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Strategies to Improve Forage Utilization by Sheep Offered Forage Mixtures

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Strategies to Improve Forage Utilization by Sheep Offered Forage Mixtures

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by

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ABSTRACT

In the first experiment, alfalfa and novel endophyte-infected tall fescue (NE+) forages were harvested after a killing frost, then mixed to formulate 4 different treatments; alfalfa alone, 67% alfalfa +33% fescue, 33% alfalfa + 67% fescue, or 100% fescue. After 3 months of storage as silage, Dorper ewe lambs (n = 20; mean BW = 34.7 ± 6.65 kg) were fed silage for ad libitum consumption, using 5 animals per treatment. Increasing the proportion of NE+ tall fescue improved ($P < 0.05$) silage total acids and lactic acid concentrations and decreased silage ammonia concentration. Digestible dry matter and organic matter intake and nitrogen utilization parameters decreased with increasing inclusion of NE+ in diet. In the second experiment, 16 Dorper ewe lambs (41.8 ± 4.61 kg BW) were assigned to 4 different treatments; alfalfa silage alone (0 g/kg; CONT) or alfalfa silage mixed with chopped sericea lespedeza (SL) hay to provide 90 (LOW), 180 (MED), or 270 g/kg SL (HIGH) on a dry matter basis in a randomized complete block design experiment with 2 period to provide 4 observations per treatment for each experimental period. Increasing the proportion of sericea lespedeza in the diet decreased dry matter and organic matter digestibility but did not affect feed intake. Fecal nitrogen (g/kg N intake) increased linearly ($P < 0.01$) while urinary N (g/ day and g/ kg of N intake) tended to decreased linearly and quadratically ($P \leq 0.1$) with increasing the proportion of SL in diet. In the third experiments, 16 gestating Dorper ewe lambs (49.1 ± 4.61 kg BW) were allocated to 4 treatments; alfalfa silage alone (0% g/kg; CONT) or alfalfa silage mixed with lablab purpureus(LP) hay to provide 90 (LOW), 180 (MED), or 270 g/kg LP (HIGH) on a dry matter (DM) basis, in a randomized complete block design experiment with a total 8 replication per treatment. Supplementation of LP in diet increased quadratically ($P \leq 0.04$) forage dry matter, organic matter intake, digestible dry matter and digestible organic matter intake ($P < 0.05$).

Nitrogen apparently absorbed and urinary N both decreased linearly ($P < 0.01$) with adding more LP hay in diet. Harvesting and mixing alfalfa and fescue after a killing frost improved silage fermentation characteristics and supplementation of tannins from SL and polyphenol from LP altered N excretion.

Key words: lablab, sericea lespedeza, alfalfa silage, nitrogen, sheep.

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CHAPTER I

INTRODUCTION

Forage-based feed source continues to occupy a large part in ruminants' production feeding systems. However, ruminants are inefficient in terms of feed conversion, especially carbon and nitrogen utilization compared to other livestock production systems. In the US, silage producers lose $2 billion each year due to poor silage management practices Grant and Adesogan, 2018. Feeding low quality silage negatively affects forage utilization by ruminants (Niyigena et al., 2019). Variation in temperature, especially during the fall season, limits the growth of forage and is associated with forage losses. In addition, harvesting forage and drying hay is challenging than silage making due to limited solar radiation and shorter days (Coblenz et al., 2016).

Silage production could be an alternative to hay making during the fall, and mixing grasses and legumes improves silage fermentation quality and utilization by ruminants (Samuil et al., 2015). Feeding a high protein diet to ruminants causes a negative effects on the environment. Agriculture accounts for 9% of total greenhouse gas emissions in the US where nitrous oxide (N_2O) makes 69% of these agricultural GHG emissions (USDA, 2016). Over a quarter of total global methane emissions is released by livestock (Ogino et al., 2007) and these gas productions cause negative impacts on animal performance (Johnson and Johnson, 1995). Different feeding strategies have been adopted, including diet manipulation, to mitigate production of these gases. Supplementation with tannins and polyphenols in the diet may improve N utilization and reduce the release of nutrients into the environment. Multiple feeding strategies to improve forage utilization in ruminants and mitigate negative impact of livestock to the environment were well documented in previous studies. However, producers are challenged to address different environmental conditions of forage production and conservation and protein utilization by ruminants. Therefore, using sericea lespedeza and lablab purpureus as tannin and phenol sources to improve forage utilization by ruminants will be worthy of investigation

OBJECTIVES OF THE STUDY

1) To determine the effect of ensiling mixtures of alfalfa and tall fescue harvested after a killing frost on silage fermentation characteristics and forage utilization by sheep

2) To study the impact of supplementation of different levels of tannins from sericea lespedeza to alfalfa silage on forage utilization and nitrogen balance by lambs

3) To investigate the influence of adding different concentrations of polyphenols from lablab purpureus to alfalfa silage on intake, digestibility and nitrogen utilization by ewes

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CHAPTER II

REVIEW OF LITERATURE

Overview of feed utilization by ruminants

The world currently has 19.6 billion chickens, 1.4 billion cattle, 1.87 billion goats and sheep, and 980 million pigs (Robinson et al., 2014). Ruminants are the main source for red meat, milk, and fiber. Production systems for ruminant animals constitute an advantage because ruminant animals use feedstuffs that are mostly inedible by humans. However, ruminants are the most inefficient production system in terms of feed conversion and carbon-nitrogen footprint compared to other meat production systems. The digestive system of ruminants is designed to digest fiber from forages, ferment feedstuffs, and obtain energy. Feeds are digested in the rumen by a diverse group of microbes, anaerobic bacteria, fungi and protozoa, which live in a symbiotic and anaerobic environment (Forsberg and Cheng, 1992). Diets of ruminants are mainly composed of carbohydrates, especially nonstructural carbohydrates and structural (cell wall) polysaccharides such as cellulose, hemicellulose and pectin. One of challenges that occur in the digestion process is the insolubility and the inaccessibility of cell wall components, which limits the rumen fermentation process (Nagaraja et al., 1997).

Ruminants do not utilize protein efficiently especially when they are offered feedstuffs with low C: N ratios. These forages usually result in greater urine N and may cause negative impacts on the environment. Rumen fermentation results in the production of carbon dioxide (**CO_2**) and methane (**CH_4**), which also reduces feed efficiency and production by animals. In order to correct this problem, the manipulation of ruminal function to improve efficiency has gained an important focus in modern livestock production. Therefore, the objective of this literature review will be to explore different management practices to improve forage utilization and conservation and the subsequent effect on animal performance. The second objective will focus on the impact of livestock on the environment, feeding strategies to improve feed efficiency, especially protein, and the role of tannins or polyphenols in ruminant nutrition.

Alfalfa

Alfalfa is the one of the most widely grown forage crops in the US and worldwide and has multiple benefits: high feed value, yield, and the capacity to fix atmospheric N into the root system. Moreover, alfalfa has high digestible energy and protein, which make it a valuable feed source for ruminants, and it can be consumed fresh or as hay or silage (Lacefield et al., 2009). Alfalfa is palatable and can be used to stimulate intake by livestock that are fed low quality forage. Supplementation of alfalfa pellets to beef cattle grazing dormant tall grass prairie increased forage dry matter intake and digestible dry matter intake (DelCurto et al., 1990).

Different factors can affect alfalfa forage quality such as maturity, forage conservation, and environmental conditions. Nutrients content of alfalfa can be affected by forage maturity, with greater nutrient value occurring during the vegetative stage. For example, in the vegetative stage, crude protein (CP) can range from 24 to 27%, neutral detergent fiber from 25 to 37%, and total digestible nutrients from 68 to 75%, while during the late bloom, crude protein can range between 9 and 13%, neutral detergent fiber between 56 to 60%, and total digestible nutrients from 50 to 57 % (Ball et al., 2007).

Packaging alfalfa hay can reduce nutrient value through leaf loss, where the effect of handling alfalfa hay can cause a dry matter loss of 34 % of the forage mass, and protein content loss of 44% of total forage protein. (Lacefield et al., 2009). Alfalfa can be preserved as silage as well, but ensiling alfalfa is very challenging because of low fermentable carbohydrates, high buffering capacity and stem structure that facilitates air absorption while packing forage in silos (Marshall et al., 1993). Producing silage can change the proportion of the forage components as well as forage utilization by the animals. According to Dewhurst and King (1998), forage composition, especially fatty acids may change with the forage conservation methods. In a study conducted to compare the effect of feeding fresh or alfalfa silage to Holstein dairy cows on milk composition, feeding fresh alfalfa lowered the saturated and increased polyunsaturated fatty acids compared with feeding alfalfa silage (Whiting et al., 2004).

Environmental conditions also can affect alfalfa forage quality and productivity. Seasonal changes can affect alfalfa yield and quality, with a sharp decline in crude protein during the late summer due the shorter days and low solar radiation which causes a fall dormancy response by plants. Hence, the plant favors root growth more than shoot growth (Putman and Ottman, 2013).

Alfalfa can be mixed with forage grass species for better forage management and utilization. Mixing alfalfa with orchardgrass at 50:50 ratio produced better quality silage than ensiling alfalfa alone (Samuil et al., 2015). Growing grass and legume forage mixtures has advantages as well. For example, growing alfalfa with ryegrass produced more yield than planting alfalfa alone (Schneider and Undersander, 2008). In another study, mixing alfalfa with tall fescue improved yields and higher-quality forage compared to growing alfalfa alone (Tracy et al., 2016).

Tall fescue

Tall fescue is a perennial, cool-season forage, which occupies over 15 million ha in the US and was first brought to the US from Europe in the 1800'S (Young et al., 2013, Ball et al., 1993). Two cultivars of tall fescue, Alta and Kentucky 31 (KY-31), were first planted extensively in the 1940s, and tall fescue gained popularity because of its resistance to pest and diseases.

Multiple factors can affect nutrient content in tall fescue. Crude protein (CP) in tall fescue can range from 14.1% in pasture but during summer it can drop down to below 10%. However, different forage management practices can change forage CP composition. Fertilization of tall fescue increases CP content and dry matter digestibility (DMD), and decreases neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations (Fribourg et al., 2009).

An endophyte fungus found in tall fescue produces ergot alkaloids, which can cause negative effects on animal production and performance such as loss of body weight, increased body temperature, agalactia, low conception rates, low survival of offspring, and fescue foot (Fribourg et al., 2009). In order to alleviate the toxicity problems, an alternative fescue was developed, novel endophyte (NE+) fescue, which contains less toxin than the endophyte-infected tall fescue. Feeding NE+ tall fescue to animals presents the advantage of improving animal performance; however, the NE+ does not appear to be as resistant to drought, overgrazing, and diseases compared to endophyte-infected tall fescue.

Several studies have been conducted comparing endophyte-infected and endophyte-free fescue. A study conducted in Georgia compared cattle performance when stocker cattle were grazing endophyte-infected (AR542 or AR502), endophyte-free (E−), or wild-type E+. Steers grazing E+ pastures had lower DMI than steers grazing AR542 and E− pastures during spring and lower DMI than steers grazing E− pastures during autumn (Parish et al., 2003). Another study was conducted at the University of Arkansas where grazing NE+ tall fescue improved calving rate, calf weaning weight, and resulted in greater economic return per acre compared to grazing E+ (Smith et al. 2012; Caldwell et al., 2013). Other studies (Washburn and Green, 1991; Coblentz et al., 2006) also found that cows grazing E+ resulted in lower calving rate than cows grazing low or endophyte-free fescue.

The accumulation of non-structural carbohydrates (NSC) in a plant occur when carbohydrates produced during the photosynthesis process exceed the required amount for plant growth and maintenance (Watts and Chatterton, 2004). Cool-season forages store a large amount of NSC as fructan in their stems (Longland and Byrd, 2006). Cool-season grasses produces more fructan and total non-structure carbohydrates compared to warm-season forages and legumes (Jensen et al., 2014). Environmental conditions can change the extent of carbohydrate storage in the plant. Previous studies have found some important variables in accumulation of carbohydrates such as seasonal variations (Shewmaker et al., 2006) and diurnal variation (Lechtenburg et al., 1972). Tall fescue stores more water soluble carbohydrates at temperatures ranging from 10 and 16^{o}C compared to the temperature range of 21 and 26^{o}C (Labhart et al. (1983). Low temperatures, especially the first killing frost, can improve the retention of carbohydrates in the stems of the plant. Low temperatures reduce plant respiration and growth and minimize plant metabolite losses such as water and CO_2 but allow photosynthesis to continue and plants to store more carbohydrates (Pollock et al., 1983).

On the other hand, higher temperatures speed the maturity of the plant, stimulate seed-head formation, and depress plant height, which in turn can have negative impacts on forage yield. Forage dry matter digestibility was reported to decline due to warmer experimental conditions (Donaghy et al., 2008).

Weather conditions that favor the production of WSC are critical in order to improve forage quality. Ensiling forage with high content of WSC improves fermentation characteristics by reducing pH through increasing lactic acid content and silage total acids (Downing et al., 2008). Under silage fermentation, some of the WSC are converted into lactic acid which is necessary for preserving silage.

Environmental concerns from agriculture

Agriculture commodities bring in $330 billion of gross income each year and livestock contributes half of this value (USDA, 2007), but changes in climate conditions alter the stability of the food supply (Hatfield et al., 2014). Agriculture accounts for 9% of total greenhouse gas emissions in the US, where nitrous oxide (**N_2O**) makes 69% of these agricultural GHG emissions (USDA, 2016).

Greenhouse gas emissions from livestock

Methane makes up to 50% of greenhouse gas (GHG) emissions from livestock (Ogino et al., 2007) and production of methane reduces feed efficiency and energy utilization by cattle (Johnson, 1995). Reducing enteric methane emissions through dietary manipulation can enhance forage utilization as well as increase production and performance by ruminants while optimizing environmental protection. Reducing methane emission by 25% can increased body weight (BW) gain of growing cattle by approximately 75 g/d or milk production of dairy cows by 1 L/d (Lee and Beauchemin, 2014).

Ruminants can convert a large part of organic N from plant biomass into the reactive and bioavailable forms, which are mainly excreted in the urine (Chadwick et al., 2018). Nitrous oxide emissions related to deposition of urine N in grazing environments are a major greenhouse emission from livestock (Selbie et al., 2015). The excess application of N in environment above the plant requirements can also result in N losses in forms of nitrate leaching (Di and Cameron, 2007), and ammonia volatilization (Burchill et al., 2017). Reducing N losses can be accomplished by using nitrification inhibitors (Hatch et al., 2005), increasing the proportion of hippuric acid in the urine (Clough et al., 2009), or limiting feeding protein above animals' requirements because increasing protein intake above the requirement can lead to an increases of N excretion in urine (Reed et al., 2015)

Different approaches have been attempted in order alter rumen function and mitigate the effect of livestock on the environment. Tannins have the affinity of binding to proteins and make them less available in the rumen, thereby changing rumen microbial populations, which can also change the proportion of rumen volatile fatty acids (Barry, 1983). Supplementation of tannins from sericea lespedeza was reported to reduce methane emission in goats (Puchala et al., 2005) and in cattle (Beauchemin et al., 2007) compared with feeding diets without tannins. However, feeding greater tannin concentrations (5-9% of total diet DM) was reported to depress intake and digestibility in lambs (Tiemann et al. 2008).

Plant secondary metabolites

Plant secondary metabolites are produced by the process of elicitation. These compounds include alkaloids, glycosides, flavonoids, volatile oils, tannins, and resins, and they improve plant survival, persistence and competitiveness (Namdeo, 2007).

Polyphenols

Polyphenols have gained much attention in recent years due to their important role in human health as anti-oxidants, and their consumption was linked to protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative disorders (Pandey and Risvi, 2015). Polyphenols are mostly found in fruits, vegetables and cereals. Some plants, such as grapes and cherries contain between 200 and 300 mg of polyphenols per 100 g of fresh weight. Polyphenols also plays an important role in ruminant nutrition, especially by improving protein utilization and N use efficiency (Broderick and Albrecht, 1997).

Type of polyphenols

There are over 8000 phenolic compounds that are found in different plant species. Polyphenols are classified in different categories based on number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes comprise phenolic acids, flavonoids, stilbenes and lignans (Spencer et al., 2008). Phenolic acids are divided into two categories, derivatives of benzoic acid and derivatives of cinnamic acid, while flavonoids are divided in six subclasses, including flavonols, flavones, flavanones, flavanols, anthocyanins, and isoflavones. Flavonoids are responsible for color attraction in fruits, leaves, and flowers.

Polyphenols in animal production

Polyphenols can bind to protein and increase rumen-escape protein and thereby have potential to have significant impacts on ruminal fermentation (Mueller-Harvey, 2019) and fecal and urine N excretion (Powell et al., 2009). Polyphenol peroxidase in red clover was reported to decrease protein degradation in silos during silage production, improve true protein content in silage, and subsequently, improve N-use efficiency when the silage was fed to ruminants (Michael and Lee, 2014). Moreover, polyphenols reduced lipolysis in silos, thereby increasing the deposition of C18 polyunsaturated fatty acid (PUFA) in animal products.

The effect of polyphenols on protein metabolism in ruminants is mainly to induce the complexing of leaf proteins, which in turn reduces the solubility and degradability of protein in the rumen. The suppression of protein degradation in the rumen increases the flow of non-ammonia-N and undegraded dietary protein to the small intestine, which in the end leads to a decline in the absorption of ammonia across the rumen wall and urinary-N excretion.

Feeding polyphenols from essential oils decreases methanogenesis by altering protein degradation in the rumen (Newbold et al., 2004). Dietary manipulation by feeding polyphenols can change the nitrogen partitioning, especially urea. Urea constitutes 78% of the total N-containing urine components on average. Other urine components include hippuric acid at 5.5% and ammonia at 2% (Dijkstra et al., 2013), and altering the proportion of these urine components has an impact on N volatilization in the environment. Urinary N is mostly lost through ammonia and nitrous oxide volatilizations, and urea is the urine constituent that is more prone to volatilization.

Previous studies have reported an increase in hippuric acid content of the urine after consumption of diets containing phenolic compounds (Toromanović et al., 2008). Increasing the concentration of hippuric acid in urine decreased nitrous oxide emissions by 65% when urine was applied to the soil (Bertram et al., 2009). Therefore, identifying different forages that contain polyphenols and understanding how best to include them in animal diets can be a sustainable approach to mitigate greenhouse gases emissions from livestock production systems.

Considerable effort has been expended to promote the use of polyphenols from plant species, but some of these forages have lower productivity and diseases resistance. *Lablab purpureus* is a forage legume that presents numerous advantages: adaptability, drought resistant, the ability to grow in a diverse range of environmental conditions, and a good source of polyphenols (Murphy and Colucci, 1999). A study conducted by Gwanzura et al. (2012) comparing nutrient composition and tannin content found that *lablab preperus* had greater total polyphenol compound content (1.24 mg/ g) than sorghum Sudanese (0.17mg/g,) cowpea (0.05 mg/g) and *mucuna pruriens* (0.35 mg/g). In addition, feeding lablab hay mixed with Katambora hay to goats reduced enteric methane emissions (Washaya et al., 2018).

Tannins

There are two main categories of tannins: proanthocyanidins (PA) or condensed tannins and hydrolyzable tannins (HT). Proanthocyanidins are flavonoid polymers. Hydrolyzable tannins are polymers of gallic or ellagic acid esterified to a core molecule, mostly glucose or a polyphenol such as catechin (Reed, 1995).

Condensed tannins

Condensed tannins (CTs) make up to 20% of the dry matter in forage legumes that are used in ruminants' diets. The CT play an important role in forage-livestock production systems by reducing bloat, improving farm profitability, controlling parasites, and reducing greenhouse gas and ammonia emissions (Mueller-Harvey et al., 2019). Another important role of CT is to protect dietary protein from excessive fermentation in the rumen. Tannins have the capacity to bind to proteins, thereby reducing rumen proteolysis, which results in greater proportion of protein escaping the rumen to the small intestine (Waghorn, 2007). However, when dietary CT are too large and protein content is too small, CT may reduce forage utilization by the animal (Cooper et al., 1988). The CT forage can be grazed but CT should be diluted with other tannin-free forages (Waghorn and Shelton, 1997). The N loss is greater in urine than in feces, generally, but supplementing CT in the diet can cause a reduction in urinary N and increase fecal N excretion and thereby reduce N loss by the animal (Terrill et al., 1994). Previous studies from an integrated farm system model have shown that a shift of N from urine to feces can reduce a quarter of N losses, thereby reducing fertilizer input (Zeller and Grabber, 2015). The CT can improve animal health by suppressing gastrointestinal nematodes. This suppression stems from the ability of CT to bind with proteins (Hoste et al., 2012) or by inhibiting key parasite enzymes such as glutathione-S-transferases that play a crucial role in detoxification (Hansen et al., 2016). Silage fermentation can reduce apparent concentration of CT by 30% (Mena et al., 2015) but sericea lespedeza silage still exhibited anthelmintic properties by decreasing fecal worm egg counts (Whitley et al.,2018). There are many different forages that contain tannins. Sericea lespedeza, a perennial woody legume that is found mostly in the western and southern US has 18% tannins (Mueller-Harvey, 2006); however, it is considered a weed species in some areas.

Conclusion

It has been documented extensively that understanding the role of different forage species and their effects on forage quality and utilization by ruminants are critical for forage production and conservation management. Ruminants are not efficient in terms of protein utilization, and N excretion from ruminants pose a threat to environment. Feeding plants that produce secondary metabolites, such as tannins and polyphenols, can improve nitrogen use and efficiency in ruminants, but further work is needed to optimize nitrogen use through determination of optimal forage combinations that have the greatest impact at the animal and landscape level.

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CHAPTER III

Intake, digestibility rumen fermentation and nitrogen balance in sheep offered alfalfa and tall fescue-mixtures harvested and ensiled after a killing frost

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ABSTRACT

The objective of this study was to determine the effect of harvesting alfalfa (*Medicago sativa*) and tall fescue *(Schedonorus arundinaceus)* after frost and ensiling mixtures of these forages on silage fermentation characteristics, intake, digestibility and ruminal fermentation by ewes. Forages were harvested in October of 2018, wilted, and blended as either alfalfa alone, 67% alfalfa + 33% novel endophyte-infected tall fescue, 33% alfalfa + 67% fescue, or 100% fescue. Twenty bins were lined with 2 plastic bags, packed (n = 5/trt) with the respective forages, stored for 3 months, and then assigned randomly to Dorper ewe lambs (n = 20; mean BW = 34.7 ± 6.65 kg) and offered for ad libitum consumption. Lambs were allowed 17 d of adaptation followed by 5 d of total feces and urine collection. Data were analyzed using PROC-MIXED of SAS and orthogonal polynomial trends were used to identify the effects of different proportions of fescue-to-alfalfa silage. Silage total acids, lactate, and acetate increased linearly ($P < 0.01$), while silage ammonia decreased linearly ($P < 0.01$) with increasing proportions of fescue in the silage. The proportions of lactate-to-total acids (mole/100 moles) increased linearly ($P < 0.01$) and quadratically ($P < 0.05$) with increasing fescue-to-alfalfa ratio. Intake (g/kg BW), digestibility (%), and intake of digestible dry matter and organic matter (g/kg BW) decreased linearly ($P < 0.01$) as fescue proportion increased in the silages. Ruminal acetate increased ($P < 0.01$) and butyrate and valerate decreased linearly ($P < 0.01$) with increasing fescue concentration in the silage. All N utilization measurements decreased with increasing fescue concentration in the silage ($P < 0.01$). Ensiling alfalfa with fescue may improve forage fermentation characteristics, but may reduce intake and digestibility by sheep compared to feeding alfalfa silage alone.

INTRODUCTION

Harvesting forages after the first killing frost is advantageous because under freezing conditions, forages reduce plant respiration (Rotz and Muck, 1994) and minimize plant metabolite losses such as water and CO_2, thereby increasing the retention of non-structural carbohydrates (Kelly, 2017). However, negative impacts on the plant that follow a killing frost can be plant leaf loss and a decline in forage yield (Andews, 1987). Additionally, harvesting forage and drying as hay in the fall season is very challenging due to short days and low intensity of solar radiation. Ensiling forages after a first killing frost can be an alternative approach to reduce the risks associated to wet and unfavorable weather conditions, but ensiling alfalfa alone is challenging due to greater buffering capacity than grasses resulting from greater concentration of organic acids and proteins (McDonald et al., 1991). Alfalfa is one the most popular forage legumes grown in the US while tall fescue occupies a large part of the grass species grown in southeastern of the part of the US. Mixing alfalfa and tall fescue after harvesting can be an alternative solution to improve silage fermentation quality. Tall fescue stores considerable concentrations of soluble carbohydrates in the leaves during the fall (Mayland et al., 2000; Lacefield et al., 2003), which should enhance silage fermentation (Downing et al., 2008). However, selected silage fermentation parameters are not necessarily good predictors of intake by ruminants (Huhtanen et al., 2007; Krizsan et al., 2007). Therefore the objective of this study was to investigate the effect of alfalfa and tall fescue mixtures harvested after a killing frost on silage fermentation characteristics, and subsequent effects on silage intake, digestibility and nitrogen balance by sheep.

MATERIALS AND METHODS

Forage harvest, silage making and storage

Alfalfa and tall fescue were both grown at the University of Arkansas North farm located in Fayetteville, AR, USA (36°4’N, 94°9’W). The tall fescue used in this study was an established stand and was infected with the MaxQ novel endophyte (NE+). Alfalfa was harvested 5 weeks from the previous forage cutting, and both NE+ fescue and alfalfa were harvested the same day at the end of October of 2017. Each forage was harvested separately and then they were mixed afterwards according to the specified proportion on a dry matter basis. Alfalfa was harvested using a plot harvester machine (Wintersteiger, Cibus S.,Wintersteiger Inc., Salt Lake City, UT), chopped at < 5 cm particle size, and was wilted on a concrete slab to reach a moisture content of 35%. Tall fescue was harvested using a mower, and was also wilted in the field to reach a 37% moisture content.

After wilting, the forages were divided into one of four different treatments based on the proportion of each forage to comprise the diet. Alfalfa and tall fescue were packaged alone or in mixtures of 67% alfalfa +33% fescue, or 33% alfalfa + 67% fescue on a dry matter (DM) basis after wilting. Dry matter content was determined using microwave oven techniques (Anderson, 2019). The mixtures were made by weighing specified amounts of each forage and blending the forage thoroughly on a concrete slab. Forages were then packed in plastic trash containers (167 l) lined with two plastic bags (3 mil) by walking on the forages as it was being placed into the containers. Five containers were packed for each treatment, with mixing and packing for all four treatments occurring simultaneously in order to minimize the effects of field or moisture variation because of time delay. A thermocouple wire was inserted geometrically in the center of each trash can in order to monitor temperature inside the containers during storage. After packing the silage into the containers, a vacuum (Wet/Dry Vacuum, Mod. L 250, Shop – Vac. Corporation, Williamsport, Pennsylvania, USA) was used to remove as much air as possible, and plastic bags were tied individually with strings. The silage containers were stored for four months in an open space building with a roof and concrete floor. Using a thermocouple thermometer, the temperature was measured and recorded daily for 30 d beginning 31 October 2017, then weekly until 15 January 2018.

Digestion study

Animal and design

The study protocol was approved by Institutional Animal Care and Use Committee at the University of Arkansas (Protocol #16031). The digestion study was carried out at the University of Arkansas North Farm located within Fayetteville, AR, USA (36°4'N, 94°9'W), in the same location as the forage was produced. Twenty Dorper ewe lambs with mean BW = 34.7 ± 6.65 kg were used in the study. Prior to the study, sheep were checked with FAMACHA score, and lambs with score ≥ 3 were dewormed with Cydectin (Bayer HealthCare, LLC, Animal Health Division, Shawnee Mission, Kansas, USA) based on animal body weight and drug recommendations. Then lambs were stratified by BW and each group was assigned randomly to treatments. Within each treatment, lambs were assigned randomly to one silage container such that each lamb in the study was offered silage from only one container for the duration of the study. Lambs were given 17 d of adaptation at ad libitum consumption followed by 5 d of total feces and urine collection. Rumen fluid was collected on the last day of the study using rumen collection tubes.

Housing and sample collection

During the digestion study, lambs were housed in a temperature-controlled facility (15 to 16 °C) with 14 h of light and 10 h of darkness each day. Lambs were housed in individual 1 × 1.5-m pens with plastic-coated, expanded metal grate flooring. After every 7 days, lambs were removed from their pens and co-mingled in a pen with a concrete floor for a 3-h exercise period with access to water only but no feed. After 17 days, prior to total collection, pens were cleaned carefully in order to remove debris occurring from the adaptation period.

Feeding and sample collection

Lambs were given access to water and feed for ad libitum consumption (10% minimum refusal; DM basis). Total daily feed amount was weighed initially, then offered in smaller portions throughout the day in order to minimize forage selectivity and spillage by animals. Daily feed was offered beginning at 1600 h; orts and feed were collected at 1600 as well. Lambs were given 30 g/day of a commercial mineral[1] that did not contain an antibiotic immediately before feeding. Two samples of each feed were collected daily beginning 2 d prior to fecal and urine collection; one sample was dried to a constant weight at 50° C, while the other feed sample was immediately frozen for later fermentation analysis. Feed refused was weighed daily beginning 1 d before fecal collection and subsamples were dried to a constant weight at 50° C.

Trays with a solid corrugated polyvinyl chloride (PVC) sheet on the underside and covered with fiberglass screening were placed underneath each individual pen. Total feces was gathered from the screens into plastic gutters at the end of each screen, then weighed and dried to a

[1] Preferred Mineral for Sheep and Goats (Ragland Mills, Inc., Neosho, MO, USA) The mineral contained 350-400 g/kg salt, 90-100 g/kg Ca, and not less than 80 g/kg P, 10 g/kg Mg, 10 g/kg K, 125 ppm Co, 150 ppm I, 5,000 ppm Fe, 10 ppm Se, 140 ppm Zn, 352,000 IU/kg of Vitamin A, 88,000 IU/kg of Vitamin D3, and 330 IU/kg of Vitamin E.

constant weight at 50° C. Total urine was collected directly into plastic containers. Hydrochloric acid (50% v/v, ~20 mL) was added to collection containers prior to urine collection in order to prevent microbial activity and ammonia volatilization. An Acumen Basic AB15 portable pH meter (Fisher Scientific, Atlanta, GA, USA) was used to verify urine pH then 10% of the urine collected daily was stored frozen (-20° C) in small jars for later lab analysis.

Rumen fluid was collected via stomach tube on the last day of the study prior to feeding (1600 h), 3 h after feeding (19000h) and 8 h after feeding (2300h). Rumen fluid was then strained through two layers of cheesecloth, and pH was measured immediately with a pH meter. The collected rumen fluid was placed immediately into 12 × 75 mm polypropylene tubes, and stored on ice during 8 hours of sample collection. Then samples were thawed, mixed well, and approximately 4 mL of fluid was transferred into 12 × 75 tubes and centrifuged at 12000 × *g* for 5 min. After centrifugation, 0.8 mL supernatant was transferred into new micro-centrifuge tubes and 0.4 mL of 12.5% m-phosphoric acid/2-ethyl butyric acid solution was added to each tube, mixed, and stored in a freezer at –20 ºC for 2 months for later volatile fatty acids analysis.

Chemical analysis

Dried samples of forage, orts, and feces were allowed to equilibrate to atmospheric moisture, then ground through a Wiley mill (Arthur H. Thomas, West Washington Square, PA, USA) to pass through a 1-mm screen. Forage, ort, and fecal sample DM was determined by drying samples at 100ºC overnight. Sample ash concentrations were determined by ashing forage samples at 500ºC in a muffle furnace, and organic matter (OM) was calculated as DM weight - ash weight. Neutral detergent fiber (aNDF) and acid detergent fiber (ADF) content in forage, orts, and feces were analyzed sequentially using a 200 Ankom Fiber analyzer (ANKOM Technology Corporation, Macedon, NY, USA; Vogel et al., 1999). Nitrogen content in feed, orts, feces and urine were measured using the Dumas total combustion method (Elementar Americas, Mt. Laurel, NJ, USA; Method 990.03; AOAC, 2000).

Silage fermentation profiles were analyzed by Cumberland Valley Analytical Services (Hagerstown, MD, USA). All frozen samples collected daily during the feeding trial were composited by animal. Silage samples (25 g) were diluted with 200 mL deionized water, allowed to sit overnight, at room temperature mixed for 2 min and filtered through coarse (20- 25 um particle retention) filter paper. Silage pH was measured directly and titratable acidity was determined by titrating samples to a pH of 6.5 with 0.1 N NaOH using a Mettler DL12 Titrator (Mettler-Toledo, Inc., Columbus, Ohio, USA). Extract was diluted 1:3 with deionized water and ammonia was determined by titrating with 0.1 N HCl using a Labconco Rapidstill II model 65200 analyzer (Labconco. Inc., Kansas City, MO, USA). Lactic acid was measured following a 1:1 dilution with deionized water using a YSI 2700 Select Biochemistry Analyzer (YSI, Inc. Yellow Springs, OH, USA). Silage acetic, propionic, butyric, and iso-butyric acids were determined by filtering 3 mL of extract through a 0.2-μm filter membrane, then injecting a 1.0-μL sub-sample into a Perkin Elmer AutoSystem gas chromatograph fitted with a Restek column packed with Stabilwax-DA (Perkin Elmer, Inc., Shelton, CT, USA).

Rumen fluid samples were analyzed for volatile fatty acid content. Samples were thawed and centrifuged at 30,000 × *g* for 5 min and then supernatant was transferred into gas chromatography vials. Volatile fatty acids (VFA) were analyzed with an automated Hewlett Packard 5890 gas chromatograph that was fitted with a NukolTM fused silica capillary column (30m × 0.25mm I.D. × 0.25um film thickness; Supelco Inc, Bellefonte PA; part# 24107).

Statistical analysis

Silage fermentation, intake, digestibility and rumen fermentation data were analyzed using PROC MIXED of SAS (SAS Institute, Cary, NC) as a completely randomized design. The proportions of alfalfa in diet (treatment) were treated as a fixed effect and animal as a random effect. Orthogonal polynomial trend analyses were used to detect the linear and quadratic trends among treatments. Rumen fermentation data were treated as repeated measures where animal within treatment was considered as the error term for treatment effects. The time of sampling was treated as a repeated measure and the statistical model included fixed effects of treatments, time of sampling and their interactions.

RESULTS

Forage composition data are presented in Table 1. Dry matter content of the silage (% of total wet weight) was not different ($P \geq 0.23$) among treatments. Organic matter, aNDF and ADF (% of DM) increased linearly ($P \leq 0.03$) with increasing proportion of fescue, while N content decreased linearly ($P < 0.01$) with greater proportion of fescue in the silage mixture.

The temperature inside silage containers was not different ($P \geq 0.63$) across fescue and alfalfa combinations (Table 2). Silage pH decreased linearly ($P < 0.01$) while silage total acids (g/kg DM) and lactic acid (g/kg DM) increased linearly ($P < 0.01$) with increasing level of fescue in the silage. The proportion of lactic acid to total acids (mole/ 100 moles total acids) increased linearly and quadratically ($P \leq 0.02$) with additional fescue in the silage, reaching a maximum concentration in the silage with 66% fescue and 34% alfalfa. Acetate concentrations (g/kg DM), increased linearly ($P < 0.01$) and propionate concentrations (g / kg DM) increased linearly and quadratically ($P \leq 0.03$) with increasing tall fescue proportion in the silages.

Ammonia-N concentrations decreased linearly and quadratically ($P < 0.01$) with increasing the proportion of tall fescue in silage and were greatest in ensiled alfalfa without fescue.

Dry matter and OM intake (g/day and g/kg BW) decreased linearly ($P < 0.01$) with increasing addition of fescue in the silages (Table 3). Digestibility of DM and OM also decreased linearly ($P < 0.01$) as the amount of fescue was increased. This resulted in decreasing digestible DM and OM intakes (g/day and g/kg BW) with increasing proportions of fescue in the silages (linear effect; $P < 0.001$). Digestibility of aNDF and ADF were not different ($P \geq 0.12$) across the different alfalfa – tall fescue combinations.

The interaction between time of sampling and treatment affected ($P \leq 0.01$) ruminal propionate concentrations and the acetate to propionate ratio, where the greatest propionate concentrations were from lambs offered alfalfa silage alone during 6 hours of sampling (Figure 1). Rumen acetate to propionate ratio deceased during the first 3 hours after feeding and spiked in lambs fed alfalfa 33% mixed with 66% fescue 6 hours after feeding (Figure 2). Ruminal pH was not different ($P \geq 0.21$) across the different silages (Table 4). Total VFAs (mM) decreased linearly and quadratically ($P \leq 0.02$) with increasing proportion of fescue in the silages with the minimum concentrations of total VFA occurring with 66% fescue and 34% alfalfa. Acetate concentrations increased ($P < 0.01$) while rumen butyrate and valerate decreased linearly ($P < 0.01$) with increasing proportion of tall fescue in the silages. Ruminal isobutyrate increased quadratically ($P < 0.05$) with increasing fescue in the silages. The total branched chain fatty acids in the rumen decreased linearly and quadratically ($P < 0.01$), when more fescue was added in the diet, with the greatest concentrations from lambs fed 66 % alfalfa and 34 % fescue.

Nitrogen intake (g/day) and fecal (g/day) and urinary N excretion (g/day) decreased linearly ($P < 0.01$) with fescue in the silage, but urine N excretion expressed as a portion of N intake (g/kg N intake) increased linearly ($P = 0.01$) and quadratically ($P = 0.04$) with increasing fescue in the silages. This resulted in linear decreases ($P < 0.01$) in N absorption (g/kg N intake and BW) and linear ($P < 0.01$) and quadratic ($P < 0.05$) declines in retained N (g /kg N intake, g/kg N absorbed) with increasing fescue in silages. These quadratic decreases in N absorption were characterized by small to moderate decreases in N retention with the initial addition of fescue with alfalfa, but a sharp decrease in N retention from silages that were entirely tall fescue.

DISCUSSION

Fiber content of silages increased while nitrogen decreased with increased proportion of NE+ tall fescue in the silage, which can be explained by a greater content of fiber in grasses and smaller protein content than in legumes. This trend is consistent with the results of Lee (2018). Lee (2018) compared nutritive values of 136 different forage species grown in 30 different countries, and determined that grasses contained the most fiber while herbaceous legumes had a larger protein content. The forages used in silage making were harvested during the fall after a killing frost, which could change the accumulation of sugars in forage. According to Pollock et al. (1983), freezing temperature reduces plant respiration but allows the accumulation of more soluble carbohydrates. Moreover, tall fescue stores more water soluble carbohydrates (WSC) in a cool environment, e.g. temperatures below 10^{o} C compared to temperatures above 21^{o} C (Labhart et al., 1983). During silage fermentation, lactic acid-producing bacteria convert WSC into organic acids, primarily lactic acids, which helps silage pH drop, suppresses proliferation of undesirable microorganisms (Pang et al., 2012) and promotes silage fermentation and preservation (Humphreys, 1994). In the present study, the total acid and lactic acid concentrations (g/kg DM, mole/100 moles total acids) increased with greater percentage of tall fescue in the silages, and these greater concentrations were probably related to a greater accumulation and fermentation of sugars from tall fescue. However, sugar content was not measured in the pre-ensiled samples. The proportion of acetate in silage increased with increasing fescue proportion, probably due to more oxygen exposure in silage while packing because packing alfalfa silage alone was easier than packing alfalfa fescue mixture or fescue alone. According to Kung and Shaver (2001), loose silage packing can lead to a greater production of acetate in silage. Ammonia-N decreased with adding more fescue in diet. The production of ammonia in silage can be attributed to metabolism of protein (McDonald et al., 1991).

The greater DMI and OMI of the alfalfa silage alone than alfalfa-fescue mixtures or fescue alone may be explained by the rapid rate of digestion of alfalfa silage compared to tall fescue due to smaller particle size. The alfalfa silage fed during this study was chopped in small particle size of 2 cm while the tall fescue was shredded. Intake measurements were not associated positively with concentration of lactic acid. The greater lactic acid concentration in this study was 7.6 g/kg DM and this value is lower than 52g/kg DM, which was reported by Krizsan and Rand (2007) to improve intake. Moreover, selected silage intake by ruminants is predicted by multiple fermentation factors (Huhtanen et al., 2007; Krizsan et al., 2007). The greater digestibility of alfalfa silage may have reduced the retention time in the rumen allowing animals to consume more feed, thereby increasing forage intake. In a study conducted with Holstein cows involving feeding different physical forms of alfalfa hay, shorter rumen retention time was related to greater feed intake (Shaver et al., 1986).

Dry matter and OM digestibilities and digestible DMI and DOMI were also greater for animals offered alfalfa silage alone or alfalfa with 34% tall fescue. These measurements decreased dramatically when tall fescue was included at greater than 34% of the silage. This trend may be attributed to the greater aNDF and ADF in tall fescue than alfalfa silage but the rate of change in digestibility with increasing proportion of tall fescue was not consistent with that of fiber concentrations. Likewise, these trends do not follow fermentation profiles as increasing the proportions of fescue in the silages improved silage fermentation characteristics. The particle size of the alfalfa was smaller compared to shredded tall fescue. This was an issue with the forage plot harvester being able to chop the alfalfa, whereas it tended to simply pull the fescue through the chopping mechanism without chopping it. According to Marsh (1978), chopping forage increases the surface area, which facilitates microbial attachment and fermentation of the forage. Similarly, in a study that involved feeding gestating sheep alfalfa silage alone, digestible DMI was positively associated with feed intake (Niyigena et al., 2019).

In the present study, the production of rumen VFA were measured over an 8-h period. Rumen total VFA were greater in sheep offered alfalfa silage alone compared with the other treatments probably due to rapid rumen fermentation and degradability of alfalfa silage compared to tall fescue. In addition, chopped alfalfa had also a greater contact surface for microbial attachment compared to shredded tall fescue. Conversely, a study conducted to investigate the rumen fermentation characteristics utilizing temperate forage legumes and grasses revealed that mixing alfalfa: tall fescue on a dry matter (DM) basis at 0:100, 25:75, 50:50 75:25 and 100:0 had no effect on total rumen VFA (Pizzola et al., 2017) but increased rumen isoacid production.

In this study, ruminal acetate was greater from sheep offered fescue silage or fescue and alfalfa mixtures compared with sheep offered alfalfa alone and this trend was similar to that of the NDF concentration in the silages evaluated in this study. Increased rumen acetate concentrations were reported in Holstein dairy cows fed a diet with greater NDF content compared with a lower NDF diet (Poorkasegran and Yansari, 2014). Rumen isobutyrate increased with increasing proportion of alfalfa in the diet, which is consistent with results of Pizzola et al. (2017). The production of isoacids, such as valerate and isobutyrate may be associated with amino acid degradation especially in alfalfa silage (Andries et al., 1987).

Nitrogen utilization measurements decreased with reduced proportion of alfalfa in silage. This trend is associated with silage composition because the N content was greater in alfalfa than in tall fescue. Urinary N excretion is normally greater in forage legumes with high rumen degradation, which produce a large amount of ammonia. A portion of the ammonia released in the rumen is absorbed and circulates to the liver, ending in the urea cycle and is excreted in urine. According to Hammond (1996), increasing the amount of protein in diet results in rise of blood urea N, which in turn increases N excretion in the urine.

Conclusion

In this study, harvesting alfalfa and tall fescue after a killing frost and mixing both forages improved silage fermentation characteristics, especially the production of lactic acid, which is critical in preserving silage. Intake, digestibility and N utilization improved with mixing alfalfa and tall fescue rather than feeding tall fescue alone. Rumen fermentation characteristics were also affected by the proportion of fescue in the diet, with a greater acetate production from animals fed the higher-fiber diet. Harvesting and ensiling alfalfa and tall fescue after a killing frost may be an alternative approach to hay making, but inclusion of tall fescue into alfalfa may have serious negative impacts on utilization by ruminants.

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TABLES AND FIGURES

Table 1. Composition of alfalfa and tall fescue silage mixtures harvested after a killing frost

	Treatments[1]					Effect[2]	
% Alfalfa	100	66	34	0			
% NE fescue	0	34	66	100	SEM	Linear	Quadratic
Item[3]							
DM, g/ kg fresh weight	670	656	634	654	13.6	0.26	0.23
			g/kg DM				
OM	890	910	910	915	10.0	< 0.01	0.05
aNDF	300	405	450	510	20.4	< 0.01	0.12
ADF	246	322	388	338	34.0	0.03	0.08
Nitrogen	35	30	28	25	4.0	< 0.01	0.14

[1]Forages were harvested in October of 2018 after a killing frost, wilted, and blended as either alfalfa alone, 66% alfalfa +34% novel endophyte infected tall fescue, 34% alfalfa + 66% fescue, or 100% fescue.

[2] Probabilities for linear and quadratic orthogonal contrasts for means across all four different treatments.

[3]DM = Dry matter, OM = Organic matter, aNDF= neutral detergent fiber inclusive of ash, ADF = acid detergent fiber.

Table 2. Silage fermentation profile of alfalfa and tall fescue harvested and ensiled separately or in mixtures after a killing frost

	Treatments[1]					Effect[2]	
% Alfalfa	100	66	34	0			
% NE fescue	0	34	66	100	SEM	Linear	Quadratic
Item[3]							
Maximum temp, °C	19.7	19.5	19.6	19.8	0.29	0.85	0.63
pH	5.8	5.6	5.6	5.5	0.03	< 0.01	0.90
TA, g/kg DM	2.9	9.0	14.0	15.2	0.27	< 0.01	0.39
Lactic, g/kg DM	1.0	4.2	7.6	7.6	0.14	< 0.01	0.29
LTA, g/kg TA	342	464	534	484	34.7	< 0.01	0.02
Ace., g/kg DM	1.9	4.8	6.4	7.5	1.40	< 0.01	0.55
Pro., g/kg DM	< 0.1	< 0.1	0.1	0.6	0.09	< 0.01	0.03
NH_3N-CPE, g/kg DM	20	10	12	14	1.2	< 0.01	< 0.01

[1]Forages were harvested in October of 2018 after a killing frost, wilted, and blended as either alfalfa alone, 66% alfalfa +34% novel endophyte infected tall fescue, 34% alfalfa + 66% fescue, or 100% fescue.

[2] Probabilities for linear and quadratic orthogonal contrasts for means across all four different treatments.

[3]Tempmax = maximum temperature reached during the fermentation process; TA = total acids; Lactic = lactic acid; LTA = lactic acid expressed in g/kg of total silage acids; Ace. = acetic acid; Pro. = propionic acid; NH3N-CPE = ammonia N expressed in crude protein equivalents**.**

Table 3. Intake and digestibility in sheep offered alfalfa or tall fescue alone or in mixtures harvested and ensiled after a killing frost

Item[3]	Treatments[1]					Effect[2]	
% Alfalfa	100	66	34	0			
% NE fescue	0	34	66	100	SEM	Linear	Quadratic
Intake							
DMI, g/day	1665	1610	1191	930	86.7	< 0.01	0.25
DMI, g/kg BW	51	43	36	27	2.8	< 0.01	0.82
OMI, g/day	1484	1460	1086	851	76.0	< 0.01	0.18
OMI, g/kg BW	45	39	33	25	2.58	< 0.01	0.72
Digestibility							
DMD, g/kg DM	720	693	649	647	7.7	< 0.01	0.12
OMD, g/kg DM	756	726	663	670	12.7	< 0.01	0.18
NDFD, g/kg DM	516	589	527	567	22.0	0.36	0.45
ADFD, g/kg DM	493	506	537	445	65.8	0.70	0.12
Digestible intake							
DDMI, g/day	1198	1114	773	603	57.5	< 0.01	0.46
DDMI, g/kg BW	39	30	24	18.0	2.1	< 0.01	0.82
DOMI, g/ day	1120	1060	722	571	54.7	< 0.01	0.42
DOMI, g/kg BW	34	29	22	17	2.0	< 0.01	0.90

[1]Forages were harvested in October of 2018 after a killing frost, wilted, and blended as either alfalfa alone, 66% alfalfa +34% novel endophyte infected tall fescue, 34% alfalfa + 66% fescue, or 100% fescue , OM = Organic matter, NDF= neutral detergent fiber.

[2] Probabilities for linear and quadratic orthogonal contrasts for means across all four different treatments.

[3] DMI= dry matter intake, OMI= organic matter intake, DMD = dry matter digestibility, OMD = organic matter digestibility, NDFD = neutral detergent fiber digestibility, ADFD= acid detergent fiber digestibility, DDMI= digestible dry matter intake, DOMI = digestible organic matter intake.

Table 4. Ruminal volatile fatty acids from sheep offered alfalfa or tall fescue alone or in mixtures harvested and ensiled after a killing frost

	Treatments[1]					Effect[2]	
% Alfalfa	100	66	34	0			
% NE fescue	0	34	66	100	SEM	Linear	Quadratic
Item[3]							
Rumen pH	7.0	7.1	7.0	7.1	0.07	0.21	0.64
Total VFA, mM	130	96	91	99	8.5	0.01	0.02
	Mole/100 mole total VFA						
Acetate	61	65	65	69	1.0	< 0.01	0.85
Propionate	25	21	22	21	1.2	0.06	0.27
Isobutyrate	1.5	1.6	1.6	1.2	0.10	0.09	0.02
Butyrate	8.9	8.4	7.9	5.9	0.61	< 0.01	0.25
Valerate	1.6	1.5	1.5	1.2	0.09	< 0.01	0.08
TBCFA	4.9	5.1	5.0	3.73	0.29	0.01	0.01

[1]Forages were harvested in October of 2018 after a killing frost, wilted, and blended as either alfalfa alone, 66% alfalfa +34% novel endophyte infected tall fescue, 34% alfalfa + 66% fescue, or 100% fescue.

[2] Probabilities for linear and quadratic orthogonal contrasts for means across all four different treatments.

[3]VFA = Volatile fatty acids, TBCFA = Total branched chain fatty acids.

Table 5. Nitrogen intake, absorption and retention in sheep offered alfalfa or tall fescue alone or in mixtures harvested and ensiled after a killing frost

	Treatments[1]					Effect[2]	
% Alfalfa	100	66	34	0			
% NE fescue	0	34	66	100	SEM	Linear	Quadratic
Item[3]							
N intake, g/day	60	49	33	23	3.0	< 0.01	0.86
Fecal N, g/day	13	11	9	8	0.97	< 0.01	0.92
N absorption, g/kg intake	790	760	720	660	2.0	< 0.01	0.44
N app. abs, g/day	47	37	24	15	2.4	< 0.01	0.80
N app. abs, g/kg BW	1.5	1.0	0.7	0.5	0.07	< 0.01	0.27
Urinary N, g/day	10	8	6	7	0.8	< 0.01	0.06
Urine N, g/kg N intake	177	173	176	301	29.6	0.01	0.04
Retained N, g/day	40	29	18	8	2.7	< 0.01	0.70
N. retained, g/kg BW	1.1	0.8	0.5	0.3	0.06	< 0.01	0.80
N retained, g/kg N intake	611	591	548	362	38.4	< 0.01	0.04
N. retained, g/kg N absorbed	775	772	756	536	44.0	< 0.01	0.02

[1]Forages were harvested in October of 2018 after a killing frost, wilted, and blended as either alfalfa alone, 66% alfalfa +34% novel endophyte infected tall fescue, 34% alfalfa + 66% fescue, or 100% fescue.

[2] Probabilities for linear and quadratic orthogonal contrasts for means across all four different treatments.

[3] N= Nitrogen, BW = body weight, N app. abs = nitrogen apparently absorbed.

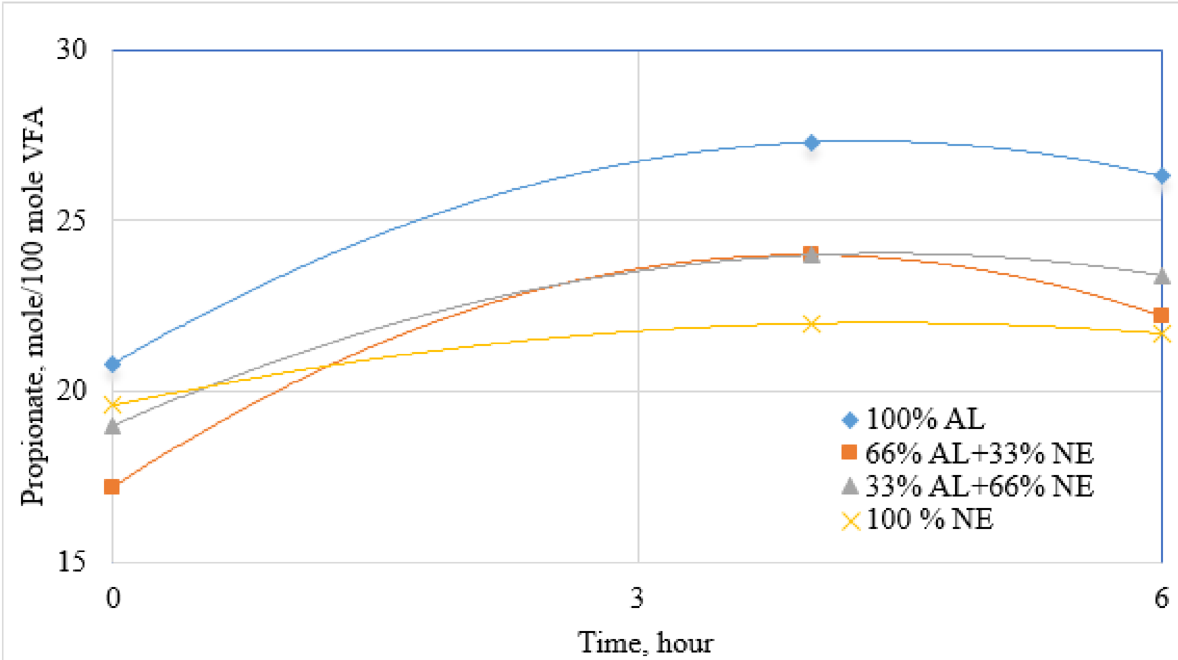

Figure 1. Forages were harvested in October of 2018 after a killing frost , wilted, and blended as either 100% alfalfa, 66% alfalfa +34% Novel Endophyte fescue, 34% alfalfa + 66% fescue, or 100% fescue. VFA = volatile fatty acids, AL= alfalfa, NE= novel endophyte infected tall fescue. Rumen fluid was collected at, 0, 3, and 6 hours after feeding. Effect of treatment ($P = 0.10$), time ($P < 0.01$) and treatment x time ($P < 0.01$).

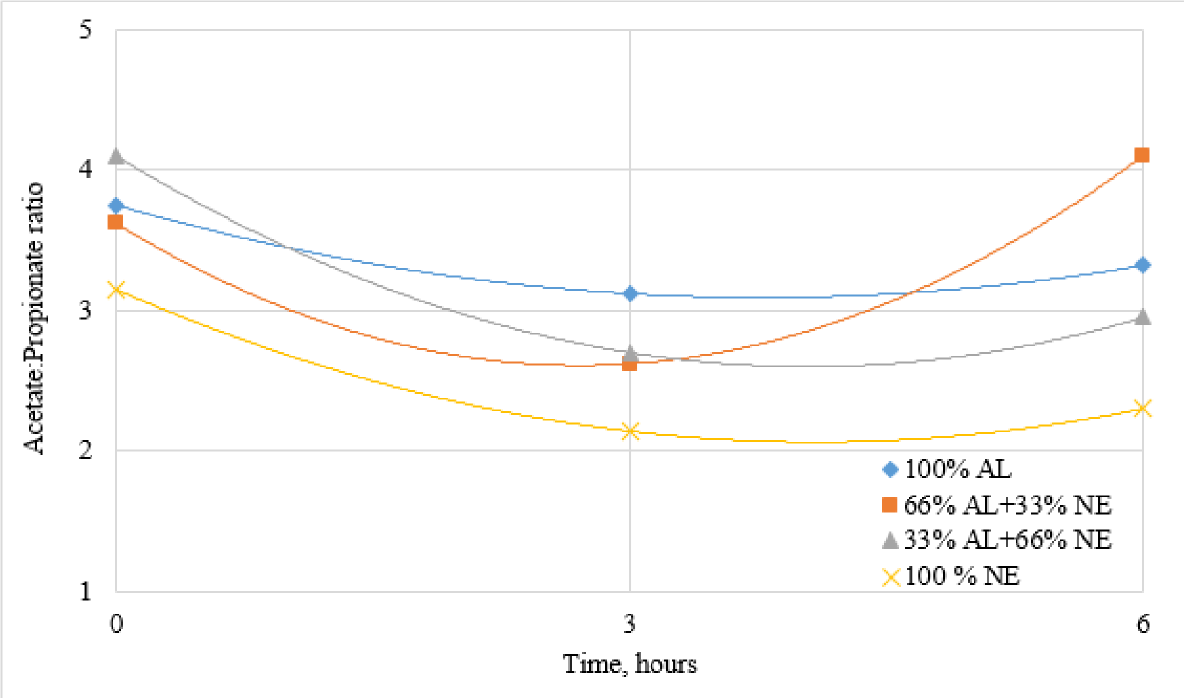

Figure 2. Forages were harvested in October of 2018 after a killing frost , wilted, and blended as either 100% alfalfa, 66% alfalfa +34% Novel Endophyte fescue, 34% alfalfa + 66% fescue, or 100% fescue. AL= alfalfa, NE= novel endophyte infected tall fescue. Rumen fluid was collected at, 0, 3, and 6 hours after feeding. Effect of treatment ($P = 0.03$), time ($P < 0.01$) and treatment x time ($P < 0.01$).

CHAPTER IV

Effect of supplementing different proportions of tannins from sericea lespedeza to alfalfa silage on intake, digestibility and nitrogen balance in sheep

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ABSTRACT

Dietary manipulations to include tannins can change the proportion and amounts of N excreted in the urine and feces as well as improve nitrogen use efficiency by the animal. This study was conducted to investigate the effects of adding different proportions of sericea lespedeza hay (SL) containing tannins to alfalfa silage. Alfalfa was harvested in June, 2018 at 75% bloom, chopped, then packed at 55% moisture into plastic containers lined with two plastic bags and allowed to ensile for 3 months. Alfalfa silage was either offered alone (0 g/kg; CONT) or mixed with chopped SL to provide 90 (LOW), 180 (MED), or 270 g/kg SL (HIGH) on a dry matter (DM) basis. These diets were offered randomly for ad libitum consumption to 16 Dorper ewe lambs (41.8 ± 4.61 kg BW) in a randomized complete block design experiment with two periods to provide four observations per treatment for each experimental period. Each period consisted of a 14-d dietary adaptation period followed by 5 d of total fecal and urine collection. Data were analyzed using PROC MIXED of SAS. Dry matter (DM) and organic matter (OM) digestibility decreased linearly and cubically ($P < 0.05$) with increasing SL and digestible DM and OM intake (g/day or g/kg BW) decreased linearly ($P < 0.05$) with increasing SL addition to the diet. Neutral detergent fiber digestibility decreased linearly and cubically ($P < 0.01$) and acid detergent fiber digestibility decreased linearly ($P < 0.01$) with increasing SL. Apparent N absorption (g/kg N intake) decreased linearly ($P < 0.05$) with increasing SL in the diet. Urinary N excretions (g/kg N intake) tended ($P = 0.10$) to decrease quadratically while fecal N (g/kg N intake) increased linearly ($P < 0.01$) with increasing SL proportion in the diet. Hippuric acid concentration (g/L) was not affected (P > 0.43) by the addition of SL in diet. Proportion of ruminal acetate increased quadratically ($P = 0.01$) and butyrate decreased ($P < 0.01$) quadratically with increasing levels of SL. In this study, supplementation with sericea lespedeza

as a tannin source to alfalfa silage decreased forage digestibility without affecting feed intake, and significantly increased N excreted in feces and tended to decrease urine N excretion. Therefore, feeding tannins in diet can alter N excretion route from urine to feces.

INTRODUCTION

Three quarters of nitrogen harvested from crops is consumed by livestock (Sutton et al., 2013). Increase in world population has also increased food demand consequently leading to the demand of using greater amount of N in agricultural systems (Groenestein et al., 2019). Feeding excessive dietary protein above requirements and high stocking rates contribute to N losses in the form of NO_2 and NH_3 in the air and nitrate leaching in soil (Tamminga, 1992). Poor N utilization by ruminants also contributes to NO_2 emissions (Dijkstra et al., 2013). Different dietary manipulation strategies have been attempted in order to reduce N losses from ruminant production systems. For instance, decreasing the crude protein (CP) content in cow diets from 19 to 10.8 % [dry matter (DM) basis], decreased N, NH_3 and P content in the resulting slurry (Van der Stelt et al., 2008). In addition, dietary manipulation by including phenolic compounds in the diet increased hippuric acid production in the urine, which is an alternative approach to mitigate NO_2 emissions (Dijkstra et al., 2011). Alfalfa is a forage legume with high protein content and high rumen degradation rate. Degradation of alfalfa (*Medicago sativa*) and other proteins in the rumen produces ammonia, which is converted to urea and a large part of the urea is lost in urine (Hammond, 1996). Previous studies have shown that supplementation with tannins reduced protein degradation in the rumen and shifted N excretion from urine to feces (Patra and Saxena, 2011). For instance, adding tannins extracted from quebracho at 2% of a forage based diet (DM basis) decreased protein digestibility in beef cattle from 63.4 to 54.5% (Beauchemin et al., 2007). Sericea lespedeza *(Lespedeza cuneate)* is a perennial forage legume with an average tannin content of 180 g/kg DM. Feeding sericea lespeza (SL) to Angora goats reduced methane emissions compared to goats that were fed tall fescue (Puchala et al., 2005). Using SL as a forage source may be beneficial to improve protein utilization. Therefore, the objective of this study was to investigate the effect of feeding alfalfa silage and SL hay mixtures on feed intake, digestibility, and N balance in sheep.

MATERIALS AND METHODS

Forage management and harvest

Sericea lespedeza hay grown in northwest Arkansas was purchased from a forage producer in August, 2018. The SL hay was harvested at full bloom and had 24 g/kg N and 644 g/kg NDF on a DM basis (Table 1). Alfalfa was harvested in June of 2018, 4 weeks after a previous forage cutting, and was at the first bloom maturity stage. Forage was fresh cut using a forage plot harvester (Wintersteiger, Cibus S.,Wintersteiger Inc., Salt Lake City, UT) that produced an average 5-cm particle size. After harvesting, the alfalfa spread onto an outdoor concrete slab and allowed to dry to 55% moisture content. Then the forage was packed in plastic containers (167 L) that were lined with a double layer of plastic bags (5 mil). Alfalfa was packed tightly by walking on the forage as it was placed into the containers. Excess air was removed using a vacuum device (Wet/Dry Vacuum, Mod. L 250, Shop – Vac. Corporation, Williamsport, Pennsylvania, USA), then plastic bags were tied. A total of 32 barrels were packed having an average weight 68 kg and the alfalfa was stored for 3 months inside an enclosed metal building to allow a good fermentation process.

Animals and design

The Institutional Animal Care and Use Committee at the University of Arkansas approved the study protocol (Protocol #18118). The digestion study was carried out at the University of Arkansas North Farm located within Fayetteville, AR, USA (36°4’N, 94°9’W) from September to October of 2018. Before the digestion study, SL was chopped to a small particle length of approximately 5 cm using commercial straw chopper (SB 5400; Harper Industries, Inc., Harper, KS, USA).

In this study, 16 Dorper ewe lambs [41.8 ± 4.61 kg body weight (BW)], were stratified by body weight and randomly assigned to 1 of 4 treatments in order to provide 4 replication/treatment in each of two periods. Treatments consisted of alfalfa silage alone (CONT) or mixed with chopped SL hay to provide 90 (LOW), 180 (MED), or 270 g/kg SL (HIGH) on a DM basis. Sericea hay contained 168 g/ kg DM of tannin content and the proportions of SL to mix with alfalfa silage was based on attempting to provide 15, 30, and 45 g/kg tannin for low, medium and high treatments, respectively.

Each period consisted 14 days of adaptation followed by 5 days of total urine and feces collection. During the second period, ewes were randomized to treatments with the condition that they were not offered the same treatment they were offered during the previous period. Ewes were removed from the facility for 5 days between periods and they were offered alfalfa silage alone.

Housing and sample collection

During the digestion study, lambs were housed in a controlled-temperature facility (15 to 16°C), with ventilation that was lighted for 14 h daily. Lambs were housed in individual 1 × 1.5-

m pens with plastic coated expanded metal grate flooring. After every 7 days, ewes were removed from their pens and co-mingled in a pen with a concrete floor for a 3-hour exercise period. Water, but no feed, was provided during that break.

Feeding and sample collection

Ewe lambs were allowed access to water and feed for ad libitum consumption. The amount of feed offered was adjusted daily in order to achieve a minimum 10% refusal (DM basis). Orts were removed and daily feed was offered beginning at 0900 h. Total daily feed was offered in different portions throughout the day in order to minimize forage spillage by animals. Fresh feed was offered each day. Diets containing both alfalfa and SL were mixed thoroughly prior to feeding. Sheep were offered 30 g of a commercial mineral[2], which did not contain an antibiotic, every day after removal of orts and before offering their respective diets. Feed collection began 2 d prior to fecal and urine collection. Feed refused was weighed daily beginning 1 d before fecal collection and subsamples were collected. Two samples of feed and orts were collected daily. One sample was dried to a constant weight at 50° C and the other sample was immediately frozen for later fermentation or tannin content analysis.

Trays in rectangular shape with solid corrugated polyvinyl chloride (PVC) sheets beneath and covered with fiberglass screening were placed underneath each individual pen. Total feces was gathered from the screens into plastic gutters at the end of each screen then weighed and removed twice daily and stored frozen (-20 °C). Total urine was collected directly into plastic containers submerged in ice packs that were changed every 8 h. The urine was then weighed,

[2] Preferred Mineral for Sheep and Goats (Ragland Mills Inc., Neosho, MO, USA) The mineral contained 350-400 g/kg salt, 90-100 g/kg Ca, and not less than 80 g/kg P, 10 g/kg Mg, 10 g/kg K, 125 ppm Co, 150 ppm I, 5,000 ppm Fe, 10 ppm Se, 140 ppm Zn, 352,000 IU/kg of Vitamin A, 88,000 IU/kg of Vitamin D3, and 330 IU/kg of Vitamin E.

and an aliquot (100 g/1000 total g) of the urine collected daily was placed in small jars and stored frozen (-20 °C) for later lab analysis. Rumen fluid was collected via stomach tube on the last day of each period immediately prior to feeding (0900 h), and 3 (1200 h) and 6 h after feeding (1500 h). The process of rumen collection, storage and analysis was discussed in chapter 3.

Chemical analysis

All collected samples were stored and analyzed in the same process as described in details in the chapter 3. Feed, orts and feces were analyzed for DM, OM, aNDF, ADF, and N content. Urine samples were analyzed for N content. Fermentation profiles in silage samples were analyzed by Cumberland Valley Analytical Services, Waynesboro, PA. Tannin concentration in feed and orts were analyzed by the University of Missouri in Columbia forage lab service.

Hippuric acid in urine was determined by a spectrophotometric procedure. After diluting 1 volume of the urine with 4 volumes of water, 0.5 mL of urine was mixed with 0.5 mL of pyridine in a centrifuge tube. Then 0.2 mL of benzenesulfonyl chloride was added, mixed for 5 seconds on a vortex, then was allowed to settle for a half hour at room temperature. The reaction was stopped by adding 5 mL of ethanol, which was followed by mixing on a vibration mixer. The samples were centrifuged at 2000 × g for 5 minutes. The supernatant was removed and placed in 1-cm cuvette and absorbance was read using a spectrophotometer set at a 410 nm wavelength.

Statistical analysis

Intake, digestibility and silage fermentation data were analyzed using PROC MIXED of SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA) as a randomized complete block design with ewe as the experimental unit. The four treatments were considered fixed effects while ewe and period were treated as random effects. Linear, quadratic, and cubic polynomial trends were used to determine the effects of different proportions of SL in the diet. Rumen fermentation data were analyzed as repeated measures with animal within treatment as the error term for treatment effects. Sampling time was treated as a repeated measure and the model included fixed effects of treatment, time of sampling, and an interaction.

RESULTS

Forage composition and silage fermentation measurements by sheep are presented in tables 1. Organic matter and N content (g/kg DM) were greater, and aNDF and ADF were lower in alfalfa silage compared to SL hay. However, NDF and ADF concentrations (% DM) were greater in SL hay compared to alfalfa silage.

Dry matter intake and OMI (g/day, and g/kg BW) were not affected ($P > 0.14$) by adding different proportion of SL in the diet (Table 2). Dry matter and NDF digestibilities decreased linearly ($P < 0.01$) and cubically ($P \leq 0.02$) with increasing the proportion of SL in the diet and ADF digestibility decreased ($P < 0.01$) linearly with increasing SL in the diet. Greater digestibility was observed in ewes that were fed alfalfa silage alone and the lowest digestibility was from sheep offered alfalfa mixed with high (270 g/kg) concentration of SL. Organic matter digestibility decreased linearly ($P < 0.01$) with greater concentration of SL in the diet, and the greater digestibility was measured in sheep fed alfalfa silage alone. Digestible DM intake, g/day or g/kg BW, and digestible OM intake decreased linearly (*P* ≤ 0.03) with addition of more SL in the diet.

Supplementation of tannins from SL to alfalfa silage did not affect (*P* ≥ 0.48) N intake (g/ day or g/ kg BW; Table 3). Nitrogen excreted in feces (g/ kg of N intake) increased linearly (P < 0.01) with increasing SL. Nitrogen absorption (g/kg N intake) decreased linearly (*P* < 0.01) with increasing SL in the diet. Urinary N (g/day) decreased linearly (*P* = 0.05) with increasing SL in the diet. Nitrogen retained (g/day, g/kg BW, g/kg of N intake) was not affected (P ≥ 0.14) increasing SL hay in the diet. However, the proportion of absorbed N that was retained tended (*P* = 0.1) to increase dramatically with 90 g/kg SL followed by small decreases with subsequent increases in SL concentration in the diet. Hippuric acid concentration in urine was not affected with addition of SL in the diet.

Rumen pH, total VFA and propionate (g/kg total VFA) were not affected (*P* ≥ 0.24) by SL hay level in the diet (Table 4). Ruminal acetate (g/kg total VFA) concentrations increased with the initial SL addition (90 g/kg SL), then decreased slightly with additional SL additions (quadratically *P* = 0.01). Ruminal butyrate concentrations decreased with the smaller concentrations of SL (90 and 180 g/kg) and then increased with the addition of 270 g/kg SL (quadratic effect *P* < 0.01). The concentrations of isobutyrate and isovalerate decreased linearly (*P* = 0.02), and that of valerate tended (*P* = 0.06) to decrease linearly with added SL in the diet.

DISCUSSION

In this study, fiber content was greater in SL hay compared to alfalfa silage. Sericea lespedeza hay used in this study was mature. The woody nature of SL reduces nutritional value of the forage due to accumulation of structural carbohydrates. The proportion of leaf mass fraction to the total plant biomass decreases with plant maturity (Haring et al., 2007), which increases the fraction of fiber content in forage. Feeding diets with greater NDF increases rumen retention time. Lignin, which is one part of NDF in forage, can limit ruminal forage cell wall digestion by preventing the enzymatic hydrolysis of polysaccharides, which thereby decreases intake and digestibility (Jung and Allen, 1995). Chemical composition of the plant also has an impact on forage palatability. Protein content in forage is positively associated with diet preference by sheep and cows, while lignin accumulation in forage is negatively associated with diet preference by animals (Heady, 1964). Moreover, regarding the palatability of different parts of the plant, animals prefer to consume leaves than stems (Waghorn and Molan, 2001).

Dry matter intake and OMI (g/day and g/kg BW) were not affected by proportion of SL in the diet. In contrast, supplementation 3% of quebracho condensed tannin extract to diets with either a high or low forage-to-concentrate ratio decreased intake of DM in dairy cattle (Dschaak et al., 2011). The inconsistency in intake response of forage containing condensed tannins in ruminants was attributed to the complexity of plants containing tannins and different responses either when CT-forage was fed solely or in combination with other plants (Harvey et al., 2019). Digestibility of DM, OM, NDF, ADF, DDMI, and DOMI decreased with increased SL in the diet. Our results are not consistent with those of Hervás et al. (2003) where merino sheep were fed alfalfa hay supplemented with different levels of condensed tannins from quebracho extract. In that study (Hervás et al., 2003) no differences were detected in DM, CP, NDF and ADF digestibilities. The difference in results may be explained by the fact that SL fed during this trial was mature. According to Cherney (1991), plant maturity increases lignification, which in turn results in reduced forage digestibility.

Nitrogen excreted in feces increased while urinary N decreased with addition of SL in the diet. These results were consistent with a previous study of Patra and Saxena (2011), who reported that supplementation of tannins in the diet reduced protein degradation in the rumen and changed N excretion from urine to feces. Alfalfa silage that was offered in this study had 24% CP. The rapid degradation of alfalfa in the rumen produces ammonia, which in turn is channeled to the liver and is partially excreted in urine as urea. Therefore, greater N excretion in urine from sheep that were fed alfalfa alone compared to N in urine from sheep that were fed alfalfa and SL hay mixtures can likely be explained by greater ruminal binding of dietary protein. The retained N (g/kg of absorbed N) tended to increase with increased SL in the diet. This improved N-use retention can be explained by the properties of tannins to bind to the protein in the rumen, thereby increasing rumen undegradable crude protein furthering absorption of protein in the small intestine and retention of N by the animal. Our results are consistent with those of Aerts et al. (1999) and Schwab (1995), who reported that feeding tannins improves protein utilization.

Rumen pH across all treatments was above 7, which creates favorable condition for microbes in the rumen to digest fiber. According to Mertens (1977), rumen pH less than 6.2 can inhibit fiber digestion. No significant variation in rumen total volatile fatty acids were detected in this study, probably because sheep were fed all fibrous forage source diets. Variation in ruminal degradation is usually greater in lower-fiber diets due to differences in retention time (Firkins, 1997). Rumen acetate increased with increased SL in the diet. Greater acetate may be related to a greater degradation of fiber, especially cellulose, which produced more acetate during rumen fermentation. When cows are fed solely a forage-based, high-fiber diet, fermenters of cellulose and hemicellulose in the rumen produces acetate as the main energy source (Kung, 2014).

Conclusion

In this study, supplementation of different levels of sericea lespedeza hay to alfalfa silage did not affect forage intake. Dry matter, organic matter, and fiber digestibilities decreased with increased concentrations of SL in the diet. Nitrogen excretion increased in feces and decreased in urine with increased SL in the diet, resulting in minimal differences in N absorption when expressed as a proportion of N ingested. Forage selectivity by sheep especially selective refusal of stems of SL hay should be taken under consideration while feeding a mature SL forage based diet. Moreover, due to the complex response of tannins and forage combinations in animals, the impact of supplementing tannins from SL on animal responses should be interpreted broadly.

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TABLES

Table 1. Chemical composition of alfalfa silage and sericea lespedeza (SL) fed to ewes[1].

Item	alfalfa	Sericea lespedeza
DM, g/ kg fresh weight	423	936
	g/kg of DM	
OM	910	872
aNDF	381	644
ADF	319	561
Nitrogen	39	24
Condensed tannins	0.0	168
Unbound condensed tannins, g/kg	0.0	24
Bound condensed tannin, g/kg	0.0	2.4
	Fermentation profile of alfalfa silage	
Total acids, g/kg DM	76	
Lactic acid, g/kg DM	55	
LTA, g/kg total silage acids	735	
Acetic acid, g/kg DM	19	
Propionic acid, g/kg DM	0.7	
NH_3N-CPE, g/kg DM	25	
Silage pH	4.6	

[1]Alfalfa silage was either offered alone (0 % CONT) or mixed with chopped SL to provide 90 (LOW), 180 (MED), or 270 g/kg SL (HIGH) on a dry matter (DM) basis

[2]DM = Dry matter, OM= organic matter, aNDF= neutral detergent fiber inclusive of ash, ADF= acid detergent fiber

LTA = lactic acid expressed in g/kg of total silage; NH_3N-CPE = ammonia N expressed in crude protein equivalents.

Table 2. Intake and digestibility by sheep offered alfalfa silage alone or alfalfa mixed with different proportions of sericea lespedeza hay

	Treatments[1]					Effect[2]		
Item[3]	Control	Low	Med	High	SEM	Linear	Quadratic	Cubic
Intake								
DMI, g/day	1320	1288	1255	1225	62.2	0.20	0.98	0.98
DMI, g/kg BW	31	31	30	30	1.5	0.75	0.75	0.89
OMI, g/day	1203	1170	1136	1105	52.6	0.14	0.98	0.97
OMI, g/kg BW	28	27	27	27	1.3	0.58	0.75	0.91
Digestibility								
DMD, g/kg DM	672	624	635	600	0.1	< 0.01	0.54	0.02
OMD, g/kg DM	697	645	653	615	0.1	< 0.01	0.43	0.01
NDFD, g/kg DM	551	470	510	428	25.5	< 0.01	0.99	< 0.01
ADFD, g/kg DM	594	523	539	493	38.0	< 0.01	0.47	0.07
Digestible intake								
DDMI, g/day	889	808	798	737	51.2	0.01	0.79	0.49
DDMI, g/kg BW	21	19	19	18	1.2	0.03	0.63	0.31
DOMI, g/ day	839	757	742	681	39.8	< 0.01	0.77	0.48
DOMI, g/kg BW	19	18	18	16	1.0	0.01	0.59	0.31

[1]Alfalfa silage was either offered alone (0 % CONT) or mixed with chopped SL to provide 90 (LOW), 180 (MED), or 270 g/kg SL (HIGH) on a dry matter (DM) basis

[2] Probabilities for linear, quadratic and cubic orthogonal contrasts for means across all four different treatments

[3] DMI= dry matter intake, OMI= organic matter intake, DMD = dry matter digestibility, OMD = organic matter digestibility, NDFD = neutral detergent fiber digestibility, ADFD= acid detergent fiber digestibility, DDMI= digestible dry matter intake, DOMI = digestible organic matter intake.

Table 3. Nitrogen balance by sheep offered alfalfa silage alone or alfalfa mixed with different proportions of sericea lespedeza hay

	Treatments[1]					Effect[2]		
Item[3]	Control	Low	Med	High	SEM	Linear	Quadratic	Cubic
N intake, g/day	48	50	47	45	3.3	0.48	0.51	0.71
N Intake, g/kg BW	1.1	1.2	1.1	1.1	0.07	0.90	0.63	0.75
Fecal N, g/day	11	13	13	13	0.9	0.08	0.20	0.26
Fecal N, g/kg N intake	229	269	270	291	69.5	< 0.01	0.18	0.07
N absorption, g/kg N intake	770	730	729	708	7.0	< 0.01	0.18	0.07
N app. abs, g/day	37	36	34	32	2.5	0.15	0.66	0.91
N app. abs, g/kg BW	86	85	83	79	5.8	0.27	0.81	0.10
Urine N, g/day	18	13	13	14	2.6	0.05	0.06	0.40
Urine N, g/kg intake	47	26	28	31	7.3	0.15	0.10	0.40
Retained N, g/day	18	23	21	18	2.7	0.81	0.16	0.59
N retained, g/kg BW	42	54	64	64	6.4	0.91	0.18	0.61
N retained, g/kg N intake	30	50	45	40	7.2	0.37	0.14	0.59
N retained, g/kg N absorbed	38	64	61	56	9.3	0.24	0.10	0.52
Hyppuric acid, g/L	2.0	1.7	1.9	2.1	0.44	0.69	0.43	0.68

[1]Alfalfa silage was either offered alone (0 % CONT) or mixed with chopped SL to provide 90 (LOW), 180 (MED), or 270 g/kg SL (HIGH) on a dry matter (DM) basis

[2] Probabilities for linear, quadratic and cubic orthogonal contrasts for means across all four different treatments.

[3] N= Nitrogen, BW = body weight, N app. abs = nitrogen apparently absorbed.

Table 5. Rumen volatile fatty acids from sheep offered alfalfa silage alone or alfalfa mixed with different proportions of sericea lespedeza hay

Item[3]	Treatments[1]					Effect[2]		
	Control	Low	Med	High	SEM	Linear	Quadratic	Cubic
Rumen pH	7.00	7.01	7.15	7.50	0.696	0.24	0.73	0.30
Total VFA, mM	80.2	77.1	78.0	80.1	5.87	0.98	0.44	0.87
ace:pro	3.5	3.6	3.6	3.6	0.09	0.60	0.34	0.76
	Mole/100 mole total VFA							
Acetate	64.0	66.0	65.5	65.2	0.45	0.05	0.01	0.57
Propionate	19.2	19.0	19.2	19.1	0.55	0.92	0.85	0.66
Butyrate	9.7	8.6	8.6	9.5	1.08	0.66	< 0.01	0.97
Isobutyrate	2.3	2.2	2.1	1.9	0.29	0.02	0.83	0.96
Valerate	1.9	1.8	1.7	1.7	0.11	0.06	0.41	0.97
Isovalerate	2.9	2.8	2.5	2.4	0.46	0.02	0.99	0.67

[1]Alfalfa silage was either offered alone (0 % CONT) or mixed with chopped SL to provide 90 (LOW), 180 (MED), or 270 g/kg SL (HIGH) on a dry matter (DM) basis

[2] Probabilities for linear, quadratic and cubic orthogonal contrasts for means across all four different treatments

[3]VFA = Volatile fatty acids, ace:pro = Acetate to propionate ratio

CHAPTER V

Effect of supplementing different proportions of polyphenols from *Lablab purpureus* hay to alfalfa silage on intake, digestibility, and nitrogen balance in gestating sheep

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ABSTRACT

The manipulation of diets by including forages containing moderate to high concentrations of polyphenolic compounds can change nutrient-use efficiency by ruminants. This study was conducted to investigate effects of adding different proportions of *Lablab purpureus* hay (LP) to alfalfa silage on the subsequent voluntary intake and digestibility by gestating sheep. Alfalfa was harvested in October 2018 at 750 plants blooming/1000 plants, chopped, and then packed at 550 g/kg moisture into plastic-lined bins, where it was allowed to ensile for 3 months. Alfalfa silage was either offered alone (Control; **C**), or mixed with 90 (low; **L**), 180 (Medium; **M**), or 270 g/kg (high; **H**) LP on a DM basis. Diets were assigned randomly and offered for ad-libitum consumption to 16 gestating ewes (49.0 ± 4.61kg BW) in a randomized complete block design experiment with two periods to provide four observations per treatment per experimental period. Each period consisted of a 14-d dietary adaptation period followed by 7 d of total fecal and urine collection. Data were analyzed using PROC MIXED of SAS and tested orthogonally for linear, quadratic and cubic trends. Dry matter (DM) and organic matter (OM) intake (g/kg BW) increased with the initial inclusion of LP, then declined with increasing inclusion of LP (quadratic response; $P < 0.05$). Digestibility of DM and OM were not affected ($P \geq 0.11$) by adding different levels of LP to the diet. Digestible DM intake (g/kg BW) tended to increase with the initial inclusion of LP, then declined with subsequent additions of LP in the diet (quadratic; $P = 0.08$). Digestible OM intake (g/kg BW) tended to increase linearly ($P = 0.09$) and quadratically ($P = 0.10$) with increasing amounts of LP in the diet. Nitrogen intake (g/kg BW), N apparently absorbed (g/kg BW) and urinary N (g/day) decreased linearly ($P < 0.01$) with increasing LP in the diet. In this study, supplementation with

90 g/kg LP as a source of phenolic-compounds improved forage DM and OM intake but did not affect DM and OM digestibilities. Therefore, adding a forage with moderate concentrations of polyphenols can improve forage utilization by ruminants.

Key words: Alfalfa, *Lablab purpureus*, Sheep

INTRODUCTION

Ruminants have the ability to convert feedstuffs with low or no value to humans into high quality human food. The consumption and demand for ruminant protein has been increasing and likely will continue increasing in the future as the world population increases where protein demand is expected to double by 2050 (Henchion et al., 2017). In past decades, the consumption of beef decreased from 43 kg in 1975 to 24 kg in 2015 per capita each year while the consumption of pork and poultry gained much popularity (USDA 2016). Feed conversion, especially protein, is more efficient in chickens than other livestock species; however, a greater part of chicken diet is from human edible food sources (Wilkinson, 2011). On the other hand, pasture and non-edible byproducts are the major feed sources for ruminants. Protein degradation in the rumen is the main factor affecting protein and feed utilization and efficiency by ruminants (Owens and Pettigrew, 1989). The rapid degradation of some forage legumes, such as alfalfa and red clover in the rumen (Messman et al., 1996) increases the synthesis of ammonia, which is excreted in urine as urea (Noeck and Russell, 1988). The consequence is reduced protein use and efficiency by ruminants. Methane production in ruminants is also a major loss of feed energy and constitutes a threat to the environment because domestic ruminants contribute emissions into the atmosphere accounting for 25% of the total anthropogenic methane in the US (EPA, 2019).

Plants that contain secondary metabolites, such as polyphenols, reduce protein degradation in the rumen. Red clover contains polyphenol oxidase (PPO) that produces equinones, which reduces forage protein degradation in silos (Fijałkowska et al., 2015) as well as protein degradation in the rumen (Broderick and Albrecht, 1997). Moreover feeding red clover silage improved milk yield more than feeding alfalfa silage (Broderick et al., 2001). Additionally, supplementation of polyphenols to a grass-based diet increased N retention and performance by lambs (Rajabi et al., 2017). The presence of polyphenols in the diet potentially enhances the concentration of hippuric acid in the urine, which reduces emissions of N_2O, a potential greenhouse gas, from soil (Van Groenigen et al., 2005; Bertram et al., 2009; Dijkstra et al., 2013).

There are multiple benefits of feeding polyphenols from different plant species to animals during production, but the low productivity and weak disease resistance in certain plant species is a challenge. It is important to investigate the use other alternative forage legumes containing polyphenols such as *Lablab purpureus* in ruminant nutrition. Lablab is resistant to drought and diseases, is a potential source of polyphenols (Murphy and Colucci, 1999), and can reduce methane emissions when fed to goats (Washaya et al., 2017). Although some progress has been made, there is a large research gap investigating the effects of mixing different forages containing phenolic compounds with other forage legumes on improvement of protein utilization and N partitioning in urine and feces of ruminants. Therefore, the objective of this study was to investigate the effect of supplementing different levels of polyphenols from *Lablab purpureus* to alfalfa silage on intake, digestibility and nitrogen balance in gestating sheep.

MATERIALS AND METHODS

The research study was conducted at the University of Arkansas North Farm located in Fayetteville, Arkansas USA. After field preparation, LP seed; Tecomate variety were planted on 1 June 2018 in a loamy soil with a seeding rate of 22 kg per hectare at 2.5 cm deep. The LP was grown in a dryland soil, the rainfall was adequate, and no irrigation was required. After 3 months of growth, LP was mowed, sun dried to attain 900 g/kg DM, baled in large round bales, and stored in a large, open barn with a concrete floor. After 4 month of storage, prior to feeding, the LP hay was chopped to a particle size of approximately 5 cm using a commercial straw chopper (SB 5400; Harper Industries, Inc., Harper, KS, USA). Alfalfa was harvested, using a plot harvester, machine chopped, and wilted on outdoor concrete flooring to reach 55% moisture during October of 2018. Alfalfa was packed in trash containers of 167 L volume, lined with 2 layers of plastic bags, and stored for 3 months. Details about silage production and storage were described in chapter 4.

Animals and feeding

In January of 2019, prior to the digestion study, blood samples were collected from a flock of 40 sheep and sent to County Service Lab in Farmington, AR USA to confirm pregnancy via blood test for pregnancy-specific protein B (Redden and Passavant, 2013). Sheep were evaluated for body condition score, FAMACHA, and number of teeth, and 16 gestating sheep that were 1 year of age and relatively similar body weight (BW) and body condition score (BCS) were selected for the digestion study. Prior to feeding each day, alfalfa silage was offered alone (C), or mixed with 90 (low; L), 180 (Medium; M), or 270 g/kg (high; H) LP on a DM basis. The amount of lablab hay to include in a diet was calculated based on the levels of sericea lespedeza that were offered in the previous experiment (chapter 4). Daily feed offered were calculated for each ewe to maintain the correct proportion of alfalfa and LP on a DM basis and to ensure a minimum of 100 g/kg refusal (DM basis). Forages were mixed thoroughly, and were offered to the 16 gestating sheep in small portions throughout the day to minimize feed wastage.

Ewes were housed in individual pens (1 × 1.5 m) with housing temperature maintained at 14^{o}C with ventilation. This provided four sheep per treatment for each of two periods. Each period consisted 14 days of adaptation and 7 days of total fecal and urine collection. Feed samples were collected daily beginning 2 days prior to fecal collection and orts samples were collected daily beginning 1 day prior to fecal collection.

Sample collection and analysis

Feed, orts, feces and urine collection procedure and chemical analysis were discussed in detail in chapter 4. Details for chemical analysis were discussed in chapter 3 where feed, orts, fecal samples were analyzed for DM, OM, NDF, ADF, N content while urine samples were analyzed for N.

Statistical analysis

Intake and digestibility data were analyzed using PROC MIXED of SAS (SAS Institute, Cary, NC) procedures for a randomized complete block design. The proportion of *Lablab purpureus* in the diet was considered a fixed effect, while period and animal were treated as random effects. Contrasts for orthogonal polynomial trend analyses were used to detect the linear, quadratic and cubic differences among treatments.

RESULTS

Forage composition data are presented in table 1and were not analyzed statistically. Neutral detergent and acid detergent fiber (aNDF and ADF, respectively) concentrations were greater in *Lablab purpureus* hay compared to alfalfa silage, but N content was greater in alfalfa silage than lablab hay.

Intake and digestibility data are presented in table 2. Dry matter and OM intake (g/d) both tended to increase quadratically ($P = 0.08$), and DM and OM intake (g/kg BW) increased quadratically ($P \leq 0.04$) with increasing level of LP in the diet. The greatest intakes were from sheep that were offered 90 g/kg lablab in the diet and the lowest intake was from sheep offered the highest proportion of LP in their diet. Dry matter and OM digestibilities were not affected ($P \geq 0.11$) by the proportion of lablab in the diet. Digestible DM and OM intakes (g/kg BW) tended ($P \leq 0.10$) to increase quadratically while digestible OM intake (g/day or g/kg BW) tended to increase linearly ($P \leq 0.09$) with greater additions of lablab hay in the diet.

Nitrogen intake (g/kg BW) decreased linearly and quadratically ($P \leq 0.04$) with greater concentrations of LP hay in the diet (Table 3). Fecal N excretion was not affected ($P \geq 0.17$) by feeding greater concentrations of LP, but N apparently absorbed (g/day and g/kg BW) decreased linearly ($P < 0.01$) with increased addition of LP in the diet. Nitrogen excreted in the urine (g/day) decreased linearly ($P < 0.01$) with increased supplementation of LP hay to the ewes. Retained N (g/day, g/kg BW, g/kg N of intake, g/kg of N absorbed) was not affected ($P \geq 0.15$) by inclusion of LB in alfalfa silage.

DISCUSSION

Lablab hay that was fed during this study was harvested at late maturity due to a rainfall pattern that did not allow favorable conditions for hay making. The concentration of NDF and ADF in this study were lower in alfalfa compared to *Lablab purpureus*, likely due to different stages of maturity at harvesting. Washaya et al. (2017) compared the variation of NDF and ADF of lablab at different maturity stages and found that the fiber concentrations were greater in post-anthesis compared to pre-anthesis stages. However, NDF and ADF values at maturity were 520 and 390 g/ kg DM, respectively, which are lower than the fiber concentrations of LP hay that was fed in the present study. Therefore, the greater fiber concentration of LP in this study may be attributed to the more advanced maturity of the forage. The N content of lablab is this study was less (13 g/kg) than that reported by Murphy et al. (1999), where the N concentration of lablab was above 33 g/kg, again indicative of a more advanced stage of maturity in the forage in the present study. Guanzura et al. (2012) compared nutrient composition of lablab with other forage species and found that the total polyphenols content in lablab was 1.24 mg/g, which is less than the total polyphenolic content (1.4 mg/g) that was fed in this study.

In the present study, dry matter and organic matter intake increased with feed containing a moderate amount of polyphenols, while feed with the greatest amount of polyphenols decreased intake. Feeding 60 mg/kg of flavonoids extract from lablab to dairy cows resulted in greater intake than feeding cows a diet supplemented with 100 mg/kg of flavonoids (Zhan et al., 2017). Contrary to the results of this study, a study involving feeding cannulated dairy cows different levels of polyphenols from propolis-based products reported no difference between treatments on dry matter intake (Aguiar et al., 2014). Increasing the amount of polyphenols in the diet depressed feeding intake by sheep during this study which is in agreement with Reed (1995) who stated that forages containing tannins or polyphenols may reduce feed intake.

In this study, DMD and OMD were not affected by supplementation of different levels of lablab. Similarly, supplementation of mulberry leaves in sheep diets as a source of flavonoids did not affect digestibility of DM and OM (Chen et al., 2016). On the other hand, feeding different concentrations of polyphenols from propolis improved digestibility in dairy cows (Aguiar et al., 2014). The responses of supplementing polyphenols in diet on intake and digestibility measurements are not consistent. Variation in phenolic compound responses on forage utilization by animals were suggested to be attributed to the differences in plant species that produce the phenolic compounds (Zhan et al., 2017).

Nitrogen intake by sheep decreased with increased LP hay in the diet. This decline can be attributed to difference in N content between the two forages where alfalfa silage had a greater concentration of N compared to LP. Fecal N was not altered with supplementation of LP in the diet. In contrast, Powell et al. (2009) reported that feeding polyphenols increased fecal N excretion in lactating Holstein dairy cows. Urinary N excretion decreased with increased LP in the diet. These results agree with those of Reed et al. (1995), who reported that increasing N intake above animal's requirement leads to an increase in urinary N excretion.

Conclusion

In this study, feeding a low concentration of *Lablab prupureus,* defined as 90 g/kg in an alfalfa silage diet improved both dry matter and organic matter intake but did not affect digestibility measures. Increasing the proportion of LP hay in the diet reduced N excretion in urine and N apparently absorbed but did not affect other N balance parameters. However, the lablab used in the present study was excessively mature due to the harvesting delays caused by weather interference which was not favorable for harvesting and hay drying. *Lablab prupureus* can be an alternative source of polyphenols to improve forage utilization, but the amount of polyphenol and forage quality should considered while supplementing lablab hay in the diet.

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TABLES

Table 1. Chemical composition of alfalfa silage and lablab purpureus (LP) hay [1]

[2]Item	Alfalfa silage	Lablab Purpureus hay
DM	40.5	90.2
OM, g/kg DM	917	895
aNDF, g/kg DM	443	631
ADF, g/kg DM	332	481
Nitrogen, g/kg DM	32	13
Total polyphenols, mg gallic acid equivalent/g	0	1.4
Antioxidant Capacity, mmol Trolox equivalent/g	0	7.3

[1]Alfalfa silage was either offered alone (CONT) or mixed with chopped LP to provide 90 (LOW), 180 (MED), or 270 g/kg (HIGH) LP on a dry matter (DM) basis,

[2] DM = dry matter, OM= organic matter, aNDF= neutral detergent fiber inclusive of ash, ADF= acid detergent fiber.

Table 2. Intake and digestibility by sheep offered alfalfa silage alone or alfalfa mixed with different proportions of lablab purpureus

	Treatments[1]					Effect[2]		
Item[3]	Control	Low	Med	High	SEM	Linear	Quadratic	Cubic
Intake								
DMI, g/day	1420	1534	1430	1284	86.3	0.12	0.08	0.60
DMI, g/kg BW	29	31	30	26	1.7	0.13	0.04	0.09
OMI, g/day	1301	1407	1311	1163	76.2	0.11	0.08	0.63
OMI, g/kg BW	26	29	27	24	1.5	0.11	0.03	0.69
Digestibility								
DMD, g/kg DM	620	612	612	611	10.0	0.51	0.71	0.86
OMD, g/kg DM	654	620	648	623	14.7	0.40	0.69	0.11
NDFD, g/kg DM	572	575	587	579	76.1	0.64	0.70	0.72
ADFD, g/kg DM	519	529	532	544	17.8	0.32	0.96	0.81
Digestible intake								
DDMI, g/day	881	940	874	786	53.5	0.12	0.14	0.64
DDMI, g/kg BW	18	19	18	16	1.0	0.13	0.08	0.70
DOMI, g/ day	852	868	849	730	48.1	0.08	0.17	0.77
DOMI, g/kg BW	17	18	18	15	0.9	0.09	0.10	0.71

[1]Alfalfa silage was either offered alone (CONT) or mixed with chopped LP to provide 90 (LOW), 180 (MED), or 270 g/kg (HIGH) LP on a dry matter (DM) basis.

[2] Probabilities for linear, quadratic and cubic orthogonal contrasts for means across all four different treatments.

[3] DMI= dry matter intake, OMI= organic matter intake, DMD = dry matter digestibility, OMD = organic matter digestibility, NDFD = neutral detergent fiber digestibility, ADFD= acid detergent fiber digestibility, DDMI= digestible dry matter intake, DOMI = digestible organic matter intake

Table 3. Nitrogen balance by lamb offered alfalfa silage alone or alfalfa mixed with different proportions of lablab purpureus hay

	Treatments[1]					Effect[2]		
Item[3]	Control	Low	Med	High	SEM	Linear	Quadratic	Cubic
N intake, g/day	47	48	43	37	3.3	< 0.01	0.09	0.60
N Intake, g/kg BW	0.94	0.98	0.89	0.75	0.066	< 0.01	0.04	0.64
Fecal N, g/kg N intake	314	336	330	337	17.1	0.17	0.48	0.36
N absorption, g/kg N intake	685	663	669	662	17.2	0.17	0.48	0.36
N app. abs, g/day	32	32	28	24	2.8	< 0.01	0.18	0.78
N app. abs, g/kg BW	0.64	0.65	0.59	0.50	0.056	< 0.01	0.10	0.83
Urine N, g/day	16	15	13	11	1.7	< 0.01	0.42	0.90
Urine N g/kg N intake	358	327	317	321	62.0	0.20	0.41	0.96
N retained g/day	16	17	15	13	4.2	0.15	0.36	0.83
N retained, g/kg BW	0.31	0.33	0.31	0.26	0.085	0.15	0.23	0.84
N retained, g/kg N intake	327	335	352	340	77.1	0.58	0.66	0.71
N retained, g/kg N absorbed	477	499	525	509	104.1	0.41	0.54	0.75

[1]Alfalfa silage was either offered alone (CONT) or mixed with chopped LP to provide 90 (LOW), 180 (MED), or 270 g/kg (HIGH) LP on a dry matter (DM) basis,

[2] Probabilities for linear, quadratic and cubic orthogonal contrasts for means across all four different treatments

[3] N= Nitrogen, BW = body weight, N app. abs = nitrogen apparently absorbed

CHAPTER VI

CONCLUSION

Harvesting and ensiling alfalfa and NE+ fescue mixtures resulted in increased silage total acids and lactic acid while ammonia decreased linearly with increasing tall fescue proportion. However adding the tall fescue decreased digestible DM and OM intake, which will affect animal performance negatively. Ruminal acetate increased and ruminal propionate and butyrate decreased with increasing fescue concentrations in the silage. The results from this experiment proved that mixing alfalfa and fescue after a killing frost improved silage fermentation characteristics which can help to avoid forage yield losses and reduce the production of ammonia which is an undesired product in silage, but mixing alfalfa and fescue can negatively affect forage utilization by ruminant animals.

Supplementation of tannins from sericea lespedeza to alfalfa silage decreased forage digestibility but did not affect feed intake while feeding low amount of *lablab purpureus* with alfalfa silage improved intake by sheep. Dietary manipulation by including tannins from sericea lespedeza and polyphenol from LP altered N excretion route, specifically reducing N excretion in urine. Feeding strategies used in these studies may be used as viable options to improve forage nitrogen utilization by ruminants and a sustainable approach to reduce negative impact of livestock production in the environment.

APPENDIX

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To:	Kenneth Coffey
Fr:	Craig Coon
Date:	May 23rd, 2018
Subject:	IACUC Approval
Expiration Date:	April 30th, 2021

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **18118:** *Effects of dietary modifications on digestion and nutrient balance in sheep and goats*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond April 30th, 2021 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Ken Coffey, Dirk Phillip, John Bignar, Colton Althaber, Valens Niyigena, and Jianchao Zhao. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Ken Coffey

FROM: Craig N. Coon, Chairman

DATE: 10/28/15

SUBJECT: IACUC Approval

Expiration Date: Nov 1, 2018

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # 16031: Nutrient balance studies with sheep and goats. The approved start date is November 2, 2015.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond Nov 1, 2018 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian