell coat polymers of some microbes in the rumen. Additionally, Tan et al. (2011) demonstrated that condensed tannins reduced the total numbers of methanogens in the rumen as well as protozoa which certain species of methanogens depend on for survival (Van Soest, 1994).

Condensed tannins also can bind with proteins through both covalent and hydrogen bonding to form complexes that are insoluble in the range of pH of 3.5 to 7.0 (Bunglavan and Dutta, 2013), which falls within the span of pH values found in the ruminal fluid. Thus, these tannin-protein complexes can protect the protein from degradation by microbes in the rumen (Perez-Maldonado and Norton, 1996). However, these complexes may disassociate once the pH drops below 3.5 in the abomasum (Getachaw et al., 2000; Barry et al., 2001) or in the duodenum (Perez-Maldonado et al., 1995) allowing the animal to digest and absorb the protein released from the complexes.

Amino acid absorption from dietary protein by ruminants is typically low because most of the protein from plants is immediately utilized by rumen microbes resulting in

the formation of ammonia and microbial protein (MacRae and Ulyatt, 1974; Beever and Siddons, 1986). Amino acids, however, are needed to convert the potentially toxic ammonia to urea in the liver (Lobley et al., 1995). In order for ruminants to gain access to amino acids from plant protein, the protein must escape degradation in the rumen so that it can be broken down and absorbed by the animals in the small intestine. By protecting plant protein from degradation in the rumen, tannin-protein complexes may allow the animals to utilize the protein provided to them in their diets more efficiently while reducing the amount of nitrogen that is excreted as a waste product of metabolism (Maamouri et al., 2011). Additionally, it has been shown that feces from animals that have consumed condensed tannins will decompose more slowly than feces from animals that have not consumed condensed tannins (Niezen et al., 2002) leading to a reduction in the amount of ammonia and nitrous oxide that volatilize from the feces.

Alternative Forage Species for Beef Production

According to MacAdam and Villalba (2015), grass species provide the soil with more organic matter, greater protection from erosion and also have a higher tolerance to grazing than legumes. In addition, the protein present in legumes is often poorly utilized by ruminants because harvest and storage conditions can cause much of the protein to be transformed into non-protein nitrogen compounds (NRC, 1989). The resulting non-protein nitrogen can usually be utilized by rumen microbes, however, if there are insufficient amounts of synchronous sources of digestible energy, then the ability of the microbes to use the non-protein nitrogen will be hindered (Broderick and Albrecht, 1997). In addition, extensive ruminal degradation to protein by microbes may release

excessive amounts of ammonia and a substantial proportion is eventually excreted into the environment in the urine (NRC, 1989).

There are many benefits, however, from feeding legumes to ruminants. Legumes typically contain less fiber than grass species at a comparable stage of maturity (Wen et al., 2002), improving the extent of forage digestion and thus leading to greater levels of intake (Van Soest, 1965). Additionally, methane production is typically lower for ruminants consuming legumes, as well as younger plants, when compared to more mature – and lower quality – forages (Benchaar et al., 2001; Waghorn et al., 2006). Most legume species are also able to form root nodules and fix their own nitrogen (de Faria et al., 1989) so they do not require nitrogen fertilization like grasses do in order to be productive. This is important because it saves the producer the additional cost of fertilizer and it lessens the potential for negative environmental impacts. On average, approximately 1.0 to 1.25 percent of applied nitrogen fertilizer ends up in the atmosphere in the form of nitrous oxide (Bouwman, 1995). Furthermore, plants can take up excess nitrogen from fertilization which leads to higher nitrogen in the diets of the consumers and higher nitrogen losses through urine and, subsequently, increased nitrous oxide emissions into the atmosphere (Monaghan et al., 2004).

Incorporating “unconventional” forages into ruminant diets, especially those that contain plant secondary compounds such as condensed tannins, have the potential to benefit animals as well as the environment. One such forage species is birdsfoot trefoil (*Lotus corniculatus* L.), a bloat free legume (Jones and Lyttleton, 1971) which typically contains 0.5 to 4.7 percent of dry matter as condensed tannins (John and Lancashire, 1981; Barry and McNabb 1999; Grabber, 2009). The condensed tannins present in this

species have been shown to increase in concentration as the plant matures (Guttek et al., 1974) and the type of condensed tannins contained in birdsfoot trefoil consists primarily of procyanidins (Mueller-Harvey, 2006).

Birdsfoot trefoil is well suited for growing under irrigation in the Intermountain West region of the United States (Williams et al., 2011a) as it can persist in areas that are cool and have alkaline soils (MacAdam and Griggs, 2006). Birdsfoot trefoil can also be tolerant of acidic soils (Waghorn and McNabb, 2003). Additionally, birdsfoot trefoil can tolerate more frequent defoliation than alfalfa (Smith and Nelson, 1967), however it does not store carbohydrates during the growing season, so care must be taken to ensure that there is sufficient photosynthetic tissue left for regrowth (Nelson and Smith, 1968).

The condensed tannins in birdsfoot trefoil are typically present at a low enough concentration that they do not constrain forage intake (Ramirez-Restrepo et al., 2006) or decrease absorption of amino acids in the small intestine (Waghorn et al., 1987). Hymes-Fecht et al. (2013), found that the condensed tannins in birdsfoot trefoil actually increased the amount of essential amino acids that were available to the animals. Birdsfoot trefoil also has a high feeding value and, from previous analysis, it is suggested that the type of condensed tannins in this forage can increase animal performance (Waghorn and McNabb, 2003).

Sainfoin (*Onobrychis viciifolia* Scop.) is another “unconventional” condensed tannin-containing legume species that has the potential for beef production. Similar to birdsfoot trefoil, sainfoin is well adapted to the Intermountain West region of the United States and the Shoshone variety was developed in this area (Gray et al., 2006). Sainfoin also performs best in alkaline soils (MacAdam and Villalba, 2015). Sainfoin typically has

a condensed tannin concentration of five to eight percent of dry matter (John and Lancashire, 1981; Scharenberg et al., 2007) consisting mainly of prodelphinidins (Mueller-Harvey, 2006). Despite the high concentrations of condensed tannins in sainfoin, it has been shown to be very palatable (Reid et al., 1974; Scharenberg et al., 2007) which may be due to the high molecular weight of its condensed tannins (Jones et al., 1976).

Similar to birdsfoot trefoil, sainfoin has a high nutritive value (Dahlberg et al., 1988) and is a non-bloating legume (Jones and Lyttleton, 1971). From a consumer standpoint, sainfoin is beneficial because it has been shown – like birdsfoot trefoil – to increase backfat thickness and marbling scores, increase concentrations of unsaturated fatty acids in the beef, as well as produce beef that is redder in color and of higher consumer preference scores than beef harvested from cattle finished on alfalfa, grass or feedlot diets (Maughan, et al., 2014; Chail et al., 2016). Additionally, Catanese et al. (2014) found that sainfoin increased total nutrients that were ingested by sheep and also reduced the anti-quality effects of the alkaloids present in tall fescue that are responsible for tall fescue toxicosis.

Cicer milkvetch (*Astragalus cicer* L.) is also a well-suited legume for the Intermountain West region of the United States; the Monarch (Townsend 1980) and Windsor (Townsend 1994) varieties were developed in this region. Cicer milkvetch is a non-bloating legume that does not contain condensed tannins (Lees et al., 1982). It is not digested as quickly as other legumes and is bloat free because of its internal leaf structure, which slows microbial access to cell contents because the epidermis remains attached to the lateral veins of the forage (Lees et al., 1982). Temperate legumes, such as

cicer milkvetch, have lower fiber content than grasses and Dahlberg et al. (1988) found cicer milkvetch to have a high nutritive value and digestibility compared to alfalfa.

Small burnet (*Sanguisorba minor* Scop.) is a tannin-containing forb (Barry and McNabb, 1999), that does well over cold winters and on soils that are well-drained (USDA NRCS, 2017), making it well suited for establishment in the Intermountain West region of the United States. Additionally, small burnet is very tolerant to drought but does not tolerate flooding (USDA NRCS, 2017). It is also important for wildlife and range livestock because it will remain green until it is covered with snow in the winter (USDA NRCS, 2017). Small burnet can contain up to 5 percent hydrolysable tannins on a dry matter basis (J. W. MacAdam, Utah State University, Logan, Utah, personal communication).

Rationale and Significance

The effects of tannin-containing forages on animal performance and environmental impacts have typically been studied under grazing conditions using fresh plants (Lassey et al., 1997; Woodward et al., 2004; Grainger et al., 2009; Maamouri et al., 2011) but there is much less information regarding the impacts of tannin-containing hays on production or environmental variables. This is likely a consequence of the assumption that tannins are labile and highly reactive molecules which are inactivated in response to the environmental conditions of the haying process. For instance, there is evidence showing that drying conditions inactivate the biological actions of condensed tannins on herbivore consumers (e.g., D'Mello and Taplin, 1978; Makkar and Singh, 1991). Nevertheless, more recent research suggests that conserved tanniferous forages

(i.e., sainfoin hay) have significant bioactive properties against gastrointestinal nematodes, similar to those observed in the fresh forage, suggesting that the biological properties of tannins remain in the hay despite the conditions experienced during the drying process (Heckendon et al., 2006). Thus, there is potential to fill this gap in knowledge and explore the benefits of “unconventional” tannin-containing hays on the nutrition and environmental impacts of livestock fed in confinement. This is particularly relevant when considering that approximately 80 percent of greenhouse gas emissions from beef production are the result of the cow-calf phase (Beauchemin et al., 2010), a period that may rely on hay feeding for a significant portion of the production cycle.

Hypothesis and Objectives

The objective of this study was to determine if feeding tannin-containing hays to mother cows and heifers influences enteric methane and nitrogen emissions relative to feeding traditional legume and grass hays. I hypothesized that because of their quality and presence of bioactive plant secondary compounds, tannin-containing legume hays would reduce the environmental impacts from beef cattle relative to more traditional grass and legume hays. To test this hypothesis, I conducted a series of trials with mature beef brood cows and heifers where I fed three tannin-containing hays (sainfoin, birdsfoot trefoil and small burnet), two legume hays without tannins (alfalfa and cicer milkvetch) and one grass hay (meadow bromegrass) to different groups of animals. I then determined intake, forage digestibility, enteric methane emissions, and nitrogen excretion from the urine and feces in response to the consumptions of the aforementioned hays.

Expected Benefits

Completion of this research will enable us to establish a more sustainable beef production system using tannin-containing legumes instead of more traditional non-tannin containing legumes or grasses for the cow-calf phase of production. Ruminants are able to digest legumes more quickly than grasses (Smith et al., 1972) so, in addition to reduced environmental impacts, this switch will allow for increased intake and production. Additionally, legumes can fix their own nitrogen (de Faria et al., 1989), so they do not need nitrogen fertilization, thus reducing nitrous oxide production. Cow-calf production in the Intermountain West primarily utilizes a system where animals consume low quality forage under grazing conditions or in confinement, so switching to high quality, perennial tannin-containing legumes will be an important option for more sustainable production in this area.

MATERIALS AND METHODS

Experimental Design and Layout

This study was conducted at the Utah State University Animal Science Farm, located in Wellsville, UT, according to procedures approved by the Utah State University Institutional Animal Care and Use Committee (Approval # 2542).

Fifteen mature beef cows with mean body weight of 676 ± 16 kg and nine yearling beef heifers with mean body weight of 464 ± 19 kg were fed three different varieties of hay during each of two consecutive trials (three hays per trial) for each age class. Hays included birdsfoot trefoil (BFT; *Lotus corniculatus* L., cv. Langille), sainfoin (SAN; *Onobrychis viciifolia* Scop., cv. Shoshone), small burnet (SML; *Sanguisorba minor* Scop., cv. Delar), alfalfa (ALF; *Medicago sativa* L., cv. Roundup Ready), cicer milkvetch (CMV; *Astragalus cicer* L., cv. Monarch), and meadow bromegrass (MB; *Bromus riparius* Rehmman, cv. Cache). Of these, BFT and SAN are tannin-containing legumes (McAllister et al., 1994; Wang et al., 2015), SML is a tannin-containing forb (Barry and McNabb, 1999), ALF and CMV are non-tannin containing legumes (MacAdam and Villalba, 2015), and MB is a grass (See Table 1 for nutritional analysis). The selection of the forages used in this study was based on their positive characteristics of adaptation, establishment and persistence relative to the environmental conditions under irrigation of the Intermountain West (MacAdam et al., 1997).

Five acres each of BFT, SAN, SML, ALF, CMV and MB were seeded in August of 2015 at the Utah State University Cache Junction Farm, UT (41° 51' N, 112° 0' W). Forages were cut, field-dried and then harvested in early June of 2016 after stands were

well-established. The 1-ton bales were then transported to the Utah State University South Farm and stored under cover.

Each trial consisted of a 14-d adaptation period to the assigned hay followed by two days for adaptation to individual pens and then a five-day sample collection period. During the adaptation periods, animals were grouped by treatment and fed their respective hays in group pens. After this adjustment period, they were randomly assigned to individual adjacent pens (2.54m x 2.36m) located inside a covered barn. Three animals per hay were fitted with halters and then tied to the corner of their stalls. They were given enough space to be able to reach their feed bins and water buckets but not enough space to be able to turn around completely. Animals were allowed an additional two-day adjustment period once in individual pens to allow for familiarization to the new environment and conditions of the study. Each animal was given *ad libitum* access to hay, water and a trace mineral salt block (mineral composition: minimum 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).

The barn where the animals were housed during collection periods was covered with an open roof, and concrete walls which provided protection from the sun and some protection from wind and rain. The barn was comprised of 12 stalls on the east side and 12 stalls on the west side of a central alleyway.

Cow Study

Trial 1

For Trial 1, 15 mature beef cows were randomly assigned to receive either BFT, CMV or MB hays (five animals/hay treatment). These groups of five cows were randomly assigned to one of three group pens and offered *ad libitum* amounts of their respective hays for 14 consecutive days. After this period, animals were moved to their individual pens for a total of seven days; two days for adaptation to the pens and experimental conditions of the study, followed by five days of sample collection. During the collection period, three of the five cows in each treatment group were used for determinations of dry matter intake, total collection of feces and urine, as well as for determinations of enteric methane production, nitrogen partitioning, and hay digestibility. The remaining two cows in each group were used only for dry matter intake estimates.

Trial 2

For Trial 2, the same 15 mature beef cows were re-randomized and assigned to either ALF, SAN or SML hay treatments (five animals/hay treatment). Each treatment group was randomly assigned to one of three group pens and offered *ad libitum* amounts of their respective hays for 14 consecutive days. After this period, they were moved to individual pens for a total of seven days and the protocol was as described for Trial 1.

Heifer Study

Trial 3

Nine heifers grazed pastures of ALF, SAN and MB at the Utah State University Pasture Facility located in Lewiston, Utah (41°56′ N, 111°52′ W), before arriving at the Utah State University Animal Science Farm. Animals were kept on the same treatments (three animals/forage) but were switched from fresh forage to hay. They were fed their respective hays in group pens for 14 days as an adaptation period. After adaptation, heifers were moved to their individual pens and the protocol was the same as described for Trial 1, except that there were no extra animals for dry matter intake measurements.

Trial 4

For Trial 4, the same nine yearling heifers were re-randomized and assigned to either BFT, CMV or SML hay so that each of the treatment groups contained three heifers. Each treatment group was randomly assigned to a group pen for 14 d to allow for adaptation to the different hays. After adaptation, heifers were moved to their individual pens and the protocol was as described for Trial 1 except that there were no extra animals for dry matter intake measurements.

Determinations

Dry matter intake was recorded for all animals used in this study. The remaining determinations were performed for three animals per treatment group.

Intake

Animals were fed once per day at 0500 h and the hay remained available to them for a full 24 h, until it was removed and replaced with new hay at 0500 h the following morning. The amount fed was weighed and recorded, and subsequent refusals were weighed, recorded, subsampled and discarded each morning. On the first day of adaptation to the individual pens, all animals were fed in amounts that represented 2.5 percent of their body weight. This amount was subsequently adjusted such that the amounts refused on a daily basis were at least 10 percent of the amount offered. Refusals were evaluated for contamination and samples were collected for both the offered hay and refused hay for each animal daily. Samples were then stored at -20°C until determination of nutritional quality.

Fecal Sampling

To minimize the risk of loss of nitrogen through volatilization, feces were collected from the cows and heifers every eight hours, at 0500, 1300 and 2100 h for five consecutive days. At these times, a flat shovel and/or a squeegee (depending on the consistency of the feces) was used to remove all of the feces from the individual pens and placed into individual buckets. The weight was recorded for determination of the total amount of feces produced in 24-h cycles. Subsamples of approximately 200 g were taken three times daily after weighing and immediately frozen at -20°C to be stored until analysis.

Urine Sampling

Urine was continuously collected for the duration of the five-day collection period using catheters. Indwelling urinary catheters were inserted on the day prior to the beginning of the collection period, and urine collected between the time of insertion and 2100 h was discarded. Catheters were removed the day following the last day of the collection period. During the collection period, the urine was continuously collected into large plastic containers via Tygo tubing connected to the catheter. Urine from each animal was weighed every eight hours for five consecutive days for determination of the total amount of urine produced in a 24-h cycle. Subsamples of approximately 30 mL were taken three times daily after weighing and immediately frozen at -20°C to avoid volatilization of nitrogen and the rest of the urine was discarded. For the 0500 h collection during the first day, one liter from each animal was weighed separately to determine the kg/L of the urine. All subsequent collections for the remainder of the five days were weighed and the kg/L determined on the first day was used as a conversion factor to convert the weights (kg) to volume (L).

Enteric Methane Emissions

Enteric methane emissions of hay-fed animals were measured using the sulfur hexafluoride tracer gas technique, which allows for the direct measurement of individual animals with minimal restriction (Johnson et al., 1994, 2007). The amounts of methane produced daily by each animal was determined by sampling air adjacent to the nostrils of cows and heifers that had each had a slow release permeation tube deposited into their rumens before the data collection periods. The permeation tubes emitted 1.01 ± 0.038 mg

of the sulfur hexafluoride marker per day, into the rumen. Each cow and heifer was fitted with a halter on the first day of the collection periods. An evacuated canister was then connected to the halter for the collection of exhaled air. Canisters were closed and changed every 24 h for the five-day collection period. Additional samples of air were taken adjacent to the animals (i.e. sample of barn air) to measure background atmospheric concentrations of methane which were used to correct values obtained from the animals (Williams et al., 2011b). Each day, the closed canisters were transported back to the laboratory where the pressure inside each canister was measured and nitrogen was added to pressurize each of them. A subsample was then obtained from each pressurized canister and placed into an evacuated vial for temporary storage.

Weight

All animals were weighed when they were brought into the barn two days prior to the start of the collection period (not fasted). They were weighed again the day after the final day of collection (not fasted). An average of the two weights was taken to determine animal body weight (kg).

Blood

On the day following the last day of collection, a blood sample was collected from every animal from the medial coccygeal vein in the tail using 10 mL serum tubes (Becton Dickinson Vacutainer System, Rutherford, NJ). The blood was allowed to clot, and serum was separated by centrifugation ($2,300 \times g$ for 25 min at 15°C), extracted from the tubes using a disposable pipette, placed into 2 mL tubes and then frozen at -20°C until urea analysis.

Chemical Analysis

Fecal and Feed Samples

After the collection period, all fecal samples for one cow or heifer were removed from the freezer and placed into a cooler to thaw slowly to reduce the amount of nitrogen lost through volatilization (Hatch et al., 1990). Once thawed, a subsample from each sample was taken and mixed together to create a composite sample for the entire five days per individual. This composite sample was thoroughly mixed and a portion of it was placed into a plastic container, weighed, and then frozen to later be freeze dried. The rest of the composite sample was weighed and then placed in a forced air oven at 60°C. This sample was stirred and weighed twice daily until it had been dried to a constant weight. This weight was recorded, and the sample was ground through a Wiley Mill to pass through a 1 mm screen. This procedure was repeated for the feces collected from each animal in the study. Additionally, offered and refused feed samples were thawed, weighed and ground through a Wiley Mill to pass through a 1 mm screen. The samples of offered and refused feed for each animal were then split in half using a Riffle Splitter so that half could be freeze dried and the other half dried in a forced air oven at 60° C. As with the feces, the feed samples were also stirred and weighed twice daily until they had been dried to a constant weight.

All oven dried fecal and feed samples were dried at 100°C for 24 h to determine their dry matter content (AOAC, 1990; Method 967.03). The samples were then placed into a furnace at 600°C for two hours for the determination of ash and organic matter content (AOAC, 1990; method 942.05). All freeze-dried samples were analyzed for

neutral detergent fiber (Van Soest et al., 1991), acid detergent fiber (AOAC, 1990; method 973.18), total nitrogen (AOAC, 1990; method 990.03), and total non-structural carbohydrates which consisted of ethanol soluble carbohydrates (Dubois et al., 1956), and starch (Hall, 2009). Data were used to calculate the apparent in vivo digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber, and crude protein from daily dry matter intakes and fecal excretions. Finally, the offered feed samples were analyzed for their condensed tannin content (Grabber et al., 2013).

Enteric Methane Emissions

Gas samples from expired air and control canisters were taken for analysis of methane and sulfur hexafluoride, by gas chromatography (Chavez et al., 2006). Enteric methane was analyzed as total number of liters produced per day, liters of methane per kg of dry matter intake, and liters of methane produced per kg of body weight.

Urine and Blood

After each collection period, all subsamples were removed from the freezer and placed into a cooler to thaw slowly to reduce the amount of nitrogen lost through volatilization (Hatch et al., 1990). Once thawed, 96% sulfuric acid was used to acidify the urine until the pH was less than 3.0. Then, all subsamples for one cow or heifer were mixed to create a composite sample of 100 mL for the entire five-day collection period per individual, which was then frozen to -20°C until analysis. This procedure was repeated for the urine collected from each animal used in the study. Urine composite samples and serum samples were analyzed for urea nitrogen concentration (Dimension Xpand Plus, Siemens Healthcare Diagnostics Inc., Newark, DE) and urine samples were

also analyzed for total nitrogen content (FP-528 Protein/Nitrogen Determinator, Leco Corporation, Saint Joseph, MI). Nitrogen retained by the animals was calculated by subtracting nitrogen in feces and urine from the nitrogen intake. A percentage of the nitrogen retained that was consumed was also calculated to determine the nitrogen balance.

Calculations

The concentration of urinary nitrogen was multiplied by the total daily urine output (L/d) to determine the grams of nitrogen excreted per day. The percentage of nitrogen that was excreted as urea was calculated by dividing the grams of urinary urea nitrogen by the grams of total urinary nitrogen. The proportion of total nitrogen excretion (total nitrogen excreted, %), as well as the proportion of nitrogen excreted with the urine (nitrogen partitioned to urine, %) or feces (nitrogen partitioned to feces, %) relative to the amount of nitrogen consumed daily with each hay were also calculated as:

Proportion of total nitrogen excretion

$$= \frac{\text{Nitrogen excreted in the urine (g/d)} + \text{nitrogen excreted in the feces (g/d)}}{\text{Nitrogen Intake (g/d)}} \times 100$$

Proportion of nitrogen excreted in the urine

$$= \frac{\text{Nitrogen excreted in the urine (g/d)}}{\text{Nitrogen Intake (g/d)}} \times 100$$

Proportion of nitrogen excreted in the feces

$$= \frac{\text{Nitrogen excreted in the feces (g/d)}}{\text{Nitrogen Intake (g/d)}} \times 100$$

The digestibilities of dry matter, organic matter, crude protein, neutral detergent fiber, and acid detergent fiber were calculated using Givens et al (2000) as:

Coefficient of digestibility

$$= \frac{\text{Nutrient consumed (g/d)} - \text{nutrient in feces (g/d)}}{\text{Nutrient consumed (g/d)}} \times 100$$

The nitrogen retained by the animals was calculated as the difference between nitrogen intake and the sum of fecal and urinary nitrogen losses (Maynard and Loosli, 1969; Owens and Bergen, 1983). A percentage of the consumed nitrogen that was retained (nitrogen balance, %) was also calculated to determine nitrogen utilization efficiency.

Statistical Analysis

Results for each study (cow, heifer) were analyzed as an incomplete block design in which three treatments were randomly assigned to each animal in two successive trials. Trial was a blocking factor and tests showed that variation attributed to trial was trivial. For each treatment, the animals were repeatedly measured for five days. All analyses were computed using a mixed-effects model (PROC MIXED; SAS Inst., Inc. Cary, NC; SAS/STAT 14.1 SAS Institute Inc.). In the model, treatment and days (with their interaction) were fixed factors, and animal and trial were random factors. The covariance structure that best fit the data was selected according to the Schwartz's Bayesian criterion (Littell et al., 1998). The model diagnostics included testing for normal distribution of the error residuals and homogeneity of variance. Differences among the means were analyzed using pairwise differences of least squares means. Linear regressions were also carried out to explore the relationship between fecal excretion of condensed tannins and

the fecal expression of nitrogen. Probability values were considered significant at $p \leq 0.10$ and a trend at $p \leq 0.20$.

RESULTS

Tannin Content and Nutritional Analysis of the Hays

The chemical compositions of the different hays used in this study are reported in Table 1. Dry matter content was similar for all hays, with slightly greater and lower dry matter contents for MB and SML, respectively. Meadow brome grass, SAN and SML showed greater values of organic matter content, whereas ALF and CMV had the greatest values of crude protein concentration. In contrast, SML and MB showed the lowest values of crude protein content. Small burnet had the lowest concentrations of neutral detergent fiber and acid detergent fiber followed by CMV, ALF, BFT and SAN, respectively. In contrast, and as expected, MB showed the highest concentration of fiber of all the hays tested. Total nonstructural carbohydrate contents were greater for SML and BFT, intermediate for MB and SAN, and lower for CMV and ALF. The concentration of condensed tannins was greater in the SAN hay ($3.0 \pm 0.35\%$) than in the BFT hay ($0.75 \pm 0.064\%$). Small burnet did not contain condensed tannins and the concentration of hydrolysable tannins in freeze-dried leaves was 5% of dry matter (J. W. MacAdam, Utah State University, Logan, Utah, personal communication; Table 1).

Table 1. Chemical composition of the hays used in the study.

| Item | ¹ Treatment | | | | | | | | | | | |
|--------------------------------------|------------------------|------------------|------|------------------|------|------------------|------|------------------|------|------------------|------|------------------|
| | ALF | ² SEM | BFT | ² SEM | CMV | ² SEM | MB | ² SEM | SAN | ² SEM | SML | ² SEM |
| Dry matter, % | 91.2 | | 90.2 | | 90.7 | | 92.6 | | 90.8 | | 89.0 | |
| Organic matter, % | 89.0 | | 89.9 | | 88.1 | | 92.5 | | 91.5 | | 91.2 | |
| Crude protein, % | 18.7 | .15 | 14.1 | .10 | 19.7 | .10 | 8.1 | .10 | 13.7 | .00 | 11.7 | .050 |
| Neutral detergent fiber, % | 38.0 | .10 | 40.3 | .20 | 32.3 | .35 | 64.3 | .10 | 42.3 | .15 | 27.9 | .25 |
| Acid detergent fiber, % | 30.6 | .050 | 31.5 | .25 | 28.3 | .15 | 41.6 | .10 | 35.7 | .35 | 24.3 | .30 |
| Total nonstructural carbohydrates, % | 7.1 | .35 | 10.2 | .050 | 7.3 | .30 | 8.5 | .15 | 9.2 | .10 | 13.6 | .00 |
| Ethanol soluble carbohydrates, % | 6.4 | .30 | 9.3 | .10 | 6.8 | .25 | 8.1 | .15 | 7.9 | .050 | 11.2 | .050 |
| Starch, % | 0.65 | .050 | 0.95 | .050 | 0.55 | .050 | 0.40 | .00 | 1.35 | .050 | 2.45 | .050 |

¹Treatment: birdsfoot trefoil (BFT), cicer milkvetch (CMV), meadow bromegrass (MB), alfalfa (ALF), sainfoin (SAN), and small burnet (SML).

²SEM: Standard error of the mean.

Cow Study

Intake and Digestibility

Dry matter intake tended to be lower for cows fed MB than for all other treatments except for SAN, and cows fed ALF tended to show greater dry matter intake than cows fed SAN ($P = 0.1669$; Table 2). Pairwise comparisons for dry matter digestibility revealed that cows consuming CMV showed greater dry matter digestibility values than cows consuming ALF ($P = 0.0798$), SAN ($P = 0.0337$), or SML ($P = 0.0226$). Cows consuming BFT also had greater digestibility values for dry matter than cows consuming SAN ($P = 0.0624$) or SML ($P = 0.0421$) and tended to show greater values than those observed for cows consuming ALF ($P = 0.1345$; Table 2). In contrast, the animals fed MB produced digestibilities for organic matter that were greater than all other treatments except for SML (Table 2), and those fed SML tended to show greater organic matter digestibilities than those fed BFT ($P = 0.1274$).

Table 2. Daily dry matter intake, digestibility of hay constituents, enteric methane emissions, and excretion of condensed tannins in the feces for beef cows in the cow study.

| COW STUDY | | | | | | | | |
|--|---------------------|---------------------|---------------------|----------------------|--------------------|---------------------|-------------------------|----------------|
| ¹ Treatment | | | | | | | | |
| Item | ALF | BFT | CMV | MB | SAN | SML | ² SEM | P-value |
| Dry matter intake (g/kg body weight) | 15.81 | 14.65 | 14.23 | 10.77 | 12.42 | 14.07 | 1.2598 | 0.1669 |
| Digestibility of hay constituents | | | | | | | | |
| Dry matter, % | 58.43 ^{ab} | 66.71 ^{bc} | 68.10 ^c | 62.76 ^{abc} | 57.32 ^a | 56.60 ^a | 3.3162 | 0.1013 |
| Organic matter, % | 58.84 ^a | 60.54 ^{ac} | 57.85 ^a | 71.11 ^b | 55.84 ^a | 67.43 ^{bc} | 2.9584 | 0.0219 |
| Crude protein, % | 73.44 ^a | 76.46 ^a | 79.44 ^a | 61.59 ^b | 63.01 ^b | 30.22 ^c | 4.2343 | <0.0001 |
| Neutral detergent fiber, % | 29.12 ^a | 48.08 ^{bc} | 36.08 ^{ab} | 56.79 ^c | 21.06 ^a | 25.98 ^a | 6.3887 | 0.0132 |
| Acid detergent fiber, % | 29.66 ^{ac} | 39.31 ^{ac} | 44.06 ^{ab} | 55.77 ^b | 20.90 ^c | 31.83 ^{ac} | 6.1137 | 0.0153 |
| Enteric methane Emissions | | | | | | | | |
| Enteric methane, L/d (L/d) | 433.15 | 461.35 | 397.49 | 388.28 | 434.85 | 312.31 | 66.7655 | 0.5045 |
| Enteric methane, L/kg of dry matter intake | 41.58 ^{ab} | 50.24 ^a | 47.60 ^a | 56.58 ^a | 54.90 ^a | 31.91 ^b | 6.3040 | 0.0548 |
| Enteric methane, L/kg body weight | 0.7032 | 0.7297 | 0.6359 | 0.5795 | 0.6963 | 0.4527 | 0.09005 | 0.1574 |
| Excretion of condensed tannins in the feces | | | | | | | | |
| Condensed tannins excreted in feces, % | ³ ND | 8.95 ^a | ³ ND | ³ ND | 14.46 ^b | ³ ND | 1.5867 | 0.0372 |
| Condensed tannins excreted in feces, g/d | ³ ND | 27.87 ^a | ³ ND | ³ ND | 47.69 ^b | ³ ND | 5.7763 | 0.0787 |

^{a-c}Means in the same row with different superscripts differ ($P \leq 0.10$)

¹Treatment: birdsfoot trefoil (BFT), cicer milkvetch (CMV), meadow brome (MB), alfalfa (ALF), sainfoin (SAN), and small burnet (SML).

²SEM: Standard error of the mean

³ND: Values were not determined for these treatments.

Crude protein digestibility was greater for cows consuming CMV, BFT, and ALF, intermediate for cows consuming SAN and MB, and lower for cows consuming SML (Table 2). Animals fed MB had neutral detergent fiber digestibility values that were greater for all other treatments except for animals fed BFT (Table 2). Cows fed CMV also tended to show greater digestibility values for neutral detergent fiber than cows fed SAN ($P = 0.1126$). Values for the digestibility of acid detergent fiber were similar with the MB treatment producing greater values than all other treatments, except for CMV (Table 2). Finally, feeding CMV tended to produce greater acid detergent fiber digestibility values than the SML ($P = 1507$) or ALF ($P = 0.1330$) treatments.

Enteric Methane Emissions

Enteric methane emissions by cows fed the different hays are shown in Table 2. There were no differences between treatments for total daily methane emissions ($P > 0.2$). However, when methane emissions were expressed as liters of methane produced per kg of dry matter intake, the SML treatment produced lower values than all other treatments except ALF (Table 2). In addition, cows fed SML tended to emit the lowest amounts of methane per kg of body weight, except for cows fed BFT ($P = 0.1574$; Table 2).

Excretion of Condensed Tannins through the Feces

Condensed tannins excreted in the feces as a percentage of fecal dry matter output was greater for cows consuming SAN hay than for cows consuming BFT hay (Table 2). This same pattern held true when condensed tannin elimination was expressed as grams of condensed tannins excreted per day ($P = 0.0787$; Table 2).

For cows ingesting condensed tannin-containing hays, a positive trend was observed for the relationship between the fecal excretion of condensed tannins and the fecal excretion of nitrogen. As the daily amounts of fecal condensed tannins increased, grams of nitrogen excreted daily in feces also tended to increase ($P = 0.1383$). Likewise, the concentration of nitrogen in the feces (expressed as a percentage of dry matter) tended to increase with increments in the fecal concentration of condensed tannins in the feces ($P = 0.1849$).

Excretion of Nitrogen in Urine and Feces and Blood Urea Nitrogen

Total daily urine output was the greatest for cows consuming CMV (Table 3). The total daily excretion of nitrogen in the urine was $ALF > CMV > SAN > BFT = MB > SML$ ($P < 0.0001$). Similarly, when expressed as grams of nitrogen excreted per liter of urine output, cows fed SML produced the lowest values, while cows fed ALF and SAN produced the greatest values ($P < 0.0001$). In contrast, the amount of nitrogen excreted in the feces was greatest for cows on the SML treatment ($P = 0.0013$; Table 3). Cows consuming SML also showed the lowest concentration of urinary urea nitrogen ($P = 0.0003$) and blood urea nitrogen ($P < 0.0001$), whereas animals fed CMV or ALF showed the greatest values for these parameters (Table 3). Likewise, when looking at the percentage of urinary nitrogen that came from urea, SML-fed cows produced lower values than all other treatments, except for SAN, and CMV-fed cows produced the greatest values for this parameter ($P = 0.0062$; Fig. 1).

Table 3. Excretion of nitrogen in urine and feces, nitrogen balance, and blood urea nitrogen for the cow study.

| COW STUDY | | | | | | | | |
|---------------------------------------|---------------------|---------------------|---------------------|--------------------|---------------------|---------------------|------------------------|----------------|
| ¹Treatment | | | | | | | | |
| Item | ALF | BFT | CMV | MB | SAN | SML | ²SEM | P-value |
| Urine Output, L/d | 13.22 ^a | 13.28 ^a | 18.89 ^b | 11.96 ^a | 10.44 ^a | 10.66 ^a | 1.9419 | 0.0743 |
| Total nitrogen in urine, g/d | 180.08 ^a | 58.38 ^b | 112.36 ^c | 56.46 ^b | 90.85 ^d | 29.40 ^e | 8.9169 | <0.0001 |
| Total nitrogen in urine, g/L of urine | 13.79 ^a | 4.71 ^b | 6.51 ^b | 4.89 ^b | 8.88 ^c | 2.34 ^d | 0.8701 | <0.0001 |
| Total nitrogen in feces, g/d | 90.67 ^a | 52.67 ^{bc} | 70.57 ^{ab} | 42.15 ^c | 75.31 ^a | 152.55 ^d | 11.2428 | 0.0013 |
| Urine urea nitrogen, g/d | 216.89 ^a | 77.18 ^b | 219.21 ^a | 84.34 ^b | 96.96 ^b | 24.15 ^c | 26.9422 | 0.0003 |
| Blood urea nitrogen, mg/dL | 15.2 ^a | 7.8 ^b | 16.0 ^a | 8.2 ^b | 11.5 ^c | 2.8 ^d | 1.2550 | <0.0001 |
| Nitrogen from urea, % | 55.36 ^{ac} | 61.61 ^{ac} | 89.86 ^b | 69.24 ^a | 49.72 ^{cd} | 37.14 ^d | 7.5440 | 0.0062 |
| Nitrogen partitioned to feces, % | 26.56 ^a | 21.51 ^a | 20.56 ^a | 38.41 ^b | 36.99 ^b | 75.34 ^c | 3.9445 | 0.0001 |
| Nitrogen partitioned to urine, % | 56.25 ^a | 23.99 ^b | 32.84 ^c | 52.68 ^a | 44.64 ^d | 14.88 ^e | 2.1165 | <0.0001 |
| Total nitrogen excreted, g/d | 283.09 ^a | 111.05 ^b | 182.93 ^c | 98.61 ^b | 166.17 ^c | 181.95 ^c | 14.4129 | 0.0002 |
| Total nitrogen excreted, % | 82.81 ^{ac} | 45.51 ^b | 53.40 ^b | 91.09 ^c | 81.62 ^a | 90.22 ^{ac} | 3.4775 | <0.0001 |
| Nitrogen retention, g/d | 58.53 ^a | 132.53 ^b | 158.51 ^c | 9.70 ^d | 37.65 ^e | 18.85 ^d | 6.1046 | <0.0001 |
| Nitrogen Balance, % | 17.19 ^{ac} | 54.49 ^b | 46.60 ^b | 8.91 ^c | 18.38 ^a | 9.78 ^{ac} | 3.4775 | <0.0001 |

^{a-e}Means in the same row with different superscripts differ (P ≤ 0.10)

¹Treatment: alfalfa (ALF), birdsfoot trefoil (BFT), cicer milkvetch (CMV), meadow bromegrass (MB), sainfoin (SAN), and small burnet (SML)

²SEM: Standard error of the mean.

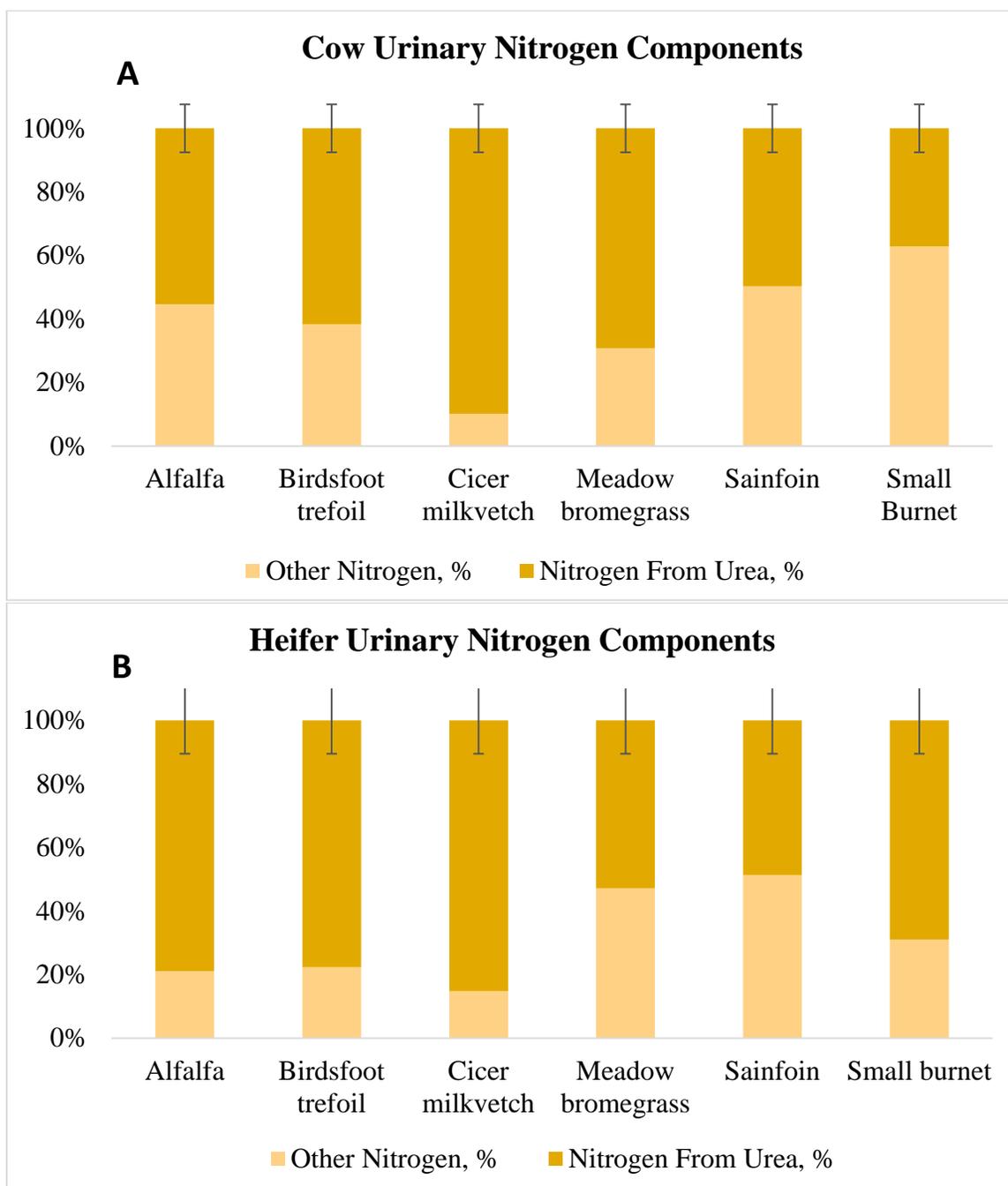
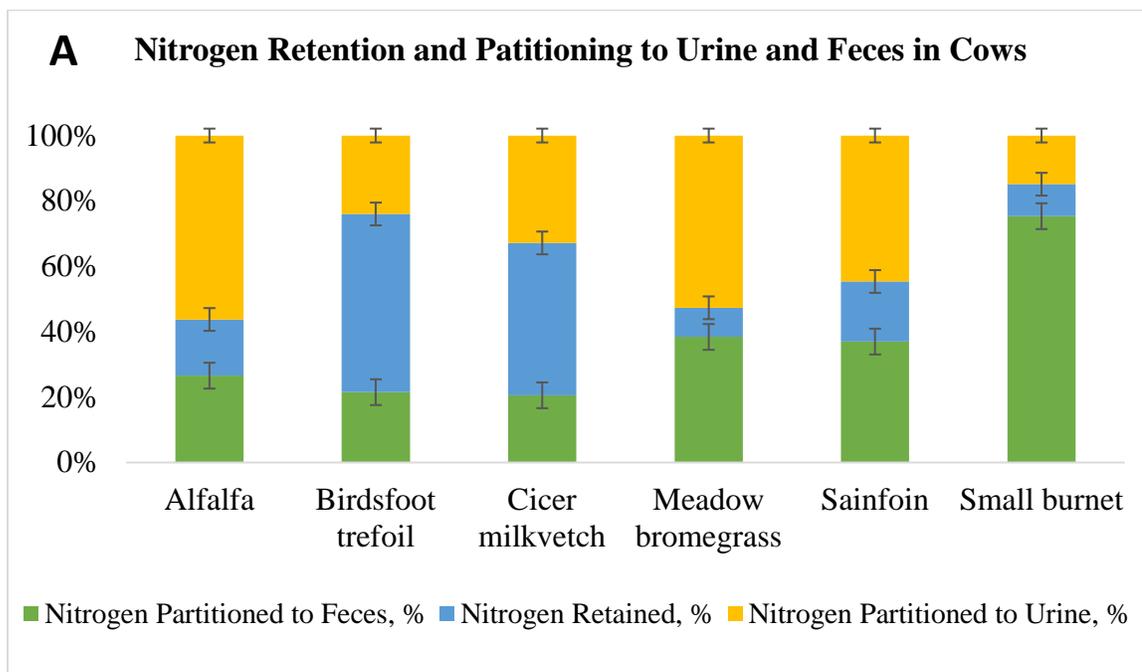


Figure 1. Percentage of urine that is composed of urea for cows and heifers. Cows consuming tannin-containing hays showed lower percentages of urea in total urinary nitrogen than cows consuming cicer milkvetch which is a non-tannin containing hay. Similarly, heifers fed sainfoin, which contains a high level of condensed tannins, showed a lower percentage of urinary nitrogen as urea than heifers fed cicer milkvetch. **A.** Cow study. **B.** Heifer Study.

Figure 2 shows the partitioning of nitrogen to the urine and feces, as well as the nitrogen that was retained by cows in each treatment. The percentage of nitrogen that was partitioned to the feces relative to the nitrogen consumed was the greatest for cows consuming SML, intermediate for cows consuming MB or SAN, and the lowest for cows consuming ALF, BFT, or CMV ($P = 0.0001$). In contrast, cows fed ALF or MB partitioned more nitrogen to the urine than cows fed tannin-containing hays or CMV ($P < 0.0001$; Fig. 2; Table 3).



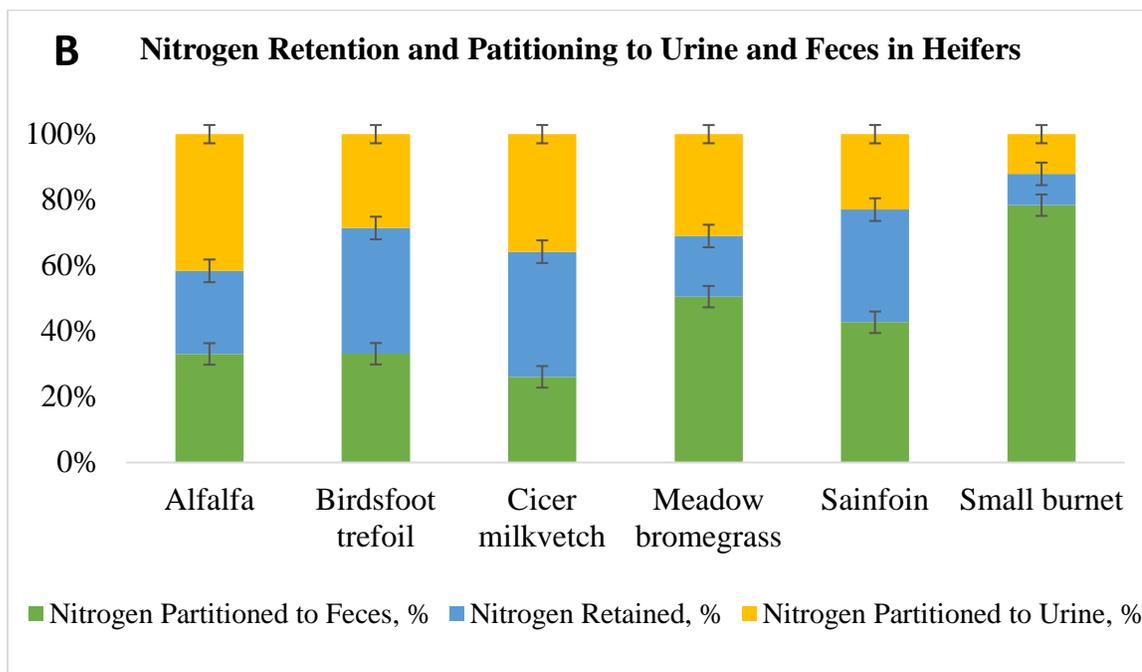


Figure 2. Nitrogen retention and partitioning of nitrogen to urine and feces for cows and heifers. Cows consuming alfalfa or meadow bromegrass partitioned a higher percentage of consumed nitrogen to the urine than cows consuming tannin-containing hays. Cows fed birdsfoot trefoil retained the most nitrogen and cows fed small burnet partitioned the most nitrogen to the feces. Heifers consuming alfalfa partitioned more nitrogen to the urine than heifers consuming tannin-containing hays. Heifers fed alfalfa or meadow bromegrass retained less nitrogen than heifers fed birdsfoot trefoil or sainfoin and those fed small burnet partitioned the greatest percentage of nitrogen to the feces. **A.** Cow study. **B.** Heifer study.

When looking at the total amount of nitrogen excreted per day, the MB and BFT treatments resulted in the lowest values observed among the hays assayed, whereas ALF hay produced the greatest values ($P = 0.0002$). However, BFT- and CMV- fed cows excreted the lowest percentage of the nitrogen consumed, which led to greater nitrogen retention values (Table 3). The total daily nitrogen retention was $CMV > BFT > ALF > SAN > SML = MB$ (Table 3). Similarly, cows that consumed BFT or CMV showed greater nitrogen balances than all other treatments ($P < 0.0001$).

Heifer Study

Intake and Digestibility

The intake of dry matter was greater for ALF than for all other hays, except for BFT, and dry matter intake was lower for SML than for all other treatments, except for MB ($P = 0.0052$; Table 4). The digestibility of dry matter was greater for heifers consuming CMV than for all other hays, except for BFT ($P = 0.0084$), and heifers consuming ALF tended to show greater dry matter digestibility values than animals offered SML ($P = 0.1156$). The digestibility of organic matter was lowest for heifers fed ALF and this treatment was not different from the SAN treatment for this parameter (Table 4).

Table 4. Daily dry matter intake, digestibility of hay constituents, enteric methane emissions, and excretion of condensed tannins in the feces for beef heifers in the heifer study.

^{a-d}Means in the same row with different superscripts differ ($P \leq 0.10$)

| HEIFER STUDY | | | | | | | | |
|--|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|------------------------|----------------|
| ¹ Treatment | | | | | | | | |
| Item | ALF | BFT | CMV | MB | SAN | SML | ²SEM | P-Value |
| Dry matter intake (g/kg body weight) | 22.53 ^a | 21.22 ^{ab} | 18.22 ^{bc} | 16.22 ^{cd} | 18.26 ^{bc} | 15.30 ^d | 1.2553 | 0.0052 |
| Digestibility of hay constituents | | | | | | | | |
| Dry matter, % | 56.30 ^{ad} | 64.03 ^{bc} | 67.11 ^b | 54.08 ^{ad} | 57.23 ^{ac} | 50.23 ^d | 2.6553 | 0.0084 |
| Organic matter, % | 53.02 ^a | 67.19 ^b | 59.83 ^{bc} | 63.23 ^{bc} | 58.14 ^{ac} | 63.91 ^b | 3.8174 | 0.0504 |
| Crude protein, % | 73.44 ^{ab} | 76.46 ^b | 79.44 ^b | 61.59 ^c | 63.01 ^{ac} | 30.22 ^d | 4.2343 | <0.0001 |
| Neutral detergent fiber, % | 28.12 ^a | 42.90 ^b | 45.28 ^b | 45.67 ^b | 28.86 ^a | 22.67 ^a | 5.1829 | 0.0259 |
| Acid detergent fiber, % | 27.73 ^a | 42.29 ^b | 49.99 ^b | 42.69 ^b | 28.88 ^a | 27.85 ^a | 5.2294 | 0.0386 |
| Enteric methane emissions | | | | | | | | |
| Enteric methane, L/d | 386.11 ^a | 382.99 ^a | 340.47 ^a | 368.08 ^a | 334.39 ^a | 269.27 ^b | 33.1954 | 0.0543 |
| Enteric methane, L/kg dry matter intake | 52.07 ^a | 40.59 ^a | 42.25 ^a | 55.11 ^b | 40.30 ^a | 39.96 ^a | 6.7996 | 0.0890 |
| Enteric methane, L/kg body weight | 0.78 | 0.89 | 0.68 | 0.80 | 0.73 | 0.62 | 0.1044 | 0.3978 |
| Excretion of condensed tannins in the feces | | | | | | | | |
| Condensed tannins excreted in feces, % | ³ ND | 12.29 ^a | ³ ND | ³ ND | 19.39 ^b | ³ ND | 2.1766 | <0.0001 |
| Condensed tannins excreted in feces, g/d | ³ ND | 43.09 | ³ ND | ³ ND | 70.60 | ³ ND | 11.9418 | 0.1529 |

¹Treatment: birdsfoot trefoil (BFT), cicer milkvetch (CMV), meadow bromegrass (MB), alfalfa (ALF), sainfoin (SAN), and small burnet (SML).

²SEM: Standard error of the mean.

Crude protein digestibility was greater for heifers that consumed CMV, BFT, or ALF and lower for heifers that consumed SML ($P < 0.0001$). Similarly, heifers offered BFT, CMV, or MB showed greater crude protein digestibilities than heifers offered SAN, ALF, or SML (Table 4). This pattern was similar for acid detergent fiber digestibility, with heifers consuming CMV, MB, or BFT showing greater digestibility values than heifers fed SAN, SML or ALF ($P = 0.0386$; Table 4).

Enteric Methane Emissions

Enteric methane emissions by heifers fed the different hays are shown in Table 4. Heifers that were offered SML produced less methane emissions (L/d) than heifers offered any of the other hays ($P = 0.0543$). When methane was expressed as liters of methane produced per kg of dry matter intake, animals fed MB produced the greatest values of this greenhouse gas ($P = 0.0890$). In contrast, there were no differences in methane emissions among the hays when analyzed as liters of methane produced per kg of body weight.

Excretion of Condensed Tannins through the Feces

Condensed tannins excreted in the feces as a percentage of fecal dry matter output was greater for heifers consuming SAN hay than for heifers consuming BFT hay ($P < 0.0001$; Table 4). Similarly, when values were expressed as grams of condensed tannins excreted in the feces per day, heifers consuming SAN tended to show greater values than heifers consuming BFT ($P = 0.1529$). Consistent with the amounts of condensed tannins excreted in feces, heifers fed SAN showed greater amounts of nitrogen partitioned to the feces than heifers fed BFT (0.0669).

For heifers consuming condensed tannin containing hays, a positive relationship was observed between the fecal excretion of condensed tannins and the fecal excretion of nitrogen. The daily excretion of condensed tannins with feces was positively correlated with the daily excretion of fecal nitrogen ($P = 0.0258$). Likewise, nitrogen excreted as a percentage of fecal dry matter, nitrogen also increased with the increasing percentage of condensed tannins in the feces ($P = 0.089$).

Excretion of Nitrogen in Urine and Feces and Blood Urea Nitrogen

Total daily urinary output was greater for heifers fed CMV than all other treatments, aside from BFT, and heifers fed SAN or MB produced the lowest amounts of urine ($P = 0.0001$; Table 5). The total amount of daily urinary nitrogen excretion was $ALF > CMV > BFT > SAN > MB > SML$ ($P < 0.0001$). Similarly, when elimination values were expressed as grams of nitrogen excreted per liter of urine output, heifers that were offered SML produced the lowest values while heifers offered ALF produced the greatest values (Table 5). Additionally, animals fed CMV ($P = 1223$) or MB ($P = 0.1059$) tended to show lower values for this parameter than animals fed SAN. Also, heifers fed SML or ALF excreted the most nitrogen through the feces whereas heifers fed MB excreted the lowest amount of nitrogen through the feces ($P < 0.0001$; Table 5). Heifers that consumed SML or MB excreted the lowest amounts of urinary urea nitrogen, whereas heifers that consumed SAN or BFT showed intermediate values, and heifers that consumed CMV or ALF excreted the greatest amounts of urinary urea nitrogen ($P < 0.0001$). Additionally, animals fed SML tended to show lower values for this parameter than animals fed MB ($P = 0.1128$). Concentrations of blood urea nitrogen among heifers

fed the different hays showed a similar pattern with $SML > MB > BFT = SAN > CMV = ALF$ ($P < 0.0001$; Table 5). When looking at the percentage of urinary nitrogen that came from urea, SAN-fed heifers tended to show lower values than CMV-fed heifers (Table 5; Fig. 1).

Table 5. Excretion of nitrogen in urine and feces, nitrogen balance, and blood urea nitrogen for the heifer study.

| HEIFERS | | | | | | | | |
|---------------------------------------|---------------------|---------------------|---------------------|--------------------|---------------------|---------------------|------------------------|----------------|
| Item | ALF | BFT | CMV | MB | SAN | SML | ²SEM | P-Value |
| Urine output, L/d | 14.81 ^{ad} | 17.72 ^{ab} | 20.82 ^b | 6.44 ^c | 5.70 ^c | 11.28 ^d | 1.7094 | 0.0001 |
| Total nitrogen in urine, g/d | 138.81 ^a | 66.07 ^b | 103.16 ^c | 29.36 ^d | 47.77 ^e | 17.09 ^f | 7.4784 | <0.0001 |
| Total nitrogen in urine, g/L of urine | 10.01 ^a | 3.93 ^b | 4.90 ^{bc} | 5.09 ^{bc} | 8.34 ^{ac} | 1.57 ^d | 1.4060 | 0.0007 |
| Total nitrogen in feces, g/d | 108.73 ^a | 80.32 ^b | 74.53 ^b | 46.13 ^c | 92.56 ^d | 102.48 ^a | 4.2607 | <0.0001 |
| Urine urea nitrogen, g/d | 224.96 ^a | 116.46 ^b | 177.81 ^a | 33.20 ^c | 46.51 ^D | 25.82 ^c | 16.3406 | <0.0001 |
| Blood urea nitrogen, mg/dL | 22.85 ^a | 14.15 ^b | 19.52 ^a | 9.48 ^c | 14.18 ^b | 2.82 ^d | 1.3796 | <0.0001 |
| Nitrogen from urea, % | 78.91 | 77.68 | 85.20 | 52.81 | 48.64 | 68.99 | 10.4800 | 0.1470 |
| Nitrogen partitioned to feces, % | 33.00 ^a | 33.21 ^a | 26.01 ^b | 50.70 ^c | 42.53 ^d | 78.77 ^e | 3.2720 | <0.0001 |
| Nitrogen partitioned to urine, % | 41.54 ^a | 28.64 ^{bc} | 35.69 ^{ac} | 31.13 ^c | 22.88 ^b | 12.16 ^d | 2.7793 | 0.0001 |
| Total nitrogen excreted, g/d | 248.62 ^a | 145.60 ^b | 177.84 ^c | 75.82 ^d | 138.68 ^b | 121.31 ^e | 9.9274 | <0.0001 |
| Total nitrogen Excreted, % | 74.75 ^a | 61.61 ^b | 62.00 ^b | 81.47 ^a | 65.84 ^b | 90.43 ^c | 3.4450 | 0.0003 |
| Nitrogen retention, g/d | 89.64 ^{ac} | 88.70 ^{ac} | 112.5 ^a | 16.44 ^b | 72.68 ^c | 10.45 ^b | 9.6673 | <0.0001 |
| Nitrogen balance, % | 25.25 ^a | 38.39 ^b | 38.00 ^b | 18.53 ^a | 34.16 ^b | 9.57 ^c | 3.4450 | 0.0003 |

^{a-f}Means in the same row with different superscripts differ (P < 0.10)

¹Treatment: birdsfoot trefoil (BFT), cicer milkvetch (CMV), meadow bromegrass (MB), alfalfa (ALF), sainfoin (SAN), and small burnet (SML).

²SEM: Standard error of the mean.

Figure 2 shows the partitioning of nitrogen to the urine and feces, as well as the nitrogen that was retained by the heifers in each treatment. The percentage of nitrogen that was partitioned to the feces relative to the nitrogen consumed was greatest for heifers offered SML or MB, intermediate for heifers offered SAN or BFT, and lowest for heifers offered ALF or CMV ($P < 0.0001$; Table 5). As expected, the pattern is reversed with regard to the nitrogen partitioned to the urine, with ALF-fed heifers showing the greatest values and the SML-fed heifers producing the lowest values of nitrogen partitioned to the urine ($P = 0.0001$; Table 5). Additionally, BFT-fed heifers tended to show lower values for this parameter than CMV-fed heifers ($P = 0.1006$).

When looking at the total amount of nitrogen excreted per day, the MB and SML treatments resulted in the lowest values of nitrogen excretion, SAN and BFT were intermediate, and feeding CMV and ALF hays led to the greatest values observed (Table 5). In contrast, heifers consuming BFT, CMV, or SAN excreted the lowest percentage of the nitrogen that was consumed ($P = 0.0003$). Total daily retention of nitrogen revealed the lowest values for animals fed SML or MB hays ($P < 0.0001$). Likewise, the nitrogen balance was lowest for heifers consuming SML, intermediate for heifers consuming ALF or MB, and greatest for heifers consuming BFT, CMV, or SAN ($P = 0.0003$; Table 5).

DISCUSSION

I hypothesized that feeding “non-traditional” tanning-containing hays to beef cattle would reduce their enteric methane emissions and nitrogen excretion. In support of this hypothesis, cows and heifers showed the lowest methane emissions when they were fed SML hay, a tannin-containing forb. My hypothesis was also supported by the low levels of blood urea nitrogen as well as by the reduced total nitrogen and urine urea nitrogen excretion in animals consuming tannin-containing hays compared with animals consuming ALF or CMV.

Intake and Digestibility

Forage intake is influenced by non-structural carbohydrates, nitrogen (Forbes, 2007), and fiber contents, which in turn impact rumen fill and passage rate (Allen, 1996). Forages with greater fiber content are digested more slowly, resulting in slow passage and low intake rates (Poore et al., 1990). Such slow rates of passage were likely responsible for the lower intake values recorded for cows and heifers consuming MB, a grass high in fiber content. In addition to fiber, high concentrations of tannins can contribute to reduced forage intake in ruminants (Barry and Duncan, 1984; Barry and McNabb, 1999). For instance, condensed tannins can reduce palatability by binding with proteins from the saliva, which causes astringency (MacAdam and Villalba, 2015). Similar to the complexes that condensed tannins form with proteins (Jones and Mangan, 1977), condensed tannins can also bind with microbes in the rumen as well as with plant fiber, slowing down digestion rates and thus intake (Reed, 1995). In addition, high tannin concentrations in feeds (above five percent of dry matter) have been shown to lower

voluntary feed intake in ruminants (Barry and Duncan, 1984; Barry and McNabb, 1999). Nevertheless, intake values for tannin- and non-tannin containing hays were comparable in the present study, except for the greater intake values observed for animals fed ALF than for those fed SAN, and for the low intake values of SML recorded for heifers, despite the high content of non-structural carbohydrates and low concentration of fiber in this hay. The former could be explained by the lower crude protein and greater fiber contents in SAN than in ALF, and the latter by the presence of hydrolysable tannins in SML which have negative impacts on forage intake (Verheyden-Tixier and Duncan, 2000; Reed, 1995). In contrast, the condensed tannins contents in BFT did not appear to have constrained intake, likely due to the low concentration of condensed tannins observed in this forage (0.75 percent condensed tannins on a dry matter basis) or to the chemical characteristics of the condensed tannins in this hay. In support of this, intake values for cows grazing BFT are typically high relative to other forages (Woodward et al., 2004).

Hay digestibility values were, in general, high for CMV and BFT, likely due to the high crude protein concentration in both of these forages, compounded with the relatively low fiber concentration in CMV and the high concentration of nonstructural carbohydrates in BFT. In addition, neutral detergent fiber digestibility in BFT was relatively high in cows and heifers, which is consistent with previous reports showing that, in general, cellulose in BFT is more digestible than cellulose in ALF (Mowat et al., 1969). However, Hunt et al. (2014) found that the thickness of the lignified xylary ring in BFT stems increases more rapidly than in ALF during plant growth, being approximately 50 percent greater at maturity in BFT than in ALF.

Digestibility values of acid detergent fiber in cows were high for MB. This is supported by the findings of Buxton et al. (1995) showing that fiber in grass species is generally less lignified and more digestible than fiber in legumes. Conversely, the low digestibility values observed for SML could be attributed to the presence of hydrolysable tannins, as it has been shown that these compounds can act as antimicrobial agents (Ekambaram et al., 2016), which reduces forage digestibility. Moreover, the low digestibility values for this hay are in contrast to its nutritional composition, with the lowest levels of fiber content and the greatest concentration of nonstructural carbohydrates of all the hays assayed in this study.

Crude protein digestibility was lower for SAN than for BFT which suggests that more protein was complexed by condensed tannins in SAN that did not disassociate in the abomasum and small intestine (Frutos et al., 2004). Alternatively, this may imply that the condensed tannin-protein complexes formed did disassociate but then reformed again before the animal had a chance to utilize the protein (McNabb et al., 1998), or that these complexes disassociated and the condensed tannins then bonded with digestive enzymes (Mole and Waterman, 1987; Silanikove et al., 1994).

Enteric Methane Production

Enteric methane emissions are a function of the quality and quantity of feed consumed, changes in the ruminal microflora, as well as of the efficiency by which an animal converts feed into meat or milk (Johnson and Johnson, 1995). High fiber content constrains passage rate, which reduces the amount of feed a cow can consume and thus also may also reduce the total amount of methane produced daily (Poore et al., 1990).

There is also evidence indicating that methane emissions per kg dry matter intake are negatively related to intake levels because augmented intake can increase the passage rate of digesta, therefore decreasing the amount of time available for microbial fermentation in the rumen (Jiao et al., 2014). However, this decline in methane production with increasing dry matter intake may be more pronounced in concentrates than in forages (Benchaar et al., 2001). This may explain why heifers in this study consumed greater amounts of ALF without significant reductions in the volume of methane produced per kg of intake. Factors other than the acceleration of rumen passage can account for decreases in methane emissions. For instance, hydrolysable tannins have been shown to reduce methane emissions (Field and Lettinga, 1987; Bhatta et al., 2009) which probably explains why cows and heifers fed SML showed the lowest daily values of methane emissions recorded and cows consuming this hay showed the lowest amounts of methane production per kg of intake. Additionally, forages with greater fiber contents also promote higher levels of methane production in cattle (Van Soest, 1994; Friggens et al., 1998), which helps to explain the greatest values for methane emissions per kg of intake observed in heifers consuming MB.

Volumes of methane production per kg of dry matter intake were, in general (e.g., for BFT, CMV, MB, SAN), greater for cows than for heifers. This is consistent with the idea that immature ruminants produce less methane per kg of dry matter intake than mature animals (Swainson et al., 2007). The reason for this difference is not clear and some studies have not found any age effects (e.g., Ramirez-Restrepo et al., 2015), but differences in methane production due to age may be at least partially explained by differences in digesta kinetics (Swainson et al., 2007).

Excretion of Condensed Tannins in the Feces, Excretion of Nitrogen in Urine and Feces, and Blood Urea Nitrogen

Both condensed tannins (Dahlberg et al., 1988; McNabb et al., 1996; Broderick and Albrecht, 1997; Abarghuei et al., 2014) and hydrolysable tannins (Driedger and Hatfield, 1972; Hervás et al., 2000; Aguerre et al., 2015) have been shown to reduce protein degradation in the rumen through the formation of tannin-protein complexes (Perez-Maldonado and Norton, 1996). The reduction in total urinary nitrogen excretions by cows and heifers consuming tannin-containing hays is likely a result of this mechanism. Blood urea nitrogen and urine urea nitrogen values result from the absorption of excess ammonia from the rumen (Lobley and Milano, 1997). In fact, the concentration of urea in blood has been regarded as an indicator of the processes of degradable protein supply to the rumen, with a significant contribution to urinary nitrogen excretion (Kebreab et al., 2004). Therefore, the lower levels of both blood urea nitrogen and urine urea nitrogen found for tannin-containing hays can also be attributed to the escape of nitrogen from the rumen through a mechanism that involves protein binding by tannins. Animals fed MB hay also showed lower values of total nitrogen, urine urea nitrogen, and blood urea nitrogen. However, this pattern can be explained by the lower dietary intake of nitrogen (Yan et al., 2007), given the low crude protein content (eight percent of dry matter) observed in MB hay.

The amounts of urine produced daily are related to the amount of minerals and protein consumed by the animal. Van Vuuren and Smits (1997) demonstrated that increases in dietary protein increase daily urine output. Both cows and heifers consuming ALF and CMV – treatment hays with high concentrations of crude protein but without

tannins – support this assertion as these treatments showed the greatest levels of daily urinary nitrogen excretions and subsequently, high amounts of urine output. Likewise, cows and heifers under the ALF treatment showed the greatest percentage of nitrogen partitioned to the urine. This can be attributed to the high concentrations of crude protein in this hay and a lack of condensed tannins to allow the nitrogen to escape degradation in the rumen, in conjunction with relatively low amounts of energy available for microbial protein synthesis in the rumen of animals fed ALF hay (Miller et al., 2001). In contrast, the SML and BFT treatments showed lower partitioning of the ingested nitrogen to urine, suggesting that the tannins contained in these hays protected the protein from degradation in the rumen. Alternatively, the high concentration of non-structural carbohydrates in the BFT hay and the low concentration of fiber in the CMV hay may have provided synchronous sources of energy-yielding substrates that contributed to the high nitrogen retention and nitrogen balance values observed (Sinclair et al., 1993).

The concentrations of condensed tannins found in BFT and SAN hays were both lower than values typically observed in fresh forages of the same species (John and Lancashire, 1981; Scharenberg et al., 2007). This is supported by the fact that condensed tannins are reactive and labile molecules so drying conditions promote the inactivation of several of their biological actions on herbivores (e.g., D’Mello and Taplin, 1978; Makkar and Singh, 1991). Nevertheless, the condensed tannins in both the BFT and SAN hays still appeared to have affected nitrogen digestion and its mode of excretion. This is supported by the fact that greater fecal excretion of condensed tannins (i.e. animals consuming SAN) was associated with greater fecal excretion of nitrogen in both cows and heifers. Additionally, lower levels of both blood urea nitrogen and urine urea

nitrogen were found for cows and heifers consuming hays containing tannins. These findings are also supported by studies showing that conserved forages containing condensed tannins, such as SAN, maintain their bioactive properties – similar to fresh forage – regarding their ability to act against gastrointestinal nematodes (Heckendon et al., 2006).

Feeding some tannin-containing hays in the present study (e. g. SAN for heifers, and SML for both cows and heifers) shifted the partitioning of nitrogen from urine to feces, consistent with studies that trace the fate of nitrogen in hydrolysable tannins (Deville et al., 2010) or condensed tannin containing diets (Ahnert et al., 2015). This result can be attributed to the formation of protein-tannin complexes in the rumen, which are stable in the pH range of 3.5 – 7 (Bunglavan and Dutta, 2013) and thus increase the metabolizable amino acid flow to the small intestine (Barry and McNabb, 1999). There are significant concerns over the potential global environmental costs associated with livestock production and its associated nitrogen losses to the environment (Pelletier and Tyedmers, 2010; Bouwman et al., 2013). A shift in nitrogen excretion from urine to feces in livestock can help ameliorate this problem as fecal nitrogen is less volatile than urinary nitrogen (de Klein and Eckard, 2008). On the other hand, urinary urea is quickly transformed to ammonia, and urinary nitrogen is a source of nitrate and nitrous oxide (Whitehead, 1995; Oenema et al., 2005). Nitrate is produced by the oxidation of ammonia and is a major pollutant of water (Eckard et al., 2010), whereas nitrous oxide is a byproduct of nitrification and denitrification in the soil (Bremmer, 1997) and is a significant greenhouse gas (Sakadevan and Nguyen, 2016). Thus, the greater levels of

urinary nitrogen in forages like ALF and CMV treatments mean greater nitrous oxide losses into the atmosphere.

Animals consuming BFT also showed lower urine urea nitrogen excretions but did not reveal any increases in fecal nitrogen excretion. This pattern can be attributed to the increased amount of nitrogen that was retained by those animals. Cows and heifers fed BFT showed high nitrogen balances which may be due to the presence of condensed tannins shifting the site of protein digestion from the rumen to the small intestine (Maamouri et al., 2011). Protein degradation and efficiency of bacterial protein synthesis have been reported to be greater in BFT than in non-tannin containing legumes like ALF (Dahlberg et al., 1988). In contrast, animals fed SML, along with animals fed MB, had very low nitrogen balances. This suggests that, for SML, the protein-tannin complexes either disassociated and the tannins then formed bonds with digestive enzymes (Mole and Waterman, 1987; Silanicove et al., 1994), they disassociated but reformed again before the animal had a chance to utilize the protein (McNabb et al., 1998), or they did not disassociate in the abomasum and small intestine (Frutos et al., 2004), therefore reducing the efficiency of nitrogen utilization by the animal. This is also supported by the fact that SML showed the lowest amounts of nitrogen retained and that a high proportion of the nitrogen consumed was excreted in the feces.

Cows and heifers fed CMV also showed high nitrogen balances, which could be attributed to an adequate supply of fermentable energy to the rumen (Miller et al., 2001), given by the high rates of digestibility and structural carbohydrate digestion observed in this species relative to other forages such as ALF (McGraw and Marten, 1986; Dahlberg et al., 1988).

Nitrogen balance was, in general, greater for heifers than for cows, in line with the idea that growing animals are more efficient at retaining nitrogen (with lower levels of nitrogen excreted in the urine) than adult animals (Blaxter, et al., 1966). However, cows consuming BFT retained more nitrogen than heifers under the same treatment. This could be due to a lower disassociation of protein-tannin complexed in the abomasum and duodenum (Waghorn et al., 1987) of heifers because of differences in pH and/or greater mastication efficiencies in heifers (Nicholson et al., 1971) which would lead to greater passage rates, all of which reduce nitrogen retention. Cows consuming CMV hay also retained more nitrogen than heifers consuming the same hay, and this could be explained by imbalances in the protein/energy ratio due to the enhanced energy requirements for growth by heifers (Chizzotti et al., 2008) that may lead to a greater extent of protein mobilization in heifers than in cows.

In summary, tannin-containing hays have the potential to reduce methane emissions and, in particular, shift the site of nitrogen excretion from the urine to the feces and increase the nitrogen balance of beef cattle. Thus, these hays can contribute to a “cleaner” cow-calf phase while maintaining or enhancing levels of animal productivity. However, certain tannin-containing hays, (e.g. SML) may have some negative impacts on nitrogen utilization, whereas other tannin-free hays (e.g. CMV) have the opposite effect and may also be able to enhance the efficiency of the cow-calf phase of production.

CONCLUSION

Per capita consumption across the globe of livestock products has greatly increased over the past few decades and with increases in human population, incomes, and urbanization, this pattern is predicted to continue to increase (Herrero et al., 2016). Within this context, environmental impacts from livestock are under high scrutiny and the attention has opened new avenues to investigate and understand how livestock greenhouse gas emissions could evolve and be better managed in the future. Moreover, greenhouse gas abatement strategies are also relevant for the overall efficiency of beef production. This is because reductions in enteric methane and nitrogen emissions represent improvements in the efficiency of nutrient use by ruminants (Huang et al., 2011) and consequently, can contribute to an increase in the cost-effectiveness of the beef production system.

In 2015, contributions from beef cattle accounted for 118.1 Tg CO₂ Eq from enteric methane emissions and 7.7 Tg CO₂ Eq from nitrous oxide from manure management (U. S. EPA, 2017a) and from this, approximately 80 percent can be attributed to emissions produced by the animals, such as mother cows, that remain in the system for a longer time (Beauchemin et al., 2010). Additionally, beef cattle are often fed cultivated forages and grains which are fertilized with manure or synthetic nitrogen containing fertilizers. This is a significant factor because agricultural soils account for approximately 251.3 Tg CO₂ Eq, or 75.1 percent of total nitrous oxide emissions in the United States (U. S. EPA, 2017a). Furthermore, a significant portion of the production cycle relies on hay as the main source of feed for these animals. Thus, the exploration of

“non-traditional” hays that may lead to more efficient nutrition as a consequence of their chemistry, could turn out to be essential in the quest for feeding systems that reduce environmental impacts from livestock. My research suggests that non-traditional tannin-containing hays have the potential to reduce enteric methane emissions from beef cattle. The main significant reduction in enteric methane emissions that I found was for cows and heifers consuming SML which contains hydrolysable tannins. Other tannin-containing hays (e.g. BFT and SAN) did yield lower volumes of enteric methane emissions from heifers but those were not significantly different from more traditional legumes like ALF. It is likely that the high variability in methane emissions observed among individuals (Garnesworthy et al., 2012) and the low sample size in the present study accounted for the lack of significant effects between ALF or grass hays and BFT or SAN.

This study suggests that the major contribution that non-traditional tannin-containing hays have on environmental impacts from ruminants is to reduce nitrogen excretion, increase nitrogen balance and shift nitrogen excretion from urine to feces. For instance, feeding SML – a hydrolysable tannin containing forb – substantially shifted the excretion of nitrogen from the urine to the feces. Organic nitrogen in feces is metabolized at a slower rate than nitrogen in urine, representing less potential for ammonia and nitrous oxide volatilization from soil and manure (de Klein and Eckard, 2008). However, despite these benefits, feeding SML was not beneficial for the nutrition of cows or heifers relative to other hays given the low levels of nitrogen retention observed for this hay (approximately 10 percent of dry matter). This was presumably due to the formation of strong protein-tannin bonds which did not disassociate in the small intestine (Frutos et al.,

2004). In contrast, the other tannin-containing hays explored in this thesis, SAN and BFT, generally led to high levels of nitrogen balance and nitrogen retention with a lesser but still significant shift in the excretion of nitrogen from the urine to the feces.

Furthermore, other non-tannin containing hays such as CMV may also contribute to a cleaner cow-calf phase by enhancing nitrogen utilization through attributes other than the presence of tannins in the plant's tissues (i.e. a greater supply of synchronous sources of fermentable energy to match the high concentration of nitrogen present in this hay). Thus, tannin-containing hays, as well as other nontraditional legume hays, such as CMV, can contribute to a "cleaner" cow-calf phase while maintaining or enhancing the levels of animal productivity.

My research also shows – as in previous studies – that volumes of methane production per kg of dry matter intake were greater for cows than for heifers. Although the reason for this difference is not clear, it appears that differences in digesta kinetics between immature and mature ruminants may help to explain such patterns (Swainson et al., 2007). In addition, nitrogen efficiency was, in general, greater for heifers, in line with the idea that growing animals are more efficient at retaining nitrogen (and will excrete less nitrogen in the urine) than adult animals (Blaxter et al., 1966). However, cows consuming BFT retained more nitrogen than heifers under the same treatment, which may be due to a lower disassociation of protein-tannin complexes in the abomasum and duodenum (Waghorn et al., 1987) of heifers because of differences in pH and/or greater efficiency of mastication (Nicholson et al., 1971) which would lead to greater passage rates in heifers than in cows, all of which reduce nitrogen retention. Cows consuming CMV hay also retained more nitrogen than heifers consuming the same hay, and this

could be explained by imbalances in the protein/energy ratio and the enhanced energy requirements for growth by heifers (Chizzotti et al., 2008) that may lead to a greater extent of protein mobilization or urea recycling in heifers compared to cows.

Results from my thesis can be utilized for obtaining a more accurate parameterization of models for life cycle assessments (e.g. Beauchemin et al., 2010), exploring budgets of greenhouse gas inputs and outputs within the beef production cycle, and the significant contribution of feeding tannin-containing legume hays to mother cows in confinement. Lower emissions observed in non-traditional hays could lead to more economic budgets regarding the efficiency of nutrient use in addition to reductions in greenhouse gas emissions.

New research should focus on exploring other non-traditional tannin-containing hays and their potential to reduce greenhouse gas emissions from cattle. More forage species could be considered relative to their agronomic characteristics and the potential adaptability to the environmental conditions present in different ecoregions across the country and worldwide. In addition, combinations of hays may be assayed in an attempt to explore complementarities among these feed resources, which could lead to synergisms and associative effects (Van Soest, 1994) with the concomitant improvement in the efficiency of nutrient utilization and reductions in carbon and nitrogen footprints. Additionally, more research should be done with regard to finding ways to increase the disassociation of protein-tannin complexes in the abomasum and small intestine to increase protein utilization of tannin-containing feeds. This could be of particular relevance for hays like SML, which yielded very low levels of nitrogen retention and a high proportion of nitrogen excreted in the feces. Furthermore, research to determine the

mechanism by which tannins reduce methane emissions would be helpful to open the doors for mitigating the reduced digestibility that sometimes accompanies the inclusion of tannins in the diet.

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APPENDIX

Methodology for Measuring Enteric CH_4 Emissions in Cattle using the SF_6 Tracer Technique

Principle: A known source of SF_6 is placed in the rumen of an animal. The expired air is sampled and a ratio of the CH_4 and SF_6 is used to estimate enteric CH_4 emissions. The source of SF_6 is a permeation tube with a known rate of release controlled by a permeable Teflon membrane.

1. Sulfur hexafluoride (SF_6) permeation tubes were prepared by transferring SF_6 gas to a threaded brass tube that had been cooled in liquid N_2 and capping the tube with a Swagelok nut with an opening for $\frac{1}{4}$ inch tubing. The nut contained a permeable Teflon disk sandwiched between 2 nylon washers. The permeation tubes were weighed before and after filling and contained at least 0.6g of SF_6 . The filled tubes were placed in an incubation oven at $39^\circ C$. Tubes were weighed weekly until an accurate loss rate was established over 6 wk. Permeation tubes were inserted into the rumen of cows and heifers using a balling gun at least 2 d prior to the first day of sample collection.
2. The apparatus used for sample collection consisted of a cannister constructed from a 4-inch schedule 40 polyvinyl chloride pipe rated for 220 psi or higher. The inside of the canisters had a volume of approximately 2.8 L. Capillary tubing with an internal diameter of 125 microns was attached to a halter so that the intake was positioned just above the nostrils and mouth of the animal. This capillary tubing was connected to the canister with polytetrafluoroethylene tubing that had a diameter of $\frac{1}{8}$ inches.

3. Prior to sample collection, canisters were tested for any leaks. This was accomplished by pressurizing the canisters with nitrogen gas to a pressure of at least 15 psi. The pressure was then checked 24 h later and any canister that had dropped by 0.5 psi or greater was considered to have a leak and was not used.
4. Usable canisters were evacuated to 0.250 psi or less in the morning prior to being placed on the animals. Actual psi was recorded for each canister.
5. Halters were placed on animals and the canisters were attached to the halters using metal clips. The capillary tubing on the halter was connected to the canister, the valve on the canister was opened, and the time was recorded.
6. Two control canisters were connected to capillary tubing and placed around the study area. The valves on the canisters were opened and the times were recorded.
7. After 24 h, the valves on the canisters were closed and removed from the animals and the closing times were recorded for each canister.
8. Canisters were immediately transported to the laboratory for subsampling.
9. Once back at the lab, pressure inside the canisters was recorded. Without releasing any of the gas inside the canisters, nitrogen gas was added to each until 16psi or greater was reached and the pressure was recorded.
10. After being pressurized, a quick connect with a septum was attached to the quick connect of the canister. The valve was then opened and a 25mL syringe with a needle was used to obtain 15-18mL of gas which was then injected into a 12mL evacuated vial.
11. Samples were then stored at room temperature until the end of the collection period and sent to a laboratory for methane analysis.

12. New canisters were put on animals, repeating steps 4-11 for five consecutive days.