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Effect of Delayed Wrapping and Wrapping Source on Intake and Digestibility of Alfalfa Silage
in Gestating Sheep

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Animal Science

by

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Bachelor of Science in Animal Science, 2012

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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Baling silage with oxygen can result in dry matter deterioration and reduce silage intake by animals. This study was conducted to investigate the effects of two different wrapping sources and time intervals between baling and wrapping on intake and digestibility of alfalfa silage. The second objective was to assess the correlation of alfalfa silage fermentation parameters with intake and digestibility parameters in gestating sheep. Alfalfa silage was baled in large round bales then wrapped with plastic either with (KURA) or without (SUN) an oxygen-limiting barrier either the day of baling or 1, 2 or 3 d after baling. Beginning in January, silages were chopped and packed into plastic-lined trash containers, then offered randomly for ad libitum consumption to 16 gestating ewes ($n = 16$; 63.5 ± 1.71 kg BW) to provide 2 observations per treatment for 3 experimental periods. Each period consisted of a 10-d dietary adaptation period followed by 7 d of total fecal and urine collection. Ewes were housed in individual 1×1.5 -m pens with plastic coated grate flooring and were re-randomized to different treatments each period such that ewes were not offered the same treatment in any period. In general, intake and digestibility measurements were not affected ($P \geq 0.15$) by wrap type. Maximum digestible DM and DOMI were from silage wrapped the day following baling ($P < 0.05$). Correlations between fermentation measurements with intake and digestibility were not strong ($r^2 < 0.42$) however lactic concentrations expressed a greater correlation value ($r^2 = 0.14$) with both DDMI and DOMI compared to other fermentation characteristics. Therefore, wrapping silage beyond 1 day after baling can have detrimental effects on energy status in gestating ewes and desirable fermentation should also result in greater intake of digestible dry and organic matter, and lactic acid concentration was not the best predictor of DDMI and DOMI in sheep.

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Chapter 1

Literature review

I. Introduction

Forage and other animal feed demands have been increasing in the last number of years as the number of animals in United States increases. Particularly, the total number of sheep in US has reached 5.28 million across all states (ASIA, 2016). Ruminants can access forage in several ways; by grazing on pasture, or by consuming silage or hay. However, pasture is a major source of feed for most cows and sheep. Ruminants require feedstuffs that are rich in degradable carbohydrates and proteins for better performance, but the supplementation of protein in animal feed has become an expensive approach. Furthermore, the restriction of protein supplements of animal origin in certain countries, such as the UK, have forced farmers to rely on home grown forage legume crops as protein supplements (Fraser et al., 2000). Forage shortage in the winter season is one of the big challenges faced by American farmers. Consequently, feed conservation through silage making was suggested to be a sustainable solution of feed shortage during the winter (Santana et al., 2015). Baled silage production has the advantages of reduced leaf losses, and shorter wilting times, thereby limiting risks of exposure to rain compared with making hay (Han et al., 2004). However, several problems may occur during silage making that can have detrimental effects on forage quality, intake and digestibility (Kung and Shaver, 2000). Therefore, a good understanding of factors that affect forage quality will help to improve livestock production through optimization of forage nutritive value and intake.

Voluntary forage intake is the major dietary factor determining level and efficiency of ruminant production (Allison, 1985). There are several factors that can affect intake such as

forage nutrient content, physical form of the feed, digestibility, chemical composition of the feed, forage species, type of forage, palatability, and forage maturity (Thornton and Minson, 1973, Allison, 1985, Bruinenberg et al., 2002). The objective of this literature review is to discuss factors that affect forage intake, digestibility and silage fermentation characteristics.

II. Factors affecting forage intake and digestibility

Forage intake and digestibility are interrelated. Poor quality feed can depress digestibility; thus, the animal needs to consume sufficient quantities of the feed to meet their requirements but the reduced digestibility limits intake as discussed in this section. Forages must be more digestible and absorbable so that ruminant animals can meet the requirements for greater growth and production.

Forage maturity: Forage mass accumulates as forages mature, but fiber content increases as well. As forages advance in maturity stage, the proportion of cell wall increases and that of cell solubles decreases (Bruinenberg et al., 2002, Akin and Burdick, 1975). A study was conducted to compare the effects of red clover vs. timothy-meadow fescue maturity at primary harvest (early vs. late cut silage) on rumen fermentation, nutrient digestion, and nitrogen metabolism using cannulated lactating cows. These animals were fed diets that consisted of grass, legume or a combination of both. Dry matter intake of grass silage slightly decreased while that of red clover slightly increased with extended maturity. Nitrogen excreted in feces and microbial non-ammonia nitrogen(NAN) decreased with advancing maturity in grass silage, but these parameters increased with advancing maturity in red clover silage (Vanhatalo et al., 2009).

When alfalfa (*Medicago sativa* L.) hay that was harvested at four different maturity stages was fed to sheep, DM intake decreased while NDF and ADF intake increased with

advancing maturity (Kawas et al., 1990). However, ensiling maize at very low maturity stage (250 g DM/kg) with a low starch content and a low starch to NDF ratio was reported to lower DM intake and milk production (Khan et al., 2015). In another study, early or late cut maize silage was fed as the sole forage or together with grass silage at average proportion of 42% DM of forage. The concentrate from distiller's grains plus rolled barley was added to the forage diet at a proportion of 60% total diet then fed to lambs. Increasing maturity at harvest tended to increase DMI by lamb (Helander et al., 2015). Other studies have also reported that forage digestibility decreases with advancing maturity (Darlington and Hershberger, 1968, McAllister et al., 1997). Therefore, maturity can negatively affect forage digestibility by decreasing rate of passage and increasing retention time, which ultimately reduces animal feed intake (Allison 1985). However, forage maturity effects can vary with forage species and forage type.

Chemical composition of the forage: Carbohydrates are key components in forages because they are used as energy sources by animals. Forage carbohydrates can be divided into nonstructural carbohydrates including monosaccharides, oligosaccharides, fructosans and starch and structural carbohydrates which consist of pectin, hemicellulose and glycoprotein (Van Soest et al., 1991).

The concentration of NDF is greater in stem than leaves (Jung and Allen 1995). The NDF fraction increases as the forage matures, and increasing NDF concentration may also increase rumen retention time. When both sheep and cattle were fed angola grass (*Digitaria decumbens*) and Rhodes grass (*Chloris gayana*) cut at 6 and 12 weeks of regrowth, DMI was greater for leaves due to shorter rumen retention time and DMI was lower in stem due to greater rumen retention time (Poppi et al., 1981). Dry matter intake was greater by Holstein cows offered a 28% NDF diet compared with those offered a 32% NDF diet (Kendall et al., 2009). Moreover

improvements in NDF digestibility were proposed to remarkably reduce rumen retention and increase intake, and milk yield (Oba and Allen, 1999). Lignin, although low in concentration in comparison to cellulose and hemicellulose, can limit ruminal forage cell wall digestion by preventing the enzymatic hydrolysis of polysaccharides leading to a decrease in intake and digestibility (Jung and Allen, 1995). In a biomass digestibility study which involved straw samples from four different *Bassica* species, the G lignin negatively affected biomass digestibility, but hemicellulosic monosaccharides positively affected biomass digestibility (Pei et al., 2016).

Physical form of the feed: Ruminant animals reduce forage particle size by mastication, rumination, and digestion. Chopping forage increases the surface area, which facilitates microbial attachment and fermentation of the forage (Marsh, 1978). Feeding shredded alfalfa hay to sheep was reported to increase intake of DM compared to non-shredded alfalfa but the rate of passage and total mean retention time of hay in the digestive tract were not affected by shredding (Hong et al., 1988). In a study utilizing alfalfa silage of different particle sizes as the main source of forage in dairy cattle diets, reducing the size of chopped alfalfa from 19 to 10 mm resulted in increased feed intake but did not affect other productive performance measurements (Kammes and Allen, 2012). In contrast, when alfalfa hay in the prebloom stage was fed to Holstein cows in long, chopped and pelleted forms, their average DM intakes were 3.75, 2.93, and 1.95% of BW, respectively and there was no effect of physical form of the forage on milk production, rumen fermentation, chewing activity and fat milk composition (Shaver et al., 1986). It should be noted that these studies evaluated forage particle size in total mixed diets for dairy cattle. The results will likely be different depending on the quality of forage and when an all-forage diet is offered.

Forage species: Forages fed to ruminant animals may be divided in four major categories, with grasses and legumes being the most common. Cell structure of legumes makes them more degradable than grasses because of the presence of high proportions of mesophyll in leaves of legumes, and parenchyma cells of legumes are completely degraded by ruminal microbes while parenchyma in grasses is poorly broken down (Akin, 1989). In a study conducted in Iran, gramineae species had a higher ADF and NDF content than leguminosae species (Amiri and Shariff, 2012). When sheep were fed different species of grasses and legumes, the voluntary intake of legumes was greater with shorter retention time in the rumen than in sheep fed grasses (Thornton and Minson, 1973). The combination of legume and grasses has considerable advantages. Supplementation of legume leaves to a grass hay diet improved total OM intake and digestible OM in growing Mpwapwabulls (Mero and Uden, 1998). In addition, compared to monoculture, planting a mixture of grasses and legumes improved DM yield, without reducing herbage digestibility and CP content (Sturludóttir et al., 2013).

Forage palatability: Palatability affects forage intake in grazing animals. Preference for particular forages can be characterized by the taste, smell, appearance, physical form of the feed and forage type. Palatability can be influenced by sensors because animals prefer food with specific odors and flavors that trigger appetite (Ball et al., 2001). Sheep were reported to like food with monosodium glutamate for the flavor and butyric acid for the odor but they dislike acetic acid (Baumont et al., 1997). When different American native legumes species were grazed by sheep in order to compare the palatability among these forages, purple prairie clover and Illinois bundle flower were most palatable and easily consumed among other native legumes but maturity, nutrient values and leaf structure were not associated with palatability (Sheaffer et al., 2009).

III. Process and Conditions of Silage Making

Silage consists of preserving green forage crops under acidic conditions ensuring they remain in a succulent and appetizing state. This process is controlled by microbes which convert carbohydrates into lactic acid and other fermentation products. Several conditions such as moisture, oxygen concentration, and type of forage affect the ensiling process.

Water content of silage can increase subsequent intake by solubilizing forage sugars and making them available for microbes. Water can also dilute undesirable fermentation products. Lactating Holstein cows were fed diets with different moisture contents (78, 64, 52, and 40% DM). The replacement of alfalfa hay by silage increased intake, but the partial substitution of corn straw by corn silage did not affect intake (Lahr et al., 1983). In a study conducted to assess the effect moisture and bale density on round bale silage, the average DMI by steers offered alfalfa hay was 17.5 kg/d. When the same steers were offered alfalfa silages with two different moisture concentrations, 512 g/kg moisture silage, and 594 g/kg moisture silage, their average DMI were 19.4 and 20.5 kg/d respectively, demonstrating that preserving alfalfa as silage improved forage intake compared with hay (Han et al., 2004). Conversely, another study was done to evaluate preservation and feeding value of alfalfa stored as hay, haylage, and direct-cut silage. Feeding values, feed intake, and digestibility coefficients were greater in cows offered alfalfa hay compared with those offered silage because of high moisture, volatile organic acids and ammonia-nitrogen in silage (Gordon et al., 1961).

Presence of oxygen in baled silage can stimulate spontaneous heat production (Coblentz et al., 2004). Exposure of ensiled or ensiling forage with air increases DM loss, acetic acid production, and undesirable microorganisms such as mold and yeasts, and also decreases the

sugar content of silage (Williams et al., 1994). When alfalfa silage was preserved in a gas tight silo, poor closing of the cap of the silo allowed exposure of silage to oxygen resulting in DM loss of alfalfa (Gordon et al., 1961). In addition, feeding silage that was exposed to air for an extended time can affect animal performance negatively. Feed intake by goats declined by up to 58% from silage that was removed from the silo for 8 d compared to fresh silage (Gerlach et al., 2013). Therefore oxygen must be prevented while baling, and monitored during feeding silage.

Forage type and species must be taken into consideration when ensiling because some forages require greater precaution in order to maintain the quality of silage. Alfalfa is very challenging to ensile because of low fermentable carbohydrates, high buffering capacity and stem structure that facilitates air absorption while ensiling (Marshall et al., 1993). Contrary to legumes, some grasses can be conserved easily as silage because they contain high concentrations of soluble carbohydrates, low to moderate buffering capacity, and low protein and mineral content. Mixing legumes with grasses has advantages on forage preservation. Ensiling alfalfa mixed with orchardgrass at 50:50 ratio produced better quality silage than ensiling alfalfa alone (Samuil et al., 2015).

Silage acids

Lactic acid plays a major role in silage quality, especially by preserving its nutrients. It is a stronger acid than other silage acids, is responsible for the drop in pH and lowers the loss in DM. Poor production of lactic acid in silage may be caused by high DM content (>50%), high butyric acid, and restricted fermentation in cold weather (Kung and Shaver, 2000). A study was conducted to evaluate the effect of inoculating lactic acid producing bacteria in alfalfa silage on aerobic stability, intake and digestibility in steers and lambs (McAllister et al., 1997). Alfalfa

silage was treated with either no inoculant or with a mixture of *Lactobacillus plantarum* and *Enterococcus faecium*, or *L. plantarum* alone. After 15 d, silage pH and temperature decreased in all silages but water soluble carbohydrate were greater in treated than in non-treated alfalfa silages. Dry matter intake by lambs was slightly greater in treated silage than non-treated silage but DM and OM digestibility did not differ across treatments. Moreover DM intake by steers was high in treated silage, leading to conclusion that these lactic acid producing bacteria were responsible for the prevention of alfalfa silage deterioration.

High concentrations of butyric acid (> 5 g/kg DM) indicates a poor fermentation of silage due to the activity of clostridial organisms and these silages are characterized by high fiber and soluble protein content, and a small amount of amine production (Kung and Shaver, 2000). Feeding silage with high concentrations of butyric acid (35.2 g/kg DM) to dairy cows increased the probability of subclinical ketosis (Vicente et al., 2014). Silages with high butyric acid concentrations are characterized by lower energy content which may cause intake depression in lactating cows (Kung and Shaver, 2000).

Ethanol formation in silage is a result of excessive yeast activity during silage fermentation. Normally, the concentration of ethanol in acceptable silage ranges between 10 and 20 g/kg DM (Kung and Shaver, 2000). Feeding silage that contains ethanol can affect animal performance. Adding 5% ethanol on bermudagrass hay was reported to increase milk and intake in lactating Holstein cows (Daniel et al., 2013). However, feeding silage that contains over 30 to 40 g/kg DM as ethanol was postulated to cause off flavors in milk (Kung and Shaver, 2000).

Factors affecting digestibility and intake of silage

The concentrations of water soluble carbohydrates in alfalfa forage were reported to be linked to the time of the day, with the greater level in the afternoon than in the morning (Ball et al., 2001). Soluble carbohydrates were greatly related to tall fescue palatability (Tava et al., 1995). Water-soluble carbohydrate can also improve preservation quality of silage during fermentation because anaerobic microbes transform water soluble carbohydrates into lactic acid which is responsible for the drop of pH (Kung and Shaver, 2000). Moreover, the presence of non-fiber carbohydrates (NFC) optimize the utilization of non-protein nitrogen by microbes in alfalfa silage (Valadares et al., 1999).

Protein content in silage can impact N apparent absorption and N balance. A study was conducted to compare intake and digestibility by lambs fed high-protein content legume silages that included late second-cut birdsfoot trefoil (*Lotus corniculatus* L.), first-cut sainfoin (*Onobrychis viciifolia*), both early and late second-cut red clover (*Trifolium pratense*) and alfalfa. Voluntary intakes were between 71 and 81 g/kg, but voluntary intake of trefoil silage was significantly greater than that of the alfalfa silages. Apparent N absorption was around 0.7 for all silages except sainfoin which had the lowest apparent N absorption due to condensed tannins. The greatest N loss in urine (g/kg of N intake) was from lamb offered alfalfa silages but the retained N was greater by lambs offered birdsfoot trefoil (16 g N/d and sainfoin (-2 g N/d) silages respectively (Fraser et al., 2000). Moreover; degradation of large amounts of protein in silage produce ammonia and that process is caused by the clostridial activity or a slow drop in pH during fermentation. Leaving spaces while packing and excessive moisture of silage (< 30% DM) are also major sources of ammonia production in silages (Kung and Shaver, 2001).

IV. Conclusion

Several factors such as forage maturity, chemical composition, particle size and forage species affect forage intake, digestibility, and animal performance. The conditions such as oxygen and humidity must be carefully monitored when harvesting and ensiling alfalfa in order to reduce dry matter loss, prevent the production of butyric acid and minimize clostridial activity while concurrently increasing the lactic acid content that enables preservation of good quality silage. In addition, alfalfa silage needs to be ensiled with caution due to high protein content and buffering capacity. However, there are challenging situations where farmers cannot avoid the exposure of silage to oxygen, particularly exposure that occurs between baling and wrapping. This exposure of silage bales to oxygen is increased by time delay between baling and wrapping. Therefore, the objective of this study was to evaluate the impacts of the effect of delayed wrapping and wrapping source on intake and digestibility of alfalfa silage in gestating sheep.

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Chapter 2

Effect of delayed wrapping and wrapping source on intake and digestibility of alfalfa silage in gestating sheep

ABSTRACT

Delays often occur between baling and wrapping bale silage that increases exposure time of the silage to oxygen. This study was conducted to investigate the effects of two different wrapping sources and time intervals between baling and wrapping on intake and digestibility of alfalfa silage by gestating sheep. Alfalfa silage was baled in large round bales then wrapped with plastic either with (KURA) or without (SUN) an oxygen-limiting barrier either the day of baling or 1, 2 or 3 d after baling. Beginning in January, silages were chopped and packed into plastic-lined trash containers, then offered randomly for ad libitum consumption to 16 gestating ewes ($n = 16$; 63.5 ± 1.71 kg BW) to provide 2 observations per treatment for 3 experimental periods. Each period consisted of a 10-d dietary adaptation period followed by 7 d of total fecal and urine collection. Ewes were housed in individual 1×1.5 -m pens with plastic coated grate flooring and were re-randomized to different treatments each period such that ewes were not offered the same treatment in any period. Data were analyzed using PROC MIXED of SAS for a 2×4 factorial treatment arrangement and orthogonal polynomial trend analyses were used to assess effects of time delay for wrapping after baling. Intake of DM and OM (g/kg BW) responded in linear and quadratic manners ($P < 0.05$) as wrapping was delayed after baling. Digestibility of DM (%) and OM (%) responded cubically ($P < 0.05$), that of NDF increased linearly ($P < 0.05$) with wrapping time delay after baling. Both digestible DM and OM intake (g/kg BW) responded linearly and quadratically ($P < 0.05$) and that of DOMI also responded cubically ($P < 0.05$) with time delay

between baling and wrapping. In general, intake and digestibility were greatest in silage wrapped the day following baling. Type of wrap tended ($P = 0.10$) to affect DM digestibility, and the wrap type \times wrapping time after baling interaction tended to affect OMD and digestible OMI ($P = 0.06$ and 0.05 , respectively), but other intake and digestibility measurements were not affected ($P \geq 0.15$) by wrap type and the interaction of wrap and wrapping time after baling. Therefore, delaying wrapping alfalfa silage bales beyond 1 d after baling may have detrimental effects on energy status in gestating ewes.

INTRODUCTION

Alfalfa is the most important forage legume grown in the US (Lacefield, 2013). Producing hay is sometimes challenging due to unfavorable weather conditions that prevent hay from drying before baling and that can negatively affect forage quality (Coblentz et al., 2004). Conservation of alfalfa as silage may be a good approach because it reduces leaf losses, and requires a shorter wilting time, thereby limiting risks of exposure to rain compared with making hay (Han et al., 2004). Recommended moisture content in baled alfalfa silage must not exceed 55% (Shinners, 2003) and 70% in chopped alfalfa silage (Muck et al., 2003). When moisture content is out of the recommended range, this can affect silage quality, fermentation characteristics and feed intake. Higher moisture content is associated with production of undesired fermentation products such as NH_3 (Muck, 1987), and clostridial activity which reduces silage quality and intake (Kung and Shaver, 2000). Delays may occur between baling and wrapping bale silage that increase exposure time of the silage to oxygen. Baling forage with oxygen was related to spontaneous heat production (Coblentz et al., 2004) forage deterioration, dry matter loss, and silage intake depression (Gordon et al., 1961; Williams et al., 1994; Gerlach et al., 2013). Baling silage with a plastic that contains an O_2 barrier reduced alfalfa silage

deterioration (Borreani and Tabacco, 2008). Moreover, increasing the number of plastic layers from 2 to 4 improved alfalfa baled silage quality and preference by cows (Hancock and Collins, 2006). Still, there are few studies reporting effects of feeding baled alfalfa silage on animal performance. Consequently; the objective of this study was to evaluate the effect of delayed wrapping and wrapping type on intake and digestibility in gestating ewes.

MATERIALS AND METHODS

Field, Storage, Silage making

The field description, storage and silage making methods were discussed in detail by Coblenz et al. (2015) and are therefore not repeated herein. Briefly, alfalfa was grown and harvested from an 8.0-ha site on the University of Wisconsin Marshfield Agricultural Research Station, Stratford, Wisconsin (44°7'N, 90°1'W). The field was planted with alfalfa in 2013 and the second cutting in 2014 was removed in August when the alfalfa was at 25% bloom. The alfalfa was baled into 64 round bales and silage treatments were then generated using a 2 × 4 factorial treatment arrangement with 2 bales/treatment combination. Main effects for treatments consisted of 1) wrapping with a plastic that had an oxygen limiting barrier (**OB**; Kuraray America Inc., Pasadena, TX) or with the same plastic that did not have an oxygen limiting barrier (**NOB**; SUNFILM; 750 mm × 1,500 m × 25 μ) and 2) wrapped the day of baling or 1, 2, or 3 d between baling and wrapping. The bales were each wrapped with seven layers of plastic at an average moisture concentration of 59.1%. Alfalfa was stored for 5 months, and in January 2015, silage was transported from Wisconsin to Arkansas to be fed to gestating sheep.

Animals and design

Experimental protocol and procedures were approved by the Institutional Animal Care and Use Committee at the University of Arkansas (Protocol #13007). The study was carried out at the University of Arkansas North Farm in Washington County, Fayetteville, AR (36°4'N, 94°9'W) from January to March 2015. Katahdin ewes (n=16; 63.5 ± 1.71 kg BW) that were confirmed as pregnant via blood test (Verden Veterinary Clinic, Verden, OK) were obtained from Lincoln University (Jefferson City, MO). Ewes were allowed 21 d to recover from shipping/receiving stress then after sorting off the sick or otherwise less desirable animals, were weighed, and then allocated randomly to treatments. Ewes were allocated to provide 2 ewes per treatment for 3 experimental periods. Each period consisted of a 10-d dietary adaptation period followed by 7 d of total fecal and urine collection. Ewes were re-randomized to different treatments each period such that ewes were not offered the same treatment in any consecutive period. Ewes were co-mingled between periods and fed a common alfalfa silage diet for 4 d.

Ewes were housed in individual 1 × 1.5-m pens with plastic coated grate flooring in an enclosed insulated metal shed with the temperature controlled at approximately 15.5°C. Ewes were given access to 14 h of light and 10 h of darkness. Ewes were removed from the individual pens and comingled on a concrete floor for a minimum of 3 h on d 10 for an exercise period and to allow for thorough pen cleaning prior to starting total collections. Ewes had access to water but no feed during this time.

Pens were fitted individually with frames underneath each individual pen with a solid corrugated PVC sheet covered with fiberglass screening to allow for separate collection of urine and feces. The end of each tray was fitted with a PVC gutter to facilitate total drainage of urine into individual plastic collection pans. Before the beginning of each collection period, trays were removed, washed, and then returned back to their initial position.

Feeding

At the beginning of each period on the day prior to the initiation of feeding, bales from one of the two field replications per treatment were opened, chopped with a commercial straw chopper (model SB 5400; Harper Industries, Inc., Harper, KS) and packed into plastic containers that were lined with 2 heavy-duty plastic trash can liners. Air was removed from the silage by walking on it as it was being placed into the trash cans. After chopping and packing, headspace air was removed to the extent possible and the plastic liners were tied individually. The containers were then stored outside because of lower ambient temperature which helped further suppress spontaneous heating until the time the silages were fed.

Water and feed were offered for ad libitum consumption. Feed was offered in equal feedings at 0800 and 1600 h daily to achieve a minimum of 10% refusal (DM basis). Orts were collected daily at 1500 h and approximately 30 g of a commercial mineral¹ that did not contain an antibiotic was offered daily, immediately after removing Orts in the feeders.

Feed sampling for the digestion portion of the study began 2 d prior to the initiation of fecal collection. Two samples/silage treatment were gathered daily; one sample was weighed and dried to a constant weight at 50°C and another was placed in plastic freezer bags and frozen for later analyses of fermentation profiles. Orts collection began 1 d before fecal collection; these samples were weighed and dried to a constant weight at 50°C. Feces were collected twice daily at 0800 and 1500 h, weighed immediately, and a sub-sample was weighed and dried to a constant weight at 50°C. Urine was collected twice daily at 0800 and 1500 h from plastic containers

¹ Preferred Mineral for Sheep and Goats (Ragland Mills Inc., Neosho, MO) 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.

placed at the end of the urine gutters and a 20% aliquot was stored frozen (-20°C) pending later analyses. 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. The urine acidity was checked using a portable pH meter to verify that the pH was at or below 2.

Chemical analysis

Daily feed samples were composited by treatment each period and orts and feces were composited by animal each period. A sub-sample was taken, and then ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas, West Washington Square, PA). Ash concentrations were determined by burning samples in a muffle furnace at 500°C for 6 h (Method 942.05; AOAC, 2000) and OM was calculated as the DM minus ash. Sequential analysis for NDF and ADF were performed with an Ankom 200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY; Vogel et al., 1999). Nitrogen was measured using the Dumas total combustion method (Elementar Americas, Mt. Laurel, NJ; Method 990.03; AOAC, 2000). Forage fermentation profiles were analyzed by Cumberland Valley Analytical Services, Hagerstown, MD.

Statistical analysis

Data were analyzed using PROC MIXED of SAS (SAS Institute, Cary, NC) for a 2 × 4 factorial treatment arrangement, using animal as experimental unit. Wrap type, wrapping time after baling and their interaction were considered fixed effects and period and animal were considered random effects. In the absence of a wrap type × time after baling interaction, linear, quadratic and cubic orthogonal polynomial trend analyses were used to assess effects of time

delay for wrapping after baling. When the wrap type \times time after baling interaction was detected ($P < 0.05$), means were compared using an F protected t-test.

RESULTS

Forage quality measurements

Forage composition results were presented in Table 1. There was an interaction ($P \leq 0.02$) between wrap type and time delay for all forage quality measurements of DM, OM, ADF, NDF, and N. Dry matter (%) concentrations were greater in silage wrapped with OB on d 2 and 3 and NOB on d 2 compared to silage wrapped with OB on d 0 and 1 and NOB on d 0, 1, or 3 after baling. Differences among treatments were detected for OM concentrations, but the overall range was less than 1 percentage unit. Concentrations of NDF and ADF (% of DM) also increased with extending the time between baling and wrapping with the greatest concentrations for silage wrapped with OB or NOB on 2 and 3 d and the lowest on silage wrapped with OB or NOB on d 0 or 1 d after baling. However, N concentration (% of DM) decreased with time delay between baling and wrapping. The greatest N concentration (% of DM) was from the silage wrapped with OB or NOB on d 0 or d 1 after baling, and the lowest from the silage wrapped OB or NOB 2 or 3 d after baling. No effect of wrap type on all forage quality measurements ($P > 0.12$) was detected.

Fermentation characteristics

Fermentation characteristics are presented Table 2. There was an interaction ($P = 0.03$) between wrap type and time after baling on lactic acid concentrations when expressed as a proportion of total silage acids, but not ($P \geq 0.17$) for other fermentation parameters. Moreover, there were no effects of wrap type ($P \geq 0.31$) on any of the silage fermentation measurements.

Silage pH was not affected ($P \geq 0.39$) by time interval between baling and wrapping, however, total silage acid concentrations (% of DM) decreased linearly ($P < 0.01$) as time delay between baling and wrapping was extended. Lactic acid concentration (% of DM) was linearly ($P < 0.01$) and cubically ($P = 0.02$) affected by time delay. Lactic acid concentrations (% of total acids) decreased with extending the time between baling and wrapping with the greatest concentrations within silage wrapped with OB or NOB on d 1 or d 0 after baling and the lowest on silage wrapped with OB or NOB 2 or 3 d after baling.

Acetate concentrations (% of DM) responded quadratically ($P = 0.03$) to time delay between wrapping and baling, and propionate concentrations (% of DM) decreased linearly ($P < 0.01$) with time delay between baling and wrapping. Butyric acid concentrations (% of DM) tended ($P = 0.06$) to decrease linearly as wrapping time delay increased, but no effect ($P \geq 0.23$) of time delay on NH_3N concentrations expressed in CP equivalents (%) was detected.

Intake and digestibility

There were no interactions ($P \geq 0.15$) between wrap type and time delay between baling and wrapping on intake parameters (Table 3). Wrap type did not affect ($P \geq 0.66$) silage DM or OM intakes when expressed as either g/d or g/kg BW. However, DMI and OMI (g/d) responded in linear, quadratic, and cubic manners ($P \leq 0.04$) where the greatest intake was observed from silage wrapped 1 d after baling and the lowest from silage wrapped 3 d after baling. When expressed per unit of BW, both intakes of DM and OM were linearly ($P = 0.03$) and quadratically ($P < 0.01$) affected by time delay between baling and wrapping, and only tended to respond cubically ($P \leq 0.09$). Again, the maximum DMI and OMI (g/kg BW) were from silage wrapped 1 d after baling and minimum on silage wrapped 3 d after baling.

Digestibility measurements are presented in Table 4. The wrap type \times time delay interaction affected ($P = 0.04$) DOMI (g/kg BW) and tended to affect OMD (%) and DDMI (g/kg BW; $P = 0.06$ and 0.07 , respectively), but did not affect ($P \geq 0.17$) other digestibility measurements. Dry matter digestibility (%) tended ($P = 0.10$) to be greater for silage wrapped with plastic that had OB compared to plastic without OB, but, OMD (%), NDFD (%), ADFD (%) were not affected ($P > 0.18$) by wrap type. Digestibility of DM ($P = 0.02$) and OM ($P = 0.03$) responded cubically with the greatest DMD and OMD from silage wrapped the day following baling and the lowest DMD and OMD were from silages baled 2 d after baling. Digestibility of NDF increased linearly ($P = 0.04$) with delaying time between baling and wrapping. Digestibility of ADF (%) tended ($P = 0.08$) to increase linearly with extending time interval between baling and wrapping. Digestible DM and digestible OM intake (g/kg BW) were not affected ($P \geq 0.79$) by type wrapping material. Digestible DMI (g/kg BW) responded linearly ($P = 0.01$) and quadratically ($P < 0.01$) with time delay between baling and wrapping. Digestible OMI g/kg BW was greatest in silage wrapped with OB or NOB on 0 or d 1 after baling compared to silage wrapped 2 or 3 d after baling. For both DDMI and DOMI (g/d or g/kg BW) the maximum digestibility values were observed from silages wrapped 1 d after baling and the minimum from silages wrapped 3 d after baling.

DISCUSSION

Wrap type effects

In the present study, the alfalfa silage was wrapped with seven layers of plastic and with two wrap types but wrap type did not affect fermentation characteristics. Borreani and Tabacco (2008) reported that the extra oxygen limiting barrier improved alfalfa silage quality by reducing

DM loss and mold spoilage when the silage bales were wrapped with 4 layers of plastic. In the current study, concentrations of lactic acid and ammonia N expressed in crude protein equivalents ($\text{NH}_3\text{N-CPE}$) were not affected by wrap type and those results are consistent with those of (Borreani and Tabacco, 2008) who reported that inclusion of OB did not affect lactic and NH_3N concentrations.

Time delay effects

The DM content of the silages was $\leq 49.6\%$. The silage wrapped with OB plastic on d 2 and 3 after baling and silage wrapped with NOB plastic on d 2 after baling contained DM concentration of greater than 45%. This is within the recommended DM range from 45 to 55% for baled silage (Shinners, 2003). The silage that was wrapped on the same day that it was baled had a lower DM concentration (41 to 43%) compared to that recommended for baled silage DM which should be below 45% (Shinners, 2003). This helps explain the greater concentration of butyrate from these particular silages which could have also reduced intake by sheep fed silage wrapped on d 0 compared on intake of silage wrapped 1 d after baling. The lack of a difference in silage pH was probably related to the buffering capacity of alfalfa silage due the presence of organic acids (McDonald et al., 1991).

Time delay between baling and wrapping increased exposure time of the silages to oxygen. The changes in fermentation, intake and digestibility measurements noticed may be attributed to air penetration in silage which possibly increased growth of aerobic bacteria, especially yeast, mold, but these were not measured in the present study. These microorganisms were reported to be responsible for silage aerobic deterioration, DM loss, and decline in intake (Scudamore and Livesey, 1998). The proliferation of those new microorganisms, especially

yeast, can ferment sugar to ethanol and CO₂ (Schlegel, 1987) thereby reducing the amount of sugars in the forage. In the present study, propionate decreased as the time between baling and wrapping increased possibly affecting silage deterioration, but this was not measured in the present study.

Dry matter and OM intakes, digestibility, and digestible intake were greatest in silages that were left unwrapped for 1 d after baling. The preference and digestibility of this particular silage treatment cannot be explained by trends from any of the fermentation measurements as most of those responses, if affected by time between baling and wrapping, responded linearly to delay between wrapping and baling. Soluble carbohydrates were reported to be greatly related to tall fescue palatability (Tava et al., 1995) which can also be a factor of greater intake in that particular silage. Additionally, concentration of water soluble carbohydrates in silage was positively correlated with DMI (Huhtanen et al., 2007). However, the trend observed for the lactic acid concentration expressed as a percentage of total acids more closely follows the trends observed intake and digestibility measurements. Therefore, direct fermentation measurements that directly relate to intake and digestibility measurements were not observed in this study.

CONCLUSION

The negative impacts of oxygen on silage fermentation and quality are well documented. This has resulted in current recommendations for almost immediate wrapping following baling to avoid further oxygen exposure. Simple observations of the fermentation measurements across the time delay between wrapping and baling would support the recommendations for wrapping as soon as possible following baling. However, based on intake and digestibility data, and particularly intake of digestible organic matter derived from this study, it is apparent that animal

performance should not be impacted until the silage wrapping was delayed for 2 to 3 days after baling. It should be further noted that silage wrap containing an oxygen-limiting barrier is not necessary if seven layers of plastic are used.

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Table 2.1. Forage composition of alfalfa silage wrapped with 2 different wrap sources when wrapping was delayed from 0 to 3 days after baling

Treatment		Item				
		DM,% ¹	OM,% DM	NDF,% DM	ADF,% DM	N,% DM
Wrap	Time delay ²					
O ₂ -limiting	0	43.4 ^{cd}	88.7 ^{abc}	44.5 ^d	36.8 ^b	3.1 ^{cd}
	1	42.3 ^{cd}	88.1 ^d	45.8 ^{cd}	37.3 ^b	3.3 ^a
	2	45.4 ^{bc}	88.2 ^{cd}	48.7 ^a	39.4 ^a	3.0 ^{cd}
	3	49.6 ^a	89.0 ^{ab}	48.5 ^a	39.7 ^a	3.1 ^{cde}
Conventional	0	41.4 ^d	88.9 ^{ab}	46.8 ^{bc}	38.8 ^a	3.1 ^{de}
	1	43.1 ^{cd}	88.5 ^{bcd}	46.0 ^c	37.3 ^b	3.3 ^a
	2	47.3 ^{ab}	89.2 ^a	47.5 ^{ab}	38.9 ^a	3.2 ^c
	3	42.8 ^{cd}	88.4 ^{bcd}	47.5 ^{ab}	38.7 ^a	3.1 ^{bc}
SEM ³		0.24	0.20	0.01	0.13	0.09
Effect						
Wrap type		0.12	0.14	0.88	0.69	0.48
Time		<0.01	0.15	<0.01	<0.01	<0.01
Wrap × time		0.01	0.01	< 0.01	<0.01	0.02

¹DM = Dry matter; OM = organic matter; NDF= Neutral detergent fiber; ADF acid detergent fiber, N = nitrogen concentration.

²Time delay from 0 to 3 days between baling and wrapping bales with O₂ limiting vs convention wrap.

³SEM = pooled standard error of the mean.

^{abcd}Means within a column without a common superscript letter differ ($P < 0.05$).

Table 2.2. Fermentation characteristics of alfalfa silage wrapped with 2 different wrap sources when wrapping was delayed from 0 to 3 days after baling

Treatment	Item							
	pH	TA, %	Lactic, %	LTA, %	Ace, %	Pro, %	But, %	NH ₃ N-CPE
Wrap Type								
O ₂ -limiting	5.4	3.61	1.45	39.83	1.59	0.12	0.35	3.77
Conventional	5.5	3.73	1.36	36.83	1.75	0.15	0.34	3.63
SEM ²	0.05	0.316	0.125	2.027	0.184	0.025	0.099	0.284
Wrapping Delay, d³								
0	5.45	5.21	2.26	44.33	2.09	0.22	0.57	4.09
1	5.54	4.1	1.91	47.83	1.95	0.14	0.42	3.79
2	5.47	2.4	0.86	38.83	1.14	0.11	0.17	3.12
3	5.47	2.91	0.60	22.33	1.87	0.06	0.23	3.81
SEM ²	0.081	0.446	0.158	2.867	0.261	0.035	0.140	0.402
Wrap × time	0.74	0.22	0.63	0.03	0.22	0.32	0.17	0.45
Contrasts⁴								
i) Delay ; Linear	0.99	< 0.01	< 0.01	< 0.01	0.35	< 0.01	0.06	0.74
ii) Delay: Quadratic	0.49	0.10	0.76	< 0.01	0.03	0.77	0.46	0.41
iii) Delay: Cubic	0.39	0.15	0.02	0.70	0.34	0.68	0.53	0.23
iv) O ₂ -limiting vs conventional	0.62	0.79	0.50	0.31	0.53	0.34	0.97	0.35

¹NH₃N-CPE = ammonia crud protein equivalent.

²SEM = pooled standard error of the mean.

³Time delay from 0 to 3 days between baling and wrapping bales with O₂ limiting vs convention wrap.

⁴Contrasts: (i) delay: linear = linear effect of wrapping delay; (ii) delay: quadratic = quadratic effect of wrapping delay, delay: cubic = effect of wrapping delay, and (IV) O₂-limiting vs conventional.

Table 2.3. Effect of delayed wrapping and wrapping source on intake of alfalfa silage

Treatment	Item			
	DMI, g/d ¹	OMI, g/d	DMI, g/kg BW	OMI, g/kg BW
<i>Wrap Type</i>				
O ₂ -limiting	1464	1292	22.8	20.2
Conventional	1466	1297	22.9	20.4
SEM ²	91.0	79.4	1.62	1.40
<i>Wrapping Delay, d³</i>				
0	1449	1284	22.8	20.2
1	1588	1399	24.6	21.7
2	1464	1296	22.8	20.2
3	1358	1200	21.3	18.9
SEM ²	93.6	81.5	1.66	1.44
Wrap × time	0.20	0.15	0.27	0.22
<i>Contrasts⁴</i>				
i) Delay ; Linear	0.02	0.02	0.032	0.034
ii) Delay: Quadratic	0.003	0.0005	0.003	0.004
iii) Delay: Cubic	0.02	0.041	0.06	0.09
iv) O ₂ -limiting vs conventional	0.96	0.86	0.73	0.66

¹DMI = dry matter intake; OMI = organic matter intake

²SEM = pooled standard error of the mean.

³Time delay from 0 to 3 days between baling and wrapping bales with O₂ limiting vs convention wrap.

⁴Probability of the wrap type × time delay between baling and wrapping interaction.

⁵Contrasts: (i) delay: linear = linear effect of wrapping delay; (ii) delay: quadratic = quadratic effect of wrapping delay, delay: cubic = effect of wrapping delay, and (iv) O₂-limiting vs conventional.

Table 2.4. Effect of delayed wrapping and wrapping source on digestibility parameters of alfalfa silage in gestating sheep

Treatment	Item					
	DMD , % ¹	OMD , %	NDFD, %	ADFD, %	DDMI, g/kg BW	DOMI, g/kg BW
Wrap Type						
O ₂ -limiting	66.0	66.7	59.1	59.1	14.9	13.3
Conventional	64.9	65.8	58.0	58.0	15.0	13.4
SEM ²	0.73	0.82	1.7	1.72	1.11	0.94
Wrapping Delay, d³						
0	65.5	66.5	57.8	57.7	15.0	13.5
1	67.0	67.5	57.7	57.7	16.5	14.6
2	64.6	65.3	58.8	58.4	14.7	13.1
3	64.9	66.0	60.5	60.5	13.8	12.3
SEM ²	0.69	0.92	1.86	1.85	1.14	0.97
Wrap × time	0.17	0.06	0.71	0.73	0.07	0.04
Contrasts⁴						
i) Delay ; Linear	0.15	0.14	0.04	0.08	0.01	0.01
ii) Delay: Quadratic	0.33	0.73	0.35	0.29	0.004	0.07
iii) Delay: Cubic	0.02	0.03	0.52	0.86	0.01	0.02
iv) O ₂ -limiting vs conventional	0.10	0.18	0.44	0.28	0.86	0.79

¹DMD = 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.

²SEM = pooled standard error of the mean.

³Time delay from 0 to 3 days between baling and wrapping bales with O₂ limiting vs convention wrap.

⁴Probability of the wrap type × time delay between baling and wrapping interaction.

⁵Contrasts: (i) delay: linear = linear effect of wrapping delay; (ii) delay: quadratic = quadratic effect of wrapping delay, delay: cubic = effect of wrapping delay, and (iv) O₂-limiting vs conventional.

Chapter 3

Correlation of fermentation characteristics with intake and digestibility of alfalfa silage in gestating ewes

ABSTRACT

Baled silage production provides benefits to farmers because it reduces leaf loss, and requires a shorter wilting time, thereby limiting risks of exposure to rain compared with making hay. However, improper fermentation can have negative impacts on acceptability by animals. Our objective was to investigate the correlation of alfalfa silage fermentation parameters with intake and digestibility in gestating ewes. Alfalfa from 3 field blocks was baled in large round bales at a mean moisture concentration of $59.1 \pm 4.30\%$ and then wrapped with plastic either the day of baling, or 1, 2 or 3 d after baling; this resulted in considerable variability in silage fermentation measurements. Following approximately 5 mo. of storage, the alfalfa was chopped, and then offered for individual ad libitum consumption by 16 gestating ewes (63.5 ± 1.71 kg avg. BW) where total feces were collected for 7 d following a 10-d dietary adaptation in each of 3 different periods. Diets were re-randomized to different ewes for each period such that ewes were not offered the same treatment in any period. Data were analyzed using PROC CORR of SAS to determine the correlation between alfalfa fermentation parameters and intake and digestibility measurements. Dry matter and OM intakes (g/d) were correlated positively ($P < 0.05$) with lactic acid (%) and negatively ($P < 0.05$) with ADF concentrations (%). Dry matter and OM intakes (g/kg BW) were correlated positively ($P < 0.05$) with water content of silage (%), total silage acids (%), lactic acid (%) and propionate (%), but negatively ($P < 0.01$) with ADF concentrations (%) in the alfalfa silage. Also, DMI (g/kg BW) was correlated positively ($P = 0.04$) with silage

butyrate concentrations (%). Dry matter and OM digestibilities (%) were correlated positively ($P < 0.05$) with lactic acid when expressed as a percentage of total silage acids, but negatively ($P < 0.05$) with NDF concentration (%). Furthermore, DMD was correlated negatively ($P < 0.05$) with ADF, and OMD (%) was correlated positively ($P < 0.05$) with silage pH. Digestibility of ADF (%) was correlated positively ($P < 0.05$) with silage pH, but negatively with water content (%), total silage acids (%), lactic acid (%) and propionate (%). Digestible DM and OM intakes (g/kg BW) were correlated positively ($P \leq 0.05$) with water content of silage (%), total silage acids (%), lactic acid (%), and propionate (%) and DOMI was correlated negatively ($P < 0.05$) with NDF (%) and ADF (%). Therefore, managing alfalfa silage to ensure more desirable fermentation should also result in greater intake of digestible organic matter which should improve overall energy status of ruminants.

INTRODUCTION

Voluntary forage intake is the major dietary factor determining level and efficiency of ruminant production (Allison, 1985). Some changes may occur during forage conservation as silage, especially chemical composition that can impact palatability and intake of silages. Some changes may occur during forage conservation as silage, especially chemical composition that can impact palatability and intake of silages. Feed intake was reported to decrease 19% in ensiled alfalfa compared to fresh alfalfa (Flores et al., 1986) and a combination of different factors such as pH, protein breakdown, and moisture content contributed to reduced silage intake (Kung and Shaver, 2000). Several previous studies were conducted to assess the relationship between fermentation parameters and intake. Moisture content of silage was reported to be negatively correlated with intake, but these were likely wet silage from Northern Europe (Wilkins et al., 1971). Similarly, when comparing alfalfa harvested then ensiled directly, and hay fed to lactating

cows, DM content was positively correlated with feed intake (Gordon et al., 1961). Moreover, concentrations of total acids in silage were proposed to be a good predictor of silage DMI, but concentrations of ammonia N were negatively correlated with intake (Huhtanen et al., 2007). Lactic acid can play a major role by minimizing the loss of DM in preserved silage and it was reported to be correlated negatively with silage pH (McDonald et al., 1991). In another study, adding lactic acid to silage reduced silage pH but intake by sheep was reduced by over 22% (McLeod et al., 1970). Delays that occur between baling and wrapping increase the exposure of silage to oxygen which also has a negative impact on silage fermentation. The presence of oxygen in silage caused DM loss (Williams et al., 1994) and reduced feed intake (Gerlach et al., 2013). Some studies reported attempts to minimize silage exposure to oxygen by wrapping silage with a plastic that contains an oxygen limiting barrier (Borreani and Tabacco, 2008) or by increasing number of plastic wraps (Hancock and Collins, 2006), but there is still a gap in information for assessing the relationship between fermentation characteristics, intake, and digestibility of silages made using varied management practices. Therefore the objective of this study was to determine the correlation between fermentation characteristics and intake and digestibility of alfalfa silage in gestating sheep.

MATERIALS AND METHODS

The feeding portion of this study was conducted at University of Arkansas farm from January until March 2015 inside a building with controlled temperature. Alfalfa was harvested and baled, then wrapped at different times up to 3 d after baling with wrapping material that either contained an oxygen-limiting barrier or no barrier. This resulted in a variety of silage fermentation profiles across the different bales. The bales were stored for 5 months and then fed to 16 gestating sheep over 3 experimental periods. Additional details about field description, hay

storage, silage making, animal and experimental design, were discussed in the second chapter. Chemical analyses, animal feeding, and digestibility procedures were conducted as described in Chapter 2 of this thesis. Fermentation characteristics of silage were analyzed by Cumberland Valley Analytical Services, Hagerstown, MD.

Statistical analysis

Data were analyzed using PROC CORR of SAS (SAS Inst., Inc., Cary, NC) to determine the correlation between alfalfa fermentation and forage quality parameters with intake and digestibility measurements. Step wise regression analysis was used (SAS Inst.) to determine the mathematical relationship between the most correlated fermentation measurements and intake of digestible DM and OM. The initial model only included silage fermentation measurements as options. A second analysis was conducted giving all forage quality and fermentation measurements as options for the relationships. Digestible DM and OM intake parameters were used as dependent variables and fermentation measurements as independent variable.

RESULTS

Correlations between silage intake measurements and fermentation measurements, fiber components, and N are presented in Table 1. Dry matter and OM intakes (g/d) were correlated positively ($P = 0.01$) with lactic acid concentrations (%), and tended to be correlated positively ($P < 0.10$) with propionate (%) and total silage acid concentrations (%). Moreover, DMI and OMI were correlated negatively ($P < 0.05$) with ADF concentrations (%) in the alfalfa silages. Dry matter and OM intakes (g/kg BW) were correlated positively ($P < 0.05$) with water content of silage (%), total acids (%), lactic acid (%), and propionate (%) but negatively ($P \leq 0.01$) with

ADF concentrations (%) of the alfalfa silages. Dry matter intake (g/kg BW) was also correlated positively ($P = 0.04$) with silage butyrate concentrations (%).

Correlations between digestibility and fermentation measurements are presented in Table 2. Dry matter and OM digestibilities were correlated positively ($P = 0.01$) with lactic acid (% of total silage acids) but negatively ($P \leq 0.01$) with NDF concentration. Digestibility of DM was correlated negatively ($P < 0.05$) with ADF content of alfalfa silage, and digestibility of OM was also correlated positively ($P = 0.02$) with silage pH. Digestibility of NDF (%) was correlated positively ($P < 0.05$) with NDF concentrations and tended ($P < 0.10$) to be correlated negatively with both lactic acid (%) and butyrate (%). Digestibility of ADF was correlated positively ($P < 0.05$) with silage pH, but negatively with water content, total silage acids, lactic acid (%), and propionate ($P < 0.05$). Digestibility of ADF also tended ($P \leq 0.10$) to be correlated negatively with silage NH_3N , acetate, butyrate, ADF, and N concentrations.

Digestible DMI and digestible OMI (g/d; Table 3) were correlated positively ($P \leq 0.02$) with lactic acid concentrations (% of total silage acids), and tended ($P \leq 0.10$) to be correlated positively with total acids (%) and propionate (%). Digestible OMI also tended ($P < 0.10$) to be correlated negatively with silage pH and NDF (%). Both DDMI and DOMI (g/d) were correlated negatively ($P \leq 0.01$) with silage ADF concentrations (%).

Digestible DM and OM intakes (g/kg BW) were correlated positively ($P \leq 0.05$) with water content of silage (%), total acids (%), lactic acid (%), and propionate concentrations (%) and tended ($P < 0.10$) to be correlated positively with lactic acid (% of total silage acids) and silage N concentrations. Digestible OMI was also correlated negatively ($P < 0.05$) with both NDF and ADF concentrations and DOMI tended ($P < 0.10$) to be correlated negatively with

NDF and ADF correlations. Intake of digestible DM and OM were not correlated ($P \geq 0.14$) with NH_3N (%) silage pH, acetate (%), or butyrate (%) concentrations.

Step wise regression analysis data were presented in Table 4. Both DDMI and DOMI were mostly correlated with lactic acid (%) compared to other fermentation measurements, but the relationship was weak ($R^2 = 0.14$). When all forage quality components and fermentation parameters were included as options, DDMI and DOMI were best explained ($R^2 > 0.39$) by variation in acetate, butyrate and ADF.

DISCUSSION

In the present study, DMI and OMI (g/d) were correlated positively with lactic acid, which is consistent with results by Wilkins et al. (1971). Conversely, in another study, intake by sheep was reduced more than 22% when lactic acid was added to silage at levels ranging from 54 to 113 g/kg DM (McLeod et al., 1970), indicating a possible difference between endogenous and exogenous lactic acid or more likely an upper threshold on lactic acid concentrations. Dry matter and OM intake (g/d and g/kg BW) were correlated negatively with ADF which can likely be explained by the presence of the least digestible fractions of the forage, particularly lignin found in ADF, which then could have reduced digestibility and intake (Jung and Allen, 1995; Pei et al., 2016). Dry matter and OM intakes (g/kg BW) were correlated positively with water content of silage. Greater concentrations of moisture were correlated negatively with intake in other studies (Gordon et al. 1961; Wilkins et al., 1971). The positive correlation of silage moisture concentration on intake in the present study is somewhat surprising, but the variation in concentrations of moisture in our silage ranged from 50.4 to 57.7%, which approximates the recommended moisture (<55%) for baled alfalfa silage (Shinners, 2003). Therefore, the effect of

moisture content of silage on intake may be more related to other reactions that occurred in the silages since moisture concentration decreased linearly as the time between baling and wrapping the silage increased (Chapter 2). Greater moisture concentrations could have facilitated the solubility of sugar, thereby diluting non-desired products in silage resulting in increased intake (Lahr et al., 1983). In the present study, DMD was correlated negatively with NDF and that is consistent with results of Jung et al. (1997) who found that digestibility and NDF were negatively correlated in both in vitro and in vivo digestibility systems when C₃ and C₄ legume and grass forages were fed to sheep.

Propionic acid was reported to be an inhibitor of aerobic silage deterioration (McDonald et al., 1991). Since DDMI and DOMI were both correlated positively with propionate, this may be because propionate was inhibiting aerobic deterioration in silage which allowed sheep to eat acceptable silage and increase their intake and digestibility. On the other hand, propionate was reported to be negatively correlated with DDMI by Merino wethers in a study that involved 20 experimental grass silages from 7 different pastures at different stages of maturity (Brown and Radcliffe, 1972). In a previous study, the concentration of ammonia N was reported to be negatively correlated with intake (Huhtanen et al, 2007). However, in this present study, NH₃N-expressed in crude protein equivalents was not correlated with any intake measurement. This may be attributed to the fact that concentration of ammonia in our silages was below 12% of the total N as NH₃N which is a good indicator that protein degradation was low (Mahanna and Chase, 2003). In addition, the concentration of moisture ($\leq 57\%$) in our silage was not sufficient to cause clostridial activities which could have led to the production of non-desired products including NH₃N.

Lactic acid was the only one of the fermentation parameters that was included in the initial run of the step-wise regression that only included fermentation measurements as options because it has the greater correlation value. When all measurements were used as options in the step-wise procedure, acetate, butyrate and ADF were chosen in the final model. Although certain components such as acetate, butyrate and ADF were related to DDMI and DOMI, the overall r^2 (≤ 0.41) was low, indicating that a sizeable portion of the variability in these components resulted from sources other than the fermentation and fiber concentrations.

CONCLUSION

In general, correlations between fermentation measurements with intake and digestibility were not strong. However, the lactic concentrations expressed a greater correlation value while the silage pH had the lowest correlation value compared to other fermentation characteristics with intake and digestibility measurements. The positive correlation of lactic acid with digestible dry matter and organic matter intakes can be a good indicator that lactic acid can play a role of preserving silage quality without decreasing silage pH.

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Table 3.1: Pearson correlation coefficients between fermentation characteristics and intake measurements of alfalfa silage in gestating sheep

		Fermentation Parameters											
Item		H ₂ O, %	¹ NH ₃ , %	TA, %	pH	Lactic, %	LTA,%	Ace,%	Pro, %	But,%	NDF,%	ADF	N
DMI, g/d	r	0.21	0.15	0.27	-0.11	0.35	0.28	0.09	0.26	0.21	-0.16	-0.34	0.14
	P-value	0.14	0.30	0.05	0.43	0.01	0.05	0.54	0.07	0.14	0.26	0.01	0.32
OMI, g/d	r	-0.20	0.14	0.27	-0.11	0.35	0.28	0.08	0.25	0.20	-0.17	-0.33	0.14
	P-value	0.17	0.34	0.06	0.43	0.01	0.05	0.57	0.08	0.16	0.25	0.02	0.32
DMI, g/ kg BW	r	0.31	0.19	0.32	-0.15	0.35	0.22	0.14	0.34	0.29	-0.22	-0.47	0.26
	P-value	0.03	0.18	0.02	0.29	0.01	0.13	0.33	0.01	0.04	0.13	<0.01	0.06
OMI, g/ kg BW	r	0.29	0.18	0.31	-0.16	0.35	0.22	0.13	0.33	0.28	-0.22	-0.47	0.26
	P-value	0.04	0.21	0.03	0.28	0.01	0.12	0.36	0.02	0.05	0.12	<0.01	0.06

¹NH₃ = ammonia crude protein equivalent, TA= Total acid, Lactic = lactic acid, LTA= proportion of lactic acid to total acid, ace = acetate, pro = propionate, but = butyrate, NDF = neutral detergent fiber, ADF= acid detergent fiber, N= Nitrogen.

²DMI= dry matter intake, OMI = organic matter intake.

Table 3.2: Pearson correlation coefficients between fermentation characteristics and digestibility measurements of alfalfa silage in gestating sheep

Item		Fermentation parameters											
		H ₂ O, %	¹ NH ₃ , %	TA, %	pH	Lactic, %	LTA,%	Ace,%	Pro, %	But,%	NDF,%	ADF	N
DMD,%	r	-0.01	-0.17	0.002	0.25	0.19	0.37	-0.22	-0.02	-0.17	-0.35	-0.30	0.12
	P-value	0.98	0.23	0.98	0.09	0.18	0.01	0.13	0.87	0.25	0.01	0.03	0.40
OMD,%	r	0.15	-0.24	-0.08	0.33	0.10	0.34	-0.25	-0.12	-0.17	-0.49	-0.19	0.06
	P-value	0.30	0.10	0.58	0.02	0.47	0.01	0.08	0.39	0.24	< 0.01	0.19	0.67
NDFD,%	r	0.04	-0.13	-0.23	0.01	-0.24	-0.16	-0.13	-0.12	-0.06	0.37	-0.06	0.04
	P-value	0.79	0.36	0.11	0.93	0.09	0.27	0.37	0.39	0.07	< 0.01	0.68	0.74
ADFD%	r	-0.30	-0.23	-0.33	0.35	-0.37	-0.12	-0.24	-0.29	-0.25	-0.1	-0.24	-0.24
	P-value	0.04	0.10	0.02	0.01	0.01	0.41	0.10	0.04	0.08	0.48	0.09	0.09

¹NH₃ = ammonia crude protein equivalent, TA= Total acid, Lactic = lactic acid, LTA= proportion of lactic acid to total acid, ace = acetate, pro = propionate, but = butyrate, NDF = neutral detergent fiber, ADF= acid detergent fiber, N= Nitrogen, ²DMD = dry matter digestibility,

OMD = organic matter digestibility, NDFD = neutral detergent fiber digestibility, ADFD = acid detergent fiber digestibility.

Table 3.3: Pearson correlation coefficients between fermentation characteristics and digestible DM and digestible OM measurements of alfalfa silage in gestating sheep

					Fermentation parameters								
Item		H ₂ O,%	¹ NH ₃ , %	TA,%	pH	Lactic,%	LTA,%	Ace,%	Pro,%	But,%	NDF,%	ADF	N
DDMI, g/d	r	0.22	0.13	0.28	-0.06	0.38	0.33	0.06	0.26	0.23	-0.22	-0.38	0.15
	P-value	0.14	0.37	0.05	0.65	< 0.01	0.02	0.66	0.07	0.12	0.14	<0.01	0.30
DOMI, g/d	r	0.18	0.11	0.26	-0.05	0.38	0.34	0.74	0.24	0.21	-0.24	-0.36	0.15
	P-value	0.21	0.46	0.07	0.07	< 0.01	0.02	0.05	0.10	0.14	0.09	0.01	0.41
DDMI, g/kg BW	r	0.31	0.17	0.32	-0.10	0.37	0.27	0.11	0.32	0.06	-0.28	-0.51	0.27
	P-value	0.03	0.25	0.02	0.47	< 0.01	0.06	0.45	0.02	0.66	0.05	0.06	0.06
DOMI, g/kg BW	r	0.28	0.14	0.30	-0.09	0.37	0.28	0.09	0.31	0.07	-0.31	-0.50	0.27
	P-value	0.05	0.33	0.04	0.52	0.01	0.05	0.51	0.03	0.60	0.03	0.01	0.06

¹NH₃ = ammonia crude protein equivalent, TA= Total acid, Lactic = lactic acid, LTA= proportion of lactic acid to total acid, ace = acetate, pro = propionate, but = butyrate, NDF = neutral detergent fiber, ADF= acid detergent fiber, N= Nitrogen, DDMI = digestible dry matter intake, DOMI = digestible organic matter intake.

Table 3.4. Step wise regression analysis between fermentation characteristics and digestible DM and digestible OM measurements in gestating sheep

Item	Coefficient	<i>P</i> value	R square
DDMI, g/kg BW			
Intercept	12.93		
Lactic acid (%) ¹	1.57	< 0.01	0.14
DOMI, g/kg BW			
Intercept	44	< 0.01	
Acetate	-1.75	0.04	0.41
Butyrate	5.14	< 0.01	
ADF ²	-0.73	< 0.01	
DDMI, g/kg BW			
Intercept	11.64		
Lactic acid (%) ¹	1.34	< 0.01	0.14
DOMI, g/kg BW			
Intercept	38	< 0.01	0.39
Acetate	-1.59	0.03	
Butyrate	4.50	< 0.01	
ADF ²	-0.62	< 0.01	

¹ Prediction equation when only fermentation parameters were included.

² Prediction equation when all fermentation parameters and fiber and nitrogen components were included as options.

Chapter 4

CONCLUSION

The purpose of this study was to evaluate different management factors that affect quality of alfalfa silage. Having an oxygen-limiting barrier in the silage wrap did not affect silage fermentation characteristics, intake and digestibility measurements. Furthermore, no interaction between wrap type and time delay between baling and wrapping was detected when 7 layers of plastic were used to wrap silage bales. Delaying wrapping of alfalfa silage beyond 1 day after baling negatively affected silage fermentation characteristics. Intake, digestibility, and intake of digestible organic matter were reduced when wrapping the silage was delayed for 2 to 3 days after baling. Lactic acid concentrations were correlated positively with digestible dry matter and organic matter intake, indicating that lactic acid plays a role of preserving silage quality and can be used as an indicator of subsequent animal performance. Therefore, producers should wrap alfalfa silage within 24 hours after baling to avoid further oxygen exposure and to avoid poor animal performance declines from the consumption of inferior silage.



MEMORANDUM

TO: Kenneth Coffey

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: September 12, 2012

SUBJECT: IACUC Protocol APPROVAL
Expiration date : **August 31, 2015**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol #13007-
"Intake and digestibility of forages by sheep". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees
such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall
under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any
changes to the protocol during the research, please notify the IACUC in writing [via the Modification
Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **08-31-
2015**, you must submit a new protocol. 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 for
research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian