EFFECT OF SAINFOIN (ONOBRYCHIS VICIIFOLIA SCOP.) VARIETY AND HARVEST MATURITY ON QUALITY, YIELD, AND CONDENSED TANNIN CONTENT

by

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ABSTRACT

Sainfoin (Onobrychis vicifolia Scop.) is a forage legume that was introduced to North America from Europe and Asia. Unlike alfalfa, sainfoin is non-bloating, lacks autotoxicity properties, and may work as an anthelmintic when consumed by livestock. With limited information available on sainfoin varieties and management, new information is necessary to provide to producers for optimal production. The objective of this study was to determine the impact of variety and maturity at harvest on forage yield, quality, and condensed tannin content. Four varieties of sainfoin (‘AAC Mountainview’, ‘Eski’, ‘Shoshone’, and ‘Delaney’) and one alfalfa variety (‘Shaw’) were planted at two locations in Montana and the same sainfoin varieties and one alfalfa variety (‘Spredor 4’) were planted in one location in Utah. Samples were taken at 10, 50 and 100% bloom and evaluated for dry matter production and nutritive quality. Variety ($P \leq 0.005$) and maturity ($P \leq 0.001$) both were found to impact production, with Shaw, AAC Mountainview and Eski varieties having higher production, as well as tonnage increasing with advancing maturity than Delaney and Shoshone. Sainfoin had greater levels of condensed tannins than alfalfa ($P \leq 0.001$), and within sainfoin, tannin content decreased with increasing maturity ($P \leq 0.003$) at all locations. Crude protein levels were significantly different for maturity ($P < 0.001$) and variety ($P \leq 0.003$), and fiber values were only affected by maturity ($P \leq 0.001$). These results demonstrate that sainfoin is a beneficial legume to feed to livestock particularly in grazing situations, with similar quality and yield compared to alfalfa, as well as a higher condensed tannin content.
CHAPTER ONE

LITERATURE REVIEW

Sainfoin

Sainfoin is a forage legume that was introduced to North America in the early 20th century from Europe and Asia (Hybner, 2014). Sainfoin is widely distributed in northern temperate regions of the world, specifically the Mediterranean and Asia. It has the ability to grow in the different climates of Europe, Asia, North America, and New Zealand, which range from temperate to desert climates. Sainfoin can be grown in dry or irrigated areas, and in neutral, slightly acidic or alkaline soils (Carbonero et al., 2011). However, sainfoin does not perform well in high salinity, in areas with high water tables, or areas that are poorly drained (Bhattarai et al., 2016.)

Sainfoin has many benefits compared to more conventional legumes such as alfalfa, including non-bloating characteristics, no potential for autotoxicity, and some preliminary evidence that it may work as an anthelmintic in consuming livestock (Panditharatne et al., 1986; Carbonero et al., 2011; Bhattarai et al., 2016). Sainfoin can be hayed or grazed, either alone or in a grass-legume mix. Resistance to drought and frost damage are some other adaptations that make sainfoin appealing to producers (Smith, 2007). Additionally, since it is a legume, sainfoin can supply nitrogen to the soil which is fixed biologically in the nodules (Carbonero et al., 2011), decreasing the need and the cost for fertilizer.

Sainfoin can supply highly nutritious forage for producing animals, with an average daily gain of 0.96 kg for growing heifers compared to 0.91 kg on alfalfa hay (Parker and Moss, 1981).
When sainfoin and alfalfa were offered free choice, sainfoin was consumed at an average of 2.3 kg/day compared to 0.47 kg/day for alfalfa (Parker and Moss, 1981), indicating its high palatability and acceptance.

**Non-bloating**

A major issue with livestock consuming alfalfa is the risk of bloat. Bloat is a common digestive disorder that occurs in ruminant animals, particularly when consuming high-quality legumes such as alfalfa, large amounts of soluble proteins, or large amounts of high-starch feeds such as mature barley forage (Wang et al., 2012). Animals experiencing bloat have excessive gas production which becomes trapped in the rumen and reticulum. There are two main types of bloat: free-gas and frothy. Free-gas bloat is caused when there is an obstruction of the esophagus. This makes it impossible for the animal to release the gas that has built up in the rumen. Frothy bloat is caused by gases being trapped in the rumen after consuming high protein, rapidly digestible feeds. The fermentation causes a foam in the rumen that prevents the animal from eructating and releasing the gases (Howarth et al., 1979; Wang et al., 2012). If the gases are unable to escape from the rumen, the pressure will build and push on the lungs making it difficult for the animal to breathe. Frothy bloat can have a negative economic impact on entire herds, and can lead to large losses, on top of forage species aversion, due to the perceived danger associated with forages such as alfalfa.

Leguminous feeds such as alfalfa and clover are associated with bloat in ruminants (McMahon et al., 1999; Wang et al. 2012). These forages are high in protein, low in fiber, and are readily digestible. Producers can use management strategies to limit the occurrence of bloat in their herds, including grazing mixed grass/legume pastures, adding “bloat blocks”
poloxalene), grazing later in the day, grazing when plants are more mature (Wang et al. 2012), or grazing high-quality legumes that contain large amounts of condensed tannins (CT), such as sainfoin.

Adding grass to the legume pasture increases the amount of fiber and decreases overall soluble protein intake, decreasing the rate of fermentation. This allows the microbes to adjust to the legumes and decreases the occurrence of bloat (McMahon el al., 1999; Wang et al. 2012; Wang et al. 2015). Bloat blocks, such as poloxalene, are oral medications that prevent bloat by eliminating the gas bubbles in the rumen. Once the bubbles collapse, the animal is able to eructate, decreasing the pressure in the rumen. Poloxalene can either be given in a molasses block or as a top dressing to prevent bloat, or in a drench to eliminate bloat once it occurs (Wang et al., 2012). Grazing time is also something to consider when trying to decrease the occurrence of bloat.

Alfalfa’s soluble protein content fluctuates during the day. In the morning, there tends to be greater levels of soluble protein compared to the afternoon (Wang et al., 2012). Fewer soluble proteins will be consumed if animals graze in the afternoon (Wang et al., 2012). Another bloat mitigation strategy, is to graze at later plant growth stages. Grazing at the vegetative stage increases the occurrence of bloat (Wang et al., 2012). As the plant matures, the leaf-to-stem ratio decreases, increasing fiber content (Wang et al., 2012). Increased fiber content decreases the rate of digestion and fermentation, lowering the risk of bloat. Sainfoin is one of the few legume forages that do not cause bloat (Wang et al., 2012).
Condensed Tannins

Sainfoin is a non-bloating forage because of the presence of CT. The CT bind to protein, making them less soluble, decreasing the production of the stable foam in the rumen (Jones and McAllister, 1994; McMahon et al., 1999; Scharenberg et al., 2009; Wang et al., 2015). Condensed tannins are made by the chloroplast-derived organelles and stored in vacuoles in plant cells. In sainfoin, CT are located throughout the plant, but the greatest concentration is in the leaves. Concentration of CT in sainfoin varies between varieties, growing conditions, and phenological stage of the plant (Wang et al., 2015). Alfalfa has such low levels of CT, that they cannot be detected in the leaves (Skadhauge et al., 1997).

Occurrence of Bloat

Pasture bloat decreases with as little as 5.0 g CT/kg DM (Li et al., 1996). Steers had a decreased risk of bloat when sainfoin was present in the diet. Steers fed a mix of 10 to 20% sainfoin with 90 and 80% chopped alfalfa, respectively, reduced bloat occurrence by 45 to 93%, compared to feeding alfalfa alone (McMahon et al., 1999). Similarly, Wang et al. (2006) found that inclusion of 35% sainfoin when grazing alfalfa decreased the occurrence of bloat from 43% to 10%. These illustrate the value of sainfoin in reducing concerns associated with feeding legumes to ruminants.

Anthelmintic Effect

Another potential benefit of sainfoin is its anthelmintic abilities. Anthelmintic refers to the ability to decrease or eliminate parasitic worms in animals. Many producers dose their animals with chemical anthelmintic, or “dewormers”, to decrease parasite loads (Jackson and Coop, 2000). In recent years, parasitic worms have developed resistance to these synthetic
anthelmintics (Jackson and Coop, 2000), suggesting that dewormers may not eliminate worms in animals, consistently leading to decreases in animal performance (Jackson and Coop, 2000). Condensed tannins decreased the proportion of hatched eggs and inhibited egg development of lung and gastrointestinal parasites in previous research (Arroyo-Lopez et al., 2014; Hoste et al., 2012; Paolini et al., 2004). Condensed tannins inhibit larvae from developing a sheath, thereby, impairing maturation, as well as reducing reproductive abilities of adult parasites (Hoste et al., 2012).

Arroyo-Lopez et al. (2014) artificially infected lambs with *Haemonchus contortus* (Barber’s pole worm) and *Trichostrongylus colubriformis* (roundworm) and observed that lambs fed sainfoin had decreased total egg counts and worms compared to lambs fed alfalfa. Similarly, goats fed sainfoin hay had lower levels of neonate egg excretion, decreased worm fertility, a reduced worm presence by 50%, and better host resilience (Paolini et al., 2004). However, lambs treated with a commercial dewormer had lower parasite counts than lambs fed or not fed sainfoin, suggesting that synthetic dewormers are the primary therapeutic for preventing internal parasites (Arroyo-Lopez et al., 2014). Forages with high CT, like sainfoin can be used in an integrated pest management approach to enhance the control of parasitic worms. Feeding forages containing naturally high levels of CT, may also decrease parasite shedding, leading to a decreased need for synthetic anthelmintics, and reduction in parasite resistance. More work is needed to determine how plant maturity and CT content impact parasite worm population in animals.
Sainfoin vs. Alfalfa

Sainfoin and alfalfa are both high-quality legumes (Bhattarai et al., 2016). Sainfoin generally is harder to establish, lower yielding, and less persistent than alfalfa (Bhattarai et al., 2016). While sainfoin is slightly more drought tolerant than alfalfa, it can perform well in places with over 36 cm of annual precipitation (Bhattarai et al., 2016).

Dry matter (DM) production is maximized when sainfoin reaches full bloom (Carlton et al., 1968). By contrast, maximum DM production occurs at 50% bloom for alfalfa (Carlton et al., 1968). Both legume species will produce higher quality forage if harvested at earlier growth stages, but DM production is reduced. Sainfoin produces maximum yield two to three weeks after alfalfa, shortly after 70% bloom (Carlton et al., 1968). Sainfoin produced 5-20% less than alfalfa in Canada (Goplen et al., 1991). Alfalfa also has potential for multiple harvests, whereas sainfoin has less regrowth potential and generally is limited to a single cutting (Goplen et al., 1991). The combination of earlier harvest and greater regrowth potential led to greater DM production by alfalfa under favorable growing conditions (Goplen et al., 1991).

Sainfoin and alfalfa are susceptible to fungal diseases, which affect the roots and crowns. These pathogens can decrease stand longevity and productivity (Bhattarai et al., 2016). Sainfoin is resistant to certain alfalfa pests. Alfalfa weevil (*Hypera postica*) causes great losses in alfalfa across the U.S., but does not feed on sainfoin (Goplen et al., 1991). Hence, sainfoin may be the superior option in areas where alfalfa weevil is prevalent.

Quality Factors

Forage quality factors include; palatability, digestibility, anti-quality factors, intake, and nutrient content. Palatability is influenced by taste, smell and texture, with low-quality forages
usually leading to decreased palatability (Ball et al., 2001). Digestibility is mostly influenced by the stem to leaf ratio and plant maturity, with digestibility decreasing due to increases in cellulose, hemicellulose and lignin at advanced growth stages (Paterson et al., 2015). Anti-quality factors are components found in the plant which cause harm to the animal or decrease the appeal to the animal, and include tannins, nitrates, alkaloids, or dust and awns in the forage (Ball et al., 2001; Nelson and Moser, 2015). Nutrient content refers to protein, energy, fiber, vitamin and mineral content (Ball et al., 2001).

Forage quality analyses include measurements of moisture, DM, crude protein (CP), total digestible nutrients (TDN), net energy, mineral content, ash, acid detergent fiber (ADF) and neutral detergent fiber (NDF). Forage DM content at harvest impacts forage quality, since forages with low moisture content can be brittle and exhibit leaf loss, which decreases palatability (Ball et al., 2001 and Shapiro, 2001).

Crude protein concentration is a measure of the nitrogen contained in feed. It includes both true protein and non-protein nitrogen, which ruminant microbes can convert to protein while non-ruminant animals cannot. Feed CP concentration is calculated by multiplying the nitrogen concentration by 6.25, a constant reflecting the percentage of nitrogen contained in most protein (i.e., 16% or 100/16; Jiang et al., 2014). Protein is a major component of all living tissue which, with carbon, oxygen, and nitrogen, form amino acids (McDonald et al., 2010 and Shapiro, 2001). Amino acids are necessary parts of enzymes that aid in digestion, hormones that regulate body functions, hair and skin pigmentations and metabolic reactions (McDonald et al., 2010 and Shapiro, 2001). Protein is required for muscle tissue and milk production, and in feeding the microbes in the rumen (McDonald et al., 2010 and Shapiro, 2001).
Carbohydrates are an important component of TDN, along with digestible fiber, protein, and lipids. Carbohydrates provide energy to animals and high levels are found in concentrates and roughages (McDonald et al., 2010 and Shapiro, 2001). Complex carbohydrates include starch and cellulose (McDonald et al., 2010 and Shapiro, 2001). Starch consists of glucose and is easily digestible, providing energy that is readily available to the animal. In contrast, cellulose comprises a large portion of the cell wall and woody fibers in plants and cannot be digested by non-ruminant animals (McDonald et al., 2010 and Shapiro, 2001). Microbes are able to digest these fibers in ruminants, providing usable energy to the animal (McDonald et al., 2010 and Shapiro, 2001).

Fiber content is reflected in both ADF and NDF concentrations of the forage. The ADF concentration indicates cellulose and lignin content of a forage and is negatively correlated with digestibility (Stokes and Prostko, 1989; Ball et al., 2001). Neutral detergent fiber concentration reflects the hemicellulose, lignin and cellulose content and is negatively correlated with intake (Stokes and Prostko, 1989; Ball et al., 2001). Both ADF and NDF concentrations are determined by boiling samples in either acid or neutral detergent solutions and measuring the remaining insoluble residue (Ball et al., 2001).

Energy in a feed is what the animal requires for function and production. Gross energy is the total amount of energy in the feed. Some energy is lost in feces, urine, digestive heat and gas (Moran, 2005), and is calculated as net energy. Total digestible nutrients refer to the total energy within a feedstuff. Total digestible nutrients are the sum of the digestible fiber, protein, lipids and carbohydrate components of the feed. Metabolizable energy is the energy available for use by the animal. Metabolizable energy can be broken down into net energy categories for maintenance,
gain, pregnancy or lactation (Moran, 2005). Testing forages for quality is critical in determining if forages are meeting the energy and nutrient requirements for a certain class of livestock or if supplements are needed. Proper nutrition optimizes the quality of life, reproduction efficiency, and overall performance of animals.

Sainfoin is considered a high-quality forage. Alfalfa had approximately 3% more CP than sainfoin at 10, 50 and 100% bloom (Carlton et al., 1968). Carlton et al. (1968) concluded that the amount of CP in sainfoin was sufficient to meet the nutritional needs of beef cattle. Moreover, sainfoin was higher in nitrogen-free extracts, TDN, and phosphorus (Carlton et al., 1968). Due to high levels of nitrogen-free extract, the energy available in sainfoin forage was equal, or superior, to alfalfa forage. Sainfoin and alfalfa had similar ADF, CP, and non-structural carbohydrate contents when harvested at three maturity stages in a separate study (Rufino-Moya et al., 2019; Table 1). Interestingly, lignin concentrations of forage increased with plant maturity in alfalfa but remained relatively constant in sainfoin (Rufino-Moya et al., 2019).

Sainfoin is highly palatable to both wild and domestic animals. Voluntary intake of sainfoin was 20-24% higher by cattle and sheep than forages (Waghorn et al., 1990), this may be due to the high levels of CT. Condensed tannins normally decrease palatability, decreasing intake. The CT in sainfoin are less bitter than CT in other forages, making the forage more palatable than other CT containing feedstuff (Jones et al., 1976).

**Autotoxicity**

There has been no reported autotoxicity properties (Khatiwada, 2018) for sainfoin. Autotoxicity refers to the inhibition of growth or reproduction of some plants by others in the same species through biochemicals released into the soil (Volenec and Johnson, 2004). Plant
stands have reduced vigor, thin overtime, and are less productive (Volene and Johnson, 2004). Alfalfa exhibits autotoxicity, forcing farmers to rotate fields where alfalfa is seeded over time (Volene and Johnson, 2004). Sainfoin does not exhibit autotoxicity, enabling farmers to interseed sainfoin into thin stands or plant sainfoin consecutively in the same field, provided there are no other production issues present, like disease or insect pressure (Volene and Johnson, 2004).

**Conclusion**

Feeding high-quality forage is important in maximizing livestock production. Feeding a high-quality forage legume like sainfoin may be a viable option for maximizing on-farm production. With its increased CT content, it provides a non-bloating and anthelmintic forage for livestock. Sainfoin is resistant to alfalfa weevil making it a beneficial forage for producers where alfalfa weevil is prevalent. Unlike alfalfa, sainfoin has no autotoxicity properties, allowing for interseeding in thin stands or planting in consecutive seasons. Sainfoin tends to be lower yielding and have lower crude protein content than alfalfa. Current literature is limited regarding best management practices for sainfoin, starting with variety selection all the way through to harvest management.

Due to the limited research of sainfoin, this research is critical in determining appropriate methods for harvest and variety selection. The objective of this research is to see how sainfoin harvested at different maturities affects quality.
CHAPTER TWO

EFFECT OF SAINFOIN (ONOBRYCHIS VICIIFOLIA SCOP.) VARIETY AND HARVEST MATURITY ON QUALITY, YIELD, AND CONDENSED TANNIN CONTENT

Abstract

Sainfoin (Onobrychis viciifolia Scop.) is a forage legume that was introduced to North America from Europe and Asia. Unlike alfalfa (Medicago sativa), sainfoin is non-bloating, lacks autotoxicity properties, and may work as an anthelmintic when consumed by livestock. Current research on sainfoin cultivar performance and management is lacking. The objective of this study was to determine the impact of cultivar selection and growth stage on forage yield, quality, and condensed tannin (CT) content. In 2018, four sainfoin cultivars (‘AAC Mountainview’, ‘Eski’, ‘Shoshone’, and ‘Delaney’) were planted across two Montana locations and one Utah location. In the Montana locations, ‘Shaw’ alfalfa was planted and ‘Spredor 4’ alfalfa was planted at the Utah location as a control. Forage dry matter (DM) production and quality was determined at 10, 50 and 100% bloom. Variety ($P \leq 0.005$) and maturity ($P \leq 0.001$) impacted DM yield, with Shaw, AAC Mountainview and Eski cultivars having higher production, as well as tonnage increasing with advancing maturity compared to Delaney and Shoshone. Sainfoin had greater levels of CT than the alfalfa ($P \leq 0.001$), and within sainfoin varieties, tannin content decreased with increasing maturity ($P \leq 0.003$). Crude protein concentrations were significantly different for maturity ($P < 0.001$) and variety ($P \leq 0.003$), while fiber concentration was only affected by maturity ($P \leq 0.001$). These results demonstrate that sainfoin is a valuable legume to feed to
livestock particularly in grazing situations, with similar quality and yield compared to alfalfa, as well as a higher condensed tannin content.

Introduction

Production and quality are important attributes when considering forage selection. Alfalfa is often selected due to ease of establishment, persistence, high quality and production considerations (Bhattarai et al., 2016). Sainfoin has advantages compared to alfalfa, including non-bloating characteristics, no potential for autotoxicity, and possible anthelmintic qualities (Panditharatne et al., 1986; Carbonero et al., 2011; Bhattarai et al., 2016). Sainfoin can be hayed or grazed, either alone or in a grass-legume mix. Sainfoin is resistant to drought and tolerates freezing temperatures, making this legume appealing to farmers in cool semi-arid regions (Smith, 2007). Like alfalfa and other legumes, sainfoin can fix nitrogen biologically (Carbonero et al., 2011), reducing or eliminating the need and cost for nitrogen fertilizer.

Research is limited on the potential of sainfoin in semi-arid regions of the western United States. Our objective was to determine the DM yield of four sainfoin cultivars and an alfalfa check harvested at three different plant growth stages. Forage quality and condensed tannin content were also determined. Our hypothesis was that DM yield would be higher for alfalfa compared to sainfoin and there would be an increase in yield with increasing maturity. We hypothesized that the quality of sainfoin and alfalfa would be similar and that there would be a decrease in quality content with increasing maturity. Lastly, we hypothesized that the CT concentration would be higher in sainfoin than in alfalfa and that there would be a decrease in CT concentration with increasing maturity.
Materials and Methods

Field experiments were established at Moccasin, Montana (MOC; 47.05°, -109.95°; Danvers-Judith clay loam, USDA); Bozeman, Montana (BZN; 45.66°, -111.07°; Turner loam and Meadowcreek loam, USDA); and Logan, Utah (UT; 41.74°, -111.91°; Ricks Gravelly loam, USDA). In Spring 2018, plots were established in a randomized complete plot design in a split-plot pattern. Cultivar comprised whole plots and included four sainfoin cultivars (‘AAC Mountainview’ [AAC], ‘Eski’ [ESK], ‘Shoshone’ [SHO], and ‘Delaney’ [DEL]. ‘Shaw’ (SHA) alfalfa was included as a control at both MT locations and ‘Spredor 4’ (SPR) alfalfa was included as a control at the UT location. Whole plots were 9-meters by 4.5-meters and randomly placed in four blocks at each location. A 3-meter alley separated adjacent blocks. Harvest timing (10%, 50%, and 100% bloom) comprised subplots. Fencing was erected around the field experiment at each location to exclude antelope and deer.

Soil samples were collected prior to seeding at each location in Spring 2018. Samples were sent to Midwest Laboratories for analysis. Pre-plant potassium (K) and phosphorus (P) fertilizer and a glyphosate were applied, as needed in BZN. No fertilizer or herbicide was needed at the MOC location, while 50 kg/ha of potassium (0-0-60) and 45 kg/ha of P (11-52-0) were applied at UT. At the BZN and MOC locations, plots were seeded at a rate of 33.6 kg pure live seed (PLS)/ha for sainfoin and 13.5 kg PLS/ha for alfalfa. At the UT location, plots were seeded at 112 kg PLS/ha for sainfoin and 14 kg PLS/ha for alfalfa. Sainfoin seeding rates were high at UT to ensure sufficient stand establishment and to eliminate the need to reseed in year two of the study. Soil samples were collected at 15 cm and 30 cm using a soil corer (Soil Sampler Inc.,
Johns Creeks, GA) in Spring 2019. No additional fertilizer was needed at any location that year, based on soil test results.

Production and quality samples were taken at all locations (Table 2). Collection dates ranged from June 7th to August 18th (Table 2) across the three locations. When each plot reached appropriate maturity, a one square meter area was hand clipped to a height of 5 cm in each subplot to evaluate herbage mass production. Clippings were immediately weighed to obtain wet weights. Subsamples were collected and weighed to obtain subsample wet weight, then subsamples were dried at 60°C and re-weighed to obtain a dry weight. Subsample wet weights and dry weights were used to determine DM percentage. Plot production was then estimated by multiplying the whole sample wet weight by the DM percentage and used to estimate production on a kg/ha basis.

Subsamples were split, and then ground at the MSU Nutrition Center. Half of the samples were ground through a 2mm screen, with the other half of the samples were ground through a 2 mm screen followed by a 1 mm screen using a Wiley Mill (Swedesboro, NJ). The 1 mm samples were then sent to the Forage Plant Physiology Lab at Utah State University to evaluate condensed tannin (CT, butanol-HCl-acetone method) content. The tannin assay solution utilized ammonium iron sulfate dodecahydrate, hydrochloric acid, butyl alcohol and acetone (Grabber et al, 2013). The 2 mm samples were sent to the Forage Physiology Lab at University of Missouri to be analyzed for crude protein (CP, Dumas high pressure combustion method), acid detergent fiber (ADF, ANKOM method) and neutral detergent fiber (NDF, ANKOM method). Crude protein was analyzed using a Dumas combustion analyzer (Elementar, Rhine Main, Germany). Analysis for NDF (AOAC, 2005) and ADF (AOAC, 2005) were completed by using an Ankon
200 Fiber Analyzer (Ankom Co., Fairport, NY). Alpha-amylase and sodium sulfite were used in the NDF procedure. Statistical analysis was completed utilizing the GLM procedure of SAS 9.4 (Cary, NC). Experimental unit was identified as individual plot. Fixed effects were variety, maturity and the variety maturity interaction. Due to environmental differences, main effects of variety, treatment, and the maturity variety interaction were analyzed within location. Statistical differences were determined at $P \leq 0.05$.

Results and Discussions

Production

Forage DM yield averaged 5,033 kg/ha at the UT location. Dry matter yield was unaffected by variety selection ($P = 0.22$) and plant maturity ($P = 0.43$), nor was an interaction detected ($P = 0.55$; Figure 1, panel F). All cultivars were harvested on the same calendar days corresponding to 10% bloom (7 June), 50% (17 June), and 100% bloom (27 June) possibly confounding an ability to detect differences between cultivars and plant maturity. Growth stage was attained by sainfoin and alfalfa on the same calendar date at other locations included in this study, but there were several exceptions (Table 2). In particular, the alfalfa cultivar reached 50% and 100% bloom at later calendar dates than the sainfoin cultivar at both MOC and BZN (Table 2).

Harvest growth stage affected forage DM production when the alfalfa check was removed from the analysis at the UT location ($P = 0.006$; Figure 1, panel E). There was a 20% increase in DM production at 50% compared to 10% bloom, with an additional 13% increase at 100% compared to 50% bloom averaged across sainfoin cultivars. The increase in DM yield with
advancing maturity is similar to that research reported by Borreani et al. (2002) and Carlton et al. (1968) for sainfoin.

There was a significant impact of maturity \((P < 0.001)\) and growth stage \((P = 0.005)\) (Figure 1, panel D) on forage DM yield at the MOC location. Similar DM yields were seen at 10 and 50% bloom. There was a 17% increase in production from 50% to 100% bloom, while the second 10% cutting produced 59% less than the first 10% bloom sampling. The alfalfa cultivar produced 28% more DM yield than the sainfoin cultivars when averaged together. This increase in DM yield with increased stage of growth, as well as higher DM yields for alfalfa is similar to results seen by Borreani et al. (2002).

Harvest growth stage affected forage DM production when the alfalfa check was removed from the analysis at the MOC location \((P < 0.001; \text{Figure 1, panel C})\). There was no statistical difference between the 10 and 50% bloom. There was a 24% increase in DM yield from 50 to 100% bloom averaged across sainfoin cultivars.

In BZN, there was an interaction between maturity and variety \((P = 0.02, \text{Figure 1, panel B})\). When evaluating the differences between varieties within each maturity, all sainfoin varieties produced similar amounts at the 10% maturity. Shoshone, SHA, and DEL had the highest production when harvested at 50% maturity, while AAC had the lowest production. Eski and SHO harvested at 100% produced more than AAC, DEL and SHA at 100% bloom. At 100% bloom, SHA had the lowest production. Damage to SHA plots by the feeding of alfalfa weevil and grazing by deer that were able to jump over the protective fence, may explain the relatively poor performance of alfalfa at the BZN location.
An interaction between plant growth stage and cultivar selection was not detected ($P = 0.29$) when SHA was removed from the analysis. Plant maturity ($P < 0.001$) and variety selection ($P = 0.004$) affected forage DM production (Figure 1, panel A). There was 29% less DM production at 10% compared to 50% bloom, with 47% less DM production at 10% bloom compared to 100% bloom when averaged across sainfoin cultivars. AAC Mountainview and DEL, when averaged, produced 21% less than the SHO variety.

This research concluded that sainfoin has lower yield potential than alfalfa in the environments evaluated. When the growing season allows for regrowth, it is possible to graze or hay that regrowth. This was particularly true for the AAC variety at the MOC location. It was also observed that sainfoin varieties at the UT location, averaging 46 cm of annual rainfall, produced as much at 50% bloom compared to 100% bloom. By harvesting at an earlier bloom, 50% in high rainfall areas, or potentially two harvests at 10% where season allows, producers would be able to obtain yields similar to 100% bloom, while also producing higher quality forages.

**Quality**

**Condensed Tannins** There was a decrease in the CT content of sainfoin forage with increasing plant maturity at BZN and UT location ($P < 0.001$), as was previously reported by Borreani et al. (2002) and Wang et al. (2015; Figure 2). This is most likely due to increases in plant stem and fiber material relative to leaf tissue as plants age, since CT content is located mainly in the leaves (Skadhauge et al., 1997). At MOC, CT content was lowest at 10% bloom, which is not consistent with the other locations or previous research (Wang et al., 2015). Possible reasons for this could be environmental factors, such as frost before sampling or significant leaf
loss. This was not seen at this location before sampling, indicating further research is needed. The CT content was 96% higher in sainfoin than alfalfa at each of the three locations, comparable to previous research (Skadhauge et al., 1997, Wang et al., 2015). These high levels of CT in sainfoin explain the lack of bloat when forage is consumed (Wang et al., 2006), supporting the grazing of sainfoin pasture by beef cattle.

At both BZN and UT, 10% bloom had significantly higher CT content than 50 and 100% bloom ($P < 0.001$; Figure 2, panel A and E). In UT, the average CT content was 23% higher at 10% bloom than at 100% bloom. In BZN, the average CT content was 4% higher at 10% bloom than at 50% and 25% higher at 10% bloom than at 100% bloom. The difference in maturities is similar to previous research conducted by Borreani et al. (2002) and Wang et al. (2015). At the MOC location, there was an effect of maturity and variety ($P < 0.0001$ and $P = 0.04$, respectively, Figure 2). Higher levels of CT in the ESK and SHO varieties than in the DEL variety were seen, while AAC had similar levels to all other varieties at the MOC location. The CT content in the 10% bloom was the lowest, while 50% and the second 10% cutting were the highest.

With the higher levels of CT present in sainfoin, producers are able to feed it alone or in a mix, with lower risk of bloat. One of the concerns with a high tannin forage is its bitter flavor. However, research indicates that voluntary intake of sainfoin was higher than alfalfa (Parker and Moss, 1981). The reason why the tannins do not affect palatability of sainfoin is unknown. By growing a highly palatable, CT-containing forage, producers are able to supply a high-quality legume forage without risk of bloat.
While not seen at all locations, higher levels of CT are noted in the beginning stages of growth at two of the locations. This decrease with maturity is associated with a reduction in CT concentrations within the leaves, where most of the CT exists (Li et al., 2014; Wang et al., 2015). Previous research has shown that CT is related to decreased incidence of bloat, as well as decrease of internal parasites in livestock (Paolini et al., 2004; Wang et al., 2006). If producers are looking to decrease risk of bloat or use sainfoin as an anthelmintic, grazing in the early stages of growth, when CT levels and quality are high is the best option.

**Acid and Neutral Detergent Fiber** At the MOC and BZN locations, there was an interaction between maturity and variety for NDF and ADF ($P < 0.002$ and $P < 0.003$, respectively, Figure 3 and 4). The alfalfa had slightly higher levels of NDF and ADF concentrations compared to the sainfoin varieties, which is in agreement with research conducted by Rufino-Moya et al. (2019). At the 10% sampling, DEL, SHA, and SHO had higher levels of NDF than AAC and ESK, while ADF was similar across all 10% cultivars at the MOC location. Similar levels of ADF and NDF were noted across all cultivars at the 50% sampling. At the 100% sampling, ESK, SHA, and SHO had higher levels of NDF than AAC and DEL and all cultivars had higher ADF levels than AAC at the MOC location. At the BZN location, SHA had higher levels of ADF and NDF than sainfoin cultivars at 50 and 100% bloom. A 10% sample of SHA was not collected due to complications with alfalfa weevil at the BZN location.

At the UT location, the NDF concentrations at 10% maturity were 10% and 8% less than the 50 and 100% maturities, respectively ($P = 0.004$; Figure 3). The concentrations for ADF were on average 14% and 10% lower at the 10% maturity than the 50 and 100% maturities, respectively ($P = 0.007$; Figure 4). This increase in ADF and NDF with maturity is similar to
research done by Rufino-Moya et al. (2019) and is expected as fiber increases with increasing maturity.

When alfalfa was removed from the analysis, there was an effect of maturity on ADF ($P = 0.003$) and NDF ($P = 0.002$) in UT (Figure 3 and 4). There was an increase of ADF and NDF with increasing maturity, where 50% and 100% were higher than the 10% bloom. At the MOC location, there was an effect of maturity on ADF ($P < 0.001$) and an interaction between maturity and variety for NDF ($P = 0.03$). For ADF, the 10% and 50% harvests were similar, while the 10% and 100% harvests were similar. The second 10% harvest had lower levels than all other harvests. For NDF, there was no difference between sainfoin varieties at 10% or 50% bloom. At the 100% harvest DEL, ESK and SHO had higher levels than AAC. At the second 10% harvest, AAC and DEL had higher levels of NDF than ESK and SHO.

In BZN, there was an interaction between maturity and variety for ADF ($P = 0.004$, Figure 4), as well as an interaction between maturity and variety for NDF ($P = 0.03$; Figure 3). There was no significant difference between the varieties 10% and 100% bloom for ADF. At 50% bloom, DEL had higher levels of ADF than all other sainfoin varieties. For NDF at the BZN location, 100% bloom was similar across all varieties. Shoshone had the highest NDF levels at 10% bloom and DEL and SHO had the highest at 50% bloom.

While there are some differences in terms of ADF and NDF between varieties, this depends on location and stage of growth. Producers should harvest earlier in the growing season if they are wanting the highest quality forage in terms of ADF and NDF. Based on what was seen in MOC, ADF and NDF levels are lower at both the 10% cuttings. If the season allows, harvesting or grazing a second cutting will produce high yields with high quality forage. With
increasing maturity there is a decrease in leave to stem ratio, meaning there is more fiber in the forage, leading to higher contents of ADF and NDF (Ball et al., 2001). By harvesting at an earlier maturity, there will be lower levels of ADF and NDF and the forage will be more digestible for the animal. This allows for better utilization of the nutrients within the forage.

**Crude Protein** At MOC and UT, there was an impact of both maturity and variety ($P < 0.001$ and $P < 0.003$, respectively; Figure 5, panel D and F) on CP content. At the BZN location, there was a maturity effect on CP ($P = 0.001$, Figure 5, panel B). There was a 9% decrease in CP from the 10% bloom harvest to the 50% harvest and a 10% decrease from the 50% harvest to the 100% sampling. At all locations, CP decreased with increasing maturity, similar to results in other research (Ball et al., 2001; Parker and Moss, 1981). At MOC, SHA had higher CP (13%) than the sainfoin varieties (11%) averaged across all maturity levels (Figure 5, panel D). This was also seen in previous research by Rufino-Moya et al. (2019). At the UT location, SHA and ESK had higher CP content than AAC, DEL and SHO (Figure 5, panel F). Alfalfa and ESK contained 10% CP and the other sainfoin varieties contained 9% CP, when averaged across all maturities. When alfalfa was removed from the analysis, there was an effect of maturity across locations ($P < 0.001$), where CP decreased with increasing maturity. At the UT location, there was also an effect of variety ($P = 0.05$) on CP content. Both ESK and AAC had higher levels than DEL and SHO.

Both alfalfa and sainfoin are good protein sources and can provide livestock with high quality protein. Proteins provide the essential amino acids livestock need for organ function, tissue, hair, skin, and enzymes. Amino acids are needed for growth, reproduction and maintenance (Shapiro, 2011). While alfalfa did have higher CP content than the sainfoin
cultivars, it should be noted that the sainfoin CP content was similar between sainfoin varieties. Harvesting at 10% maturity would provide the highest CP containing forage for livestock.
CHAPTER THREE

CONCLUSION

Sainfoin is a legume forage that has many benefits compared to more conventional legumes such as alfalfa, including non-bloating characteristics, no potential for autotoxicity, as well as some preliminary evidence that it may work as an anthelmintic in consuming livestock. This study evaluated the effect of maturity and variety on forage quality and yield.

It was concluded that alfalfa had a higher yield potential across harvest timings compared to sainfoin, which was hypothesized at the beginning of this study. Sainfoin had much higher CT content than alfalfa, allowing for livestock consumption with a lower chance of bloat occurring. The alfalfa did have higher levels of CP than the sainfoin varieties, however all sainfoin varieties had similar levels when compared across similar maturities. Neutral detergent fiber and ADF content depended on location, cultivar and stage of maturity. There were variations between locations, indicating environment plays a significant factor in the quality and yield potential of sainfoin. When the growing season allows for regrowth, farmers can harvest sainfoin twice at 10% bloom and have high quality forage for grazing or haying.

Even though sainfoin had lower yields and CP content, it can still be a viable option for many producers, including those who are looking for a forage that doesn’t cause bloat and has no potential for autotoxicity. If alfalfa weevil is a concern, sainfoin would provide producers a high-quality leguminous forage source that does not have a risk of being impacted by alfalfa weevil.

One of the main concerns a producer will have to anticipate with growing sainfoin is wildlife. At the BZN location, there was a problem with deer, despite the deer fence surrounding the plots. At the MOC location, electric fence was used and seemed to be more beneficial at
keeping out wildlife. This is something that needs to be addressed and will be an added expense when sainfoin is grown and utilized.

Sainfoin provides many benefits to livestock. This research indicates that sainfoin can be a replacement to alfalfa in some cases. Further research is needed to continue to evaluate the yield and quality potential of sainfoin, as well as best management recommendation for growers and livestock producers.
Table 1. Chemical composition and plant components of alfalfa and sainfoin at three stages of maturity described by Rufino-Moya et al. (2019).

<table>
<thead>
<tr>
<th>Item</th>
<th>Alfalfa</th>
<th>Sainfoin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vegetative Flowering</td>
<td>Late Flowering</td>
</tr>
<tr>
<td>Quality, g/kg</td>
<td>249 261 333 241</td>
<td>262 224</td>
</tr>
<tr>
<td>DM</td>
<td>103 111 98 83</td>
<td>113 82</td>
</tr>
<tr>
<td>Ash</td>
<td>227 207 116 204</td>
<td>201 181</td>
</tr>
<tr>
<td>CP</td>
<td>336 352 405 313</td>
<td>324 394</td>
</tr>
<tr>
<td>ADF</td>
<td>201 230 276 199</td>
<td>213 264</td>
</tr>
<tr>
<td>NDF</td>
<td>45 53 66 78</td>
<td>76 76</td>
</tr>
<tr>
<td>Lignin</td>
<td>18 10 16 22</td>
<td>15 11</td>
</tr>
<tr>
<td>Ether extract</td>
<td>317 320 316 379</td>
<td>347 332</td>
</tr>
<tr>
<td>NSC</td>
<td>59.3 53.7 42.7 66.3</td>
<td>56.0 38.5</td>
</tr>
<tr>
<td>Plant components, %</td>
<td>40.7 43.7 51.3 33.7</td>
<td>40.8 44.6</td>
</tr>
</tbody>
</table>

Abbreviations: ADF- acid detergent fiber; CP- crude protein; DM- Dry matter; NDF- neutral detergent fiber; NSC- nonstructural carbohydrates.
Table 2. Sampling dates for sainfoin varieties and alfalfa check at three locations in 2019.

<table>
<thead>
<tr>
<th>Maturity</th>
<th>Bozeman, MT</th>
<th>Moccasin, MT</th>
<th>Logan, UT</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% (first cutting)</td>
<td>7/1/19 (AAC, DEL, SHO)</td>
<td>6/14/19 (all varieties)</td>
<td>6/7/19 (all varieties)</td>
</tr>
<tr>
<td></td>
<td>7/5/19 (ESK)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>7/11/19 (AAC, SHO, ESK)</td>
<td>6/27/19 (DEL, AAC, SHO)</td>
<td>6/17/19 (all varieties)</td>
</tr>
<tr>
<td></td>
<td>7/16/19 (DEL)</td>
<td>7/1/19 (ESK)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7/24/19 (SHA)</td>
<td>7/8/19 (SHA)</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>7/24/19 (AAC, SHO, DEL)</td>
<td>7/1/19 (DEL, AAC, SHO)</td>
<td>6/26/19 (all varieties)</td>
</tr>
<tr>
<td></td>
<td>7/31/19 (ESK, SHA)</td>
<td>7/8/19 (ESK)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7/15/19 (SHA)</td>
<td></td>
</tr>
<tr>
<td>10% (second cutting)</td>
<td>NA</td>
<td>8/18/19 (all varieties)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: AAC - AAC Mountainview, DEL - Delaney, ESK - Eski, SHA - Shaw, SHO - Shoshone
Figure 1. Production (kg/ha) of four varieties of sainfoin and one alfalfa variety at three locations. Panel A: Bozeman location without SHAW; Panel B: Bozeman location with SHAW; Panel C: Moccasin location without SHAW; Panel D: Moccasin location with SHAW; Panel E: Utah location without Spredor 4; and Panel F: Utah location with Spredor 4. m-mMeans within maturity with differing superscripts are significantly different (P ≤ 0.05). x-xMeans within variety with differing superscripts are significantly different (P ≤ 0.05).
Figure 2. Condensed tannin content (mg/g) of four varieties of sainfoin and one alfalfa variety at three locations. Panel A: Bozeman location without SHAW; Panel B: Bozeman location with SHAW; Panel C: Moccasin location without SHAW; Panel D: Moccasin location with SHAW; Panel E: Utah location without Spredor 4; and Panel F: Utah location with Spredor 4. a-g Means within variety*maturity with differing superscripts are significantly different ($P \leq 0.05$). m-o Means within maturity with differing superscripts are significantly different ($P \leq 0.05$). x-z Means within variety with differing superscripts are significantly different ($P \leq 0.05$).
Figure 3. Neutral detergent fiber content (%) of four varieties of sainfoin and one alfalfa variety at three locations. Panel A: Bozeman location without SHAW; Panel B: Bozeman location with SHAW; Panel C: Moccasin location without SHAW; Panel D: Moccasin location with SHAW; Panel E: Utah location without Spredor 4; and Panel F: Utah location with Spredor 4. a–h Means within variety&maturity with differing superscripts are significantly different ($P \leq 0.05$). m–n Means within maturity with differing superscripts are significantly different ($P < 0.001$).
Figure 4. Acid detergent fiber content (%) of four varieties of sainfoin and one alfalfa variety at three locations. Panel A: Bozeman location without SHAW; Panel B: Bozeman location with SHAW; Panel C: Moccasin location without SHAW; Panel D: Moccasin location with SHAW; Panel E: Utah location without Spredor 4; and Panel F: Utah location with Spredor 4. a-g Means within variety*maturity with differing superscripts are significantly different ($P \leq 0.05$). m-MMeans within maturity with differing superscripts are significantly different ($P \leq 0.05$).
Figure 5. Crude protein content (%) of four varieties of sainfoin and one alfalfa variety at three locations. Panel A: Bozeman location without SHAW; Panel B: Bozeman location with SHAW; Panel C: Moccasin location without SHAW; Panel D: Moccasin location with SHAW; Panel E: Utah location without Spredor 4; and Panel F: Utah location with Spredor 4. "m" means within maturity with differing superscripts are significantly different (P ≤ 0.05). "x" means within variety with differing superscripts are significantly different (P ≤ 0.05).
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