

UTILIZING GENE SUPPRESSION TECHNOLOGY AND HAY  
STORAGE TECHNIQUES TO IMPROVE FORAGE  
QUALITY AND ANIMAL PERFORMANCE

by

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DEDICATION

To my fiancé, Kevin, and to my family, for which I would not be where I am without your kindness, love, and endless support.

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## ABSTRACT

Utilizing technologies such as genetic modification and forage management techniques are two ways to improve forage quality. The objective of the first study in this thesis was to determine the differences in forage quality between reduced-lignin and conventional alfalfa. To test these differences, twenty-four Crossbred Angus heifers were selected to participate in this study and their performance was evaluated based on changes in BW, ADG, DMI, and G:F. *In situ* digestibility was determined using four ruminally cannulated Hereford cows. Hay samples were collected and used to determine forage quality and leaf-to-stem ratio. There were no differences ( $P \geq 0.05$ ) in forage quality between treatments, except for DM ( $P = 0.01$ ). Means did not differ by treatment for percent leaf ( $P = 0.06$ ) but did differ for leaf-to-stem ratio ( $P = 0.04$ ). There were no treatment or treatment by day interactions ( $P \geq 0.05$ ) for BW, ADG, DMI, or G:F. There were no treatment or treatment by time interactions ( $P \geq 0.05$ ) for *in situ* digestibility. Overall, the results of this study suggested no difference in forage quality between reduced-lignin and conventional alfalfa. Additionally, animal performance did not differ for crossbred Angus heifers consuming reduced-lignin or conventional alfalfa. The objective of the second study in this thesis was to quantify DM and forage quality losses associated with three different methods of outdoor round bale hay storage at two different sites in Montana. Large round bales consisting of 100% grass hay wrapped in plastic net wrapping were placed into one of four storage systems at both the Bozeman Agricultural Research and Teaching farm (BART) and the Northern Agricultural Research Center (NARC). The four storage systems were: single-stack (SS), pyramid (PYR), mushroom (MSH), and inside stored bales (INSIDE). Results indicated that DM and forage quality losses differed based on geographic location in Montana. This study suggested that DM and forage quality losses differ by location and that bale placement, rather than hay storage formation, is more important for changes in DM and quality for bales stored in Montana over the winter months.

## CHAPTER ONE

## GENERAL INTRODUCTION

Forages are one of the most important nutrient sources available to ruminants and commonly serve as the foundation for energy in livestock diets (Givens et al., 2000; Krause et al., 2003). Ruminants depend on carbohydrates found in forage cell walls to provide over one-half the energy necessary for maintenance, growth, and production (Nafikov and Beitz, 2007). The amount of cell wall found in forage ranges from 20-80% of forage dry weight and is composed of structural polysaccharides, lignin, hydroxycinnamic acids, protein, ions, and water (Wilson, 1994; Hatfield et al., 1999). Interactions between these components influence forage quality and consequently, animal performance (Hatfield et al., 1999).

Unfortunately, the fraction of cell wall in forage that is readily digested and utilized by the animal is less than 50% (Hatfield et al., 1999). This is because land plants have evolved over time to develop a rigid cell wall that provides mechanical strength and hydrophobicity, as well as resistance to degradation by herbivores, micro-organisms, and enzymes (Wilson, 1994; Theodorou et al., 1996; Hatfield et al., 1999). Research aimed at improving and maximizing energy availability from forage cell walls is an ongoing battle. Maximizing production efficiency of domestic ruminants for human consumption requires improvements in both animal genetics and forage cell walls. Animal producers are constantly improving genetic selection by choosing superior animals capable of

creating offspring that will maximize production, while forage researchers are constantly working to provide ruminants access to nutrients that would otherwise be inaccessible.

Understanding cell wall structure, digestion, and absorption characteristics, coupled with an understanding of the components of animal efficiency are essential for manipulating the production and quality of agricultural foods. The importance of finding the molecular mechanism that limits cell wall degradability increases in priority as the world population grows, causing a concurrent increase in consumer demands. The current world population is 7.3 billion and is expected to rise to 8.5 billion by 2030 (DESA, 2015). This rise in population is going to require agriculture to surpass current production levels on the same, or maybe even less, acreage. Although there have been significant advances in agricultural development, there is still considerable room for improvements.

Improving both animal genetics and forage digestibility is necessary to develop an animal production system that can produce more while using less resources. Providing the agricultural community with innovative science and technology is going to be of upmost importance in meeting and satisfying rising consumer demands.

## CHAPTER TWO

### LITERATURE REVIEW

#### The Relationship Between Forage Quality and Animal Performance

The quality of a forage is a function of nutrient composition, chemical and physical characteristics of the cell wall, palatability, and associative interactions between dietary ingredients. Together, these constituents determine the availability of a forage for utilization by the consuming animal. Improving forage quality is one of the best options for optimizing animal productivity and improving overall feed efficiency. This section is dedicated to discussing ruminant anatomy and function, forage quality, and factors affecting intake and digestibility in the ruminant.

#### Basic Ruminant Anatomy and Function

The ruminant digestive system is capable of fermenting and digesting high roughage, plant-based material as a usable energy source. This digestive system is unique in that it eliminates the need to supplement ruminants with external sources of B-vitamins and amino acids, and it allows ruminant livestock to utilize feedstuffs considered useless by monogastric mammals. Ruminants are especially important to humans because they do not directly compete with humans for resources and they provide humans with a vital source of consumable and non-consumable products. The expected rise in human population by 2030 (DESA, 2015) is going to increase consumer demands for ruminant production and ruminant products. Increasing this production will require scientists to

utilize the newest technologies to improve the quality of forage. Successfully achieving this goal requires an understanding of the impact of forage quality on ruminant function.

Anatomy and Function. The function and anatomy of the rumen and the ability to retain ingesta for an extended period of time are the reasons ruminants are thought to be more efficient than non-ruminants at utilizing fibrous feedstuffs (Van Soest, 1982). Animals lack the enzymes necessary for hydrolysis of the carbohydrate polymers cellulose, hemicellulose, and pectin, but microorganisms in the rumen are capable of hydrolyzing and fermenting these polymers into usable energy sources for the ruminant (Krause et al., 2003). The end products of fermentation are volatile fatty acids (VFAs), which provide the animal with metabolic fuel, and microbial cells, which are a major source of protein and amino acids after absorption in the lower digestive tract (Krause et al., 2003).

The ruminant foregut is divided into four compartments: the rumen, reticulum, omasum, and abomasum. Most dietary carbohydrate fermentation by microorganisms occurs in the foregut. The first two compartments of the digestive sequence are the rumen and reticulum (reticulo-rumen) and are often considered one single organ. These two compartments together predominate in size and hold up to 53 gallons of ingesta in cattle (Van Soest, 1982). Most fermentative activity and absorption of nutrients occur in the reticulo-rumen. Microbes in the reticulo-rumen ferment fiber, starch, sugar, and protein into VFAs and microbial protein. The third compartment, the omasum, absorbs water and nutrients and pumps ingesta from the reticulo-rumen to the abomasum. The fourth compartment is the abomasum, also known as the “true stomach” of the ruminant.

Enzymes and acid in the abomasum digest feed prior to passage into the small intestine (Van Soest, 1982).

Digestion of roughages occurs in the following four steps: 1) particle size reduction, 2) enzymatic hydrolysis of cell wall polysaccharides by rumen microbes, 3) fermentation of ingesta to VFAs, and 4) absorption of fermentation end-products (Jung et al., 2012). Ingested feed is mixed with saliva to form a bolus, which moves from the mouth to the esophagus and then to the reticulo-rumen for fermentation and rumination. The esophagus is bidirectional and allows the animal to regurgitate larger particles for further chewing. This process is called rumination, and promotes the turnover of indigestible feed (Van Soest, 1982). During rumination, the salivary glands, tongue, teeth, and esophagus work together to regurgitate and break down indigestible fiber present in the feed. The reticulum functions to catch small digesta particles and the liquid portion of regurgitated feed, which will be passed to the omasum, and then to the abomasum. The solid portion of regurgitated feed slowly moves into the rumen for fermentation. This portion forms a dense mat in the rumen for fermentation by microorganisms such as bacteria, protozoa, and fungi. Approximately 70-75% of forage is digested in the rumen within 40-60 hours after consumption (Mertens and Ely, 1979). The remaining 10-25% of forage fiber can remain in the rumen for more than 70 hours after consumption (Mertens and Ely, 1979).

Postruminal digestion is comparable to that of the non-ruminant. Ingesta enter the small intestine after passing through the abomasum. Only about 5-20% of consumed dietary carbohydrates are digested in the small intestine (Nafikov and Beitz, 2007). The

ingesta, which are acidic after being in the abomasum, are neutralized by mixing with biliary and pancreatic secretions in the small intestine. These secretions include amylolytic, tryptic, and lipolytic enzymes, which hydrolyze starch, protein, and triglycerides. Starch, which is hydrolyzed by pancreatic amylase, forms the end-product glucose (Keomanivong, 2016). Glucose is then transferred from the lumen into the bloodstream (Harmon, 2009; Keomanivong, 2016). Protein entering the small intestine is supplied as rumen undegradable protein, microbial cells, and endogenous secretions (Keomanivong, 2016). Tryptic enzymes are responsible for breaking down protein components into peptides and amino acids for absorption through the small intestine (Keomanivong, 2016). Lipids that have not been hydrolyzed and bio-hydrogenated in the rumen reach the small intestine in the form of saturated non-esterified fatty acids, triglycerides, or bacterial phospholipids (Keomanivong, 2016). These lipids are processed in the small intestine and broken down by lipolytic enzymes into fatty acids capable of being absorbed through the small intestine (Bauchart et al., 1996).

Ingesta that have progressed through the small intestine have passed all the secretion and absorption sites that involve enzymatic activity. At this point the ingesta move to the large intestine, another site for fermentation of structural carbohydrates, starches, and proteins that have escaped ruminal fermentation (Drackley, 2011). Water, mineral, nitrogen (N), and VFA absorption occurs in the large intestine. Material left undigested after fermentation will be excreted as feces from the rectum.

Optimizing Rumen Function with Fiber. The capacity of the ruminant to retain ingesta long enough to extract nutrients is critical, especially for fibrous feedstuffs.

Selective passage in the reticulo-rumen and omasum promotes maximal retention and utilization of the plant cell wall as a substrate for the available energy generated through rumen microorganisms (Van Soest, 1982). Forages that have lower concentrations of fermentable fiber, such as alfalfa, are eaten and fermented rapidly because of their smaller, more digestible particle size (Van Soest, 1982; Varga, 2006). Coarse, fibrous feeds, such as grasses and crop residue, are eaten and fermented slowly because of their high cell wall content and lower digestibility. The fibrous portion of feeds cannot pass through the reticular-omasal orifice because of their large particle size and must be selectively regurgitated for further rumination. Fiber particles must be reduced to less than 1.18 mm before they can readily pass from the rumen (Poppi et al., 1985). Fiber particles larger than 1.18 mm require prolonged retention in the rumen and more chewing to be reduced to a bolus. Prolonged retention in the rumen will generally limit animal intake because of rumen distension and can result in lower animal performance (Wilson, 1994).

Fiber is necessary for rumination because it stimulates chewing activity and salivary secretions. Saliva consists of bicarbonate and phosphate buffers that neutralize the acids produced during fermentation (Allen, 1997). The balance between these salivary buffers and the acidic products of fermentation control rumen pH, and thus, the type of microbes present in the rumen. At a normal rumen pH level between 6.4-6.8, fibrolytic microbes predominate (Jasmin et al., 2011). Lowered pH in the rumen occurs when animals are fed high concentrate feeds consisting of rapidly fermented carbohydrates that require less chewing. Reducing the time spent chewing diminishes

buffering capacity and can shift the microbial population from fibrolytic to amylolytic (Jasmin et al., 2011). Shifting the microbial population by lowering ruminal pH can negatively impact animal performance by reducing dry matter intake (DMI), fiber digestibility, and microbial yield (Allen, 1997). If rumen pH falls below 5.5 animals become susceptible to subacute rumen acidosis (Jasmin et al., 2011).

The major role of microorganisms in the rumen is to adhere to structural and non-structural carbohydrates and catalyze their degradation to produce VFA's (Krause et al., 2003), which are the ruminant's main source of energy (Nafikov and Beitz, 2007). Rumen microorganisms are assigned to groups based on shape, size, and structure, and are classified based on their utilization of cellulose, hemicellulose, starch, sugars, intermediate acids, protein, lipid, or methane production (Yokoyama and Johnson, 1988). The three major VFA's found in the rumen include acetate, propionate, and butyrate. Acetate is primarily utilized for muscle and fat synthesis, propionate for glucose synthesis, and butyrate as an energy source for colonic ruminal epithelia (Van Houtert, 1993). The relative production of each VFA, and total VFA production depend on the digestibility of an animal's diet (Russell, 1998) and changes in the ratio of VFA's impact animal performance (Russell, 1998). Generally, the acetate to propionate ratio is lower in animals consuming concentrate diets compared to forage diets, and for animals consuming more digestible forages (Russell, 1998; Cantalapiedra-Hijar et al., 2009). Reducing the acetate to propionate ratio reduces methane production, and allows for increased energy retention in ruminants (Russell, 1998).

Large amounts of starch in high-concentrate diets may yield more lactic acid production and lower ruminal pH, influencing the microbial population and VFA production (Cantalapiedra-Hijar et al., 2009). The results of a study comparing cattle consuming a 100% forage diet compared to a 90% cereal grain and 10% forage diet, found that cattle receiving the concentrate diet had lower ruminal pH values (6.22 vs. 6.86), higher VFA concentrations (85 vs. 68 mM), and lower acetate to propionate ratios (2.24 vs. 4.12; Russell, 1998). Digestibility of an animal's diet is another factor that influences VFA production. Cantalapiedra-Hijar et al. (2009) found that alfalfa-based diets in goats promoted greater total VFA concentrations, greater molar proportions of propionate and valerate, and less proportions of butyrate when compared to grass-based diets.

Feeding more degradable forages to ruminants and lowering the acetate to propionate ratio in their rumen reduces methane production and allows for improved energy retention. Improving alfalfa digestibility by reducing the lignin content in alfalfa has the potential to impact DMI, VFA production, and animal performance.

### Forage Quality

Forage quality is a function of nutrient concentration, intake, digestibility, and partitioning of metabolized products within the animal, and is determined by evaluating animal performance after livestock have consumed forage (Buxton, 1996; Ball et al., 2001). Factors that influence year-to-year variability in forage quality are chemical composition, plant maturity, environment, and soil fertility (Buxton, 1996).

Chemical Composition and Plant Maturity. Chemical composition influences the rate of biodegradation by rumen microbes and utilization of feed by the ruminant (Van Soest, 1982). Both leaves and stems consist primarily of structural polysaccharides, made up of individual monosaccharides, bonded together by glycosidic linkages (Albrecht et al., 1987; Hatfield et al., 1999). The primary and secondary cell walls of forage are composed of cellulose, hemicellulose, and pectin (Wilson, 1994). Lignin, which is controlled by plant development and maturation, develops in the secondary cell wall (Wilson, 1994).

The relationship of forage cell wall and animal intake and digestibility depends on associated plant structure (Van Soest, 1982). To explain this concept in more detail, let's compare the differences in the cell walls of legumes and grasses. Legumes have lower cell wall content (namely cellulose and hemicellulose), higher lignin content, and thick, pectin-rich primary cell walls, which are extensively digestible (Jung and Deetz, 1993; Wilson, 1994; Jung et al., 2012). Legumes also have natural points of breakage at the angular vein junctions due to weaker vascular bundles that are not surrounded or capped by layers of sclerenchyma (Wilson, 1994; Jung et al., 2012). Grasses have higher cell wall content, lower lignin content, contain very little pectin, and do not have similar points of breakage. The nature of the cell wall of legumes (lower cell wall content and higher lignin content) allows for a more rapid rate of digestion, quicker passage of digesta from the rumen, and faster rumen emptying, explaining why animals exhibit increased intake when consuming legumes compared to grasses (Jung et al., 2012).

Cell wall content becomes limiting to the animal when it reaches 50-60% of forage DM (Van Soest, 1982). Chemical composition influences intake, the time a ruminant spends eating and ruminating, and affects the ability of the ruminant to metabolize and utilize absorbed nutrients for production (Varga, 2006). Animals consuming forages with lower cell wall content and higher digestibility require less intake to meet energy requirements (Van Soest, 1965).

Plant maturity impacts forage quality and digestibility more than any other factor, except perhaps species, because as plants mature their leaf-to-stem ratio decreases, neutral detergent fiber (NDF) and lignin increase, and neutral detergent fiber digestibility (NDFD) decreases (Fick et al., 1994; Buxton, 1996). Plants with a low leaf-to-stem ratio are less digestible than plants with a high leaf-to-stem ratio because of chemical differences between leaves and stems. Leaves are the most digestible portion of the plant, and so as the stem increases and accumulates fiber, the proportion of the highly digestible leaves decrease (Jung and Deetz, 1993; Wilson, 1994).

During maturation, associated changes in digestibility occur due to changes in proportion and composition of leaves and stems (Albrecht et al., 1987; Wilson, 1994). Research conducted by Albrecht et al. (1987) found that as alfalfa matured from the vegetative to the early-pod stage, leaf-to-stem ratio declined from 1.30 to 0.50 and concentration of the cell-wall material increased from 350 g kg<sup>-1</sup> during the vegetative stage to 540 g kg<sup>-1</sup> during the early-pod stage, with the greatest change in cell wall concentration occurring before mid-flower. Their research suggested that during maturation, stem hemicellulose concentration decreased, cellulose concentration

remained the same, and lignin concentration increased. They also found that concentrations of in vitro digestible dry matter (IVDDM) were always greater in leaves than in stems, with leaves consisting of higher hemicellulose concentrations, lower cellulose concentrations, and similar lignin concentrations than that of stems. These results correspond to the results found by Jung et al. (2012).

Environment. Environmental conditions also influence plant development and forage quality. Inadequate growing conditions, such as drought, temperature, and solar radiation, cause plants to down-regulate their photosynthetic genes, resulting in lower leaf-to-stem ratio and altered plant development and chemical composition (Van Soest et al., 1978; Buxton, 1996; Chaves et al., 2009).

The results of research examining the effects of drought on alfalfa forage quality conclude that following adaptation to drought, forage quality of alfalfa improves compared to non-stressed plants (Halim et al., 1989). Drought slows the declining leaf-to-stem ratio associated with advancing maturity and causes alfalfa to develop shorter shoots, resulting in better overall forage quality.

Temperature influences fiber levels and forage quality. Cool-season species harvested at their optimal temperature of 20°C tend to have higher NDFD, lower NDF, and lower lignification than plants harvested outside their optimal temperature range (Buxton, 1996). First and last cuttings of alfalfa are generally grown during optimal growing temperature in the spring and fall and are more likely to have favorable quality, high fiber digestibility, and more protein than middle cuttings of alfalfa if harvested at the same maturity (Buxton, 1996). Temperatures below 20°C inhibit the rate of

photosynthesis in alfalfa, which slows sugar production and mobilization of nutrients (Van Soest et al., 1978; Buxton, 1996). Increased temperatures within the optimal range tend to promote plant growth, reducing leaf-to-stem ratio and digestibility (Buxton, 1996). Wilson (1982) found that for every 1°C increase in temperature (above the optimal temperature), the digestibility of cool-season forages was reduced by 3-7 g kg<sup>-1</sup> (Buxton, 1996). Reductions in digestibility associated with elevated temperatures are due primarily to lower leaf-to-stem ratio, higher NDF content, and increased lignification (Buxton, 1996).

Diurnal variation and photoperiod influence forage quality. The concentration of nonstructural carbohydrates in forage varies based on sunlight, with values being lowest before sunrise and highest in the afternoon (Norton et al., 1991; Buxton, 1996). One study found the digestibility of alfalfa harvested later in the afternoon to be 16 g kg<sup>-1</sup> DM greater than alfalfa harvested in the early morning (Lechtenberg et al., 1971).

Lengthening photoperiod during the spring and summer also have positive effects on forage quality, whereas shortening photoperiod during late summer and fall have negative effects (Buxton, 1996). Deinum et al. (1981) found that for every 1 hour increase in day length, digestibility increased by about 2 g kg<sup>-1</sup>. This is most likely due to increases in soluble carbohydrates and apparent decreases in the cell wall fraction during the day.

Soil Fertility. Soil fertility effects forage yield much more than it does quality (Assefa and Ledin, 2001). However, balancing soil fertility does help to avoid potential mineral imbalances in animals consuming forage. Nutrient deficiencies affect microbial growth and fermentation, which can reduce animal performance (Assefa and Ledin,

2001). Fertilizing soil should be managed based on site-specific soil analyses. Important nutrients to consider when choosing fertilizer include nitrogen, phosphorus, and potassium (Jacobsen et al., 2003). These nutrients are important for both forage yield and quality.

### Factors Affecting Intake and Digestibility

Animal nutrition is dependent upon 3 basic factors: 1) animal nutrient requirements, 2) forage quality, and 3) animal intake (Allison, 1985). The energy value of a feed is expressed in the Nutrient Requirements of Beef Cattle (NRC, 2016) as total digestible nutrients (TDN). The TDN value is the sum of digestible carbohydrates, crude protein (CP), and fat (Hersom, 2007). The net energy system (NE), another estimate of energy availability, is used to assign an energy value to a feed based on its ability to support the energy demands of an animal during maintenance, growth, lactation, or pregnancy (Hersom, 2007). TDN and NE values can help producers predict potential nutrient digestibility and the ability of an animal to effectively metabolize and absorb energy from feed for maintenance and production (Voigt et al., 2000). Animal nutrient requirements vary based on age and stage of production. It is important to remember this when providing feedstuffs to animals.

Variation in voluntary forage intake is the major factor determining the efficiency of ruminant production (Allison, 1985). DMI is unique for each individual animal and is limited by chemical composition of forage, along with animal physiology and environment (Van Soest, 1982; NRC, 2016). Cell wall content and structural volume influence DMI, whereas cell wall content and lignin content influence digestibility (Van

Soest, 1982). Evaluating total plant cell wall content is the most consistent explanation of intake (Van Soest, 1982), however, most research suggests that cellulose is more closely associated with intake and lignin is more closely associated with digestibility (Van Soest, 1982). Cell wall concentration and digestibility limit intake potential and energy availability of forages (Jung and Allen, 1995). Forages that are more digestible disappear from the rumen faster and stimulate intake. This creates space for consecutive meals sooner, allowing the animal to meet nutritional requirements (Varga, 2006).

Lignin limits the digestion potential of forage (Van Soest, 1982). NDF, which accounts for lignin, is inversely related to DMI because fiber and lignin ferment slower and stay in the rumen longer than non-fibrous components of the feed (Varga, 2006). Less digestible components of forage remain in the rumen for extended periods of time, causing distension and limiting intake (Varga, 2006). Feeds that are high in NDF content are less digestible and require more time for rumination, more total chewing time, and longer rates for digestion and passage (Shaver, 1988). When animals are fed high NDF, low energy, less digestible diets, distension and passage control intake (NRC, 2016). Feeds with low NDF content may be more digestible and can ferment and pass through the rumen quicker (Varga, 2006). This creates space for more digesta and stimulates intake (Varga, 2006). Dry matter intake for animals consuming feedstuffs with low NDF content is limited by energy demand and metabolism rather than fill (Shaver et al., 1988; Dado and Allen, 1995; NRC, 2016).

Beef cattle do not have a specific requirement for intake; however, consuming enough feed to meet energy requirements is crucial for maintenance and production.

Maintenance is defined as “the amount of feed energy intake that will result in no net loss or gain of energy from the tissues of the cow’s body” (Hersom, 2007). Cows are never really in a state of maintenance; rather, they are always adding or subtracting energy from their tissues. This ongoing addition and subtraction of energy generates the energy requirements of the cow.

Body weight and stage of production influence feed intake and animal performance. Body weight and body condition score are important to consider because cattle of different sizes vary in digestive efficiency. Generally, cows are fed to improve their condition at calving (Garnsworthy and Topps, 1982). Fat cows have been shown to eat less than thin cows, however, accumulation of body fat inhibits food intake after calving (Bines et al., 1969). Adipose tissue generates feedback signals that regulate intake (NRC, 2016). For every 1% increase in body fat content over the range of 21.3 to 31.5% body fat, DMI decreases by 2.7% in beef cattle (Fox et al., 1988). Dairy cows with lower body condition scores at calving have been found to produce more milk directly from food rather than from body fat and are in positive energy balance earlier in lactation and are more biologically efficient than cows with higher body condition scores (Garnsworthy and Topps, 1982).

Stage of production influences DMI because higher producing animals require more nutrients to maximize production. Improving fiber digestibility may increase DMI, depending on energy demands and stage of lactation of the cow (Oba and Allen, 1999). Lactating animals may need 35 to 50% more DMI than non-lactating animals being fed the same diet (ARC, 1980). Oba and Allen (1999) found that lactating dairy cattle

consumed 0.17 kg more DM and produced 0.25 kg more 4% fat-corrected milk when the NDFD of forage was increased by just one unit. Varga (2006) found that cows in early lactation fed highly digestible forages consumed 1.18 kg/day more DM and produced 1.22 kg/day more fat-corrected milk than cows fed a diet lower in digestibility. In this study, DMI for mid-lactation cows was not affected by forage digestibility. Fiber that is more digestible generally stimulates intake as it disappears from the rumen, however, cows in mid-lactation in this study did not consume more. This was most likely due to higher metabolic and nutrient requirements for cows in mid-lactation. Rather than DMI being limited by fill, the DMI for these cows was limited by metabolic processes. The results of Oba and Allen (1999) and Varga (2006) indicate that producers should allocate more digestible forages to higher producing cows to maximize production.

Environmental conditions such as temperature, wind, and precipitation change the ambient temperature and have the potential to influence intake. The ability of an animal to respond or adapt to changing environmental conditions will determine the effect of environment on intake. The thermoneutral zone for healthy beef cattle is between 0° and 25° C. Cattle exposed to temperatures within this range do not have to expend energy to maintain normal body temperature. Houseal and Olson (1995) concluded that animals exposed to lower critical temperatures (-13° to -47° C; depending on size, sex, age, plane of nutrition, and previous acclimatization) for prolonged periods of time have the potential to become cold stressed and must increase metabolic heat production to maintain homeostasis. Research indicates that prior to altering intake behavior, cattle subjected to cold stress will exhibit increased ruminal motility and passage of digesta

(NRC, 2016). Generally, intake increases when the temperature falls below an animal's thermoneutral zone and decreases when ambient temperature is elevated to maintain metabolic homeostasis (NRC, 2016).

### Reduced-lignin Alfalfa

#### Alfalfa

Alfalfa is a valuable forage used in animal production. Alfalfa is used as a rotation crop because of its ability to add N back into the soil and its extensive root system, which improves soil structure. It is also used for pasture improvement because of its high nutritive value and good seedling vigor. Alfalfa can be grazed, harvested as hay, or used for seed production. High quality and yield potential are two of the most favorable agronomic characteristics of alfalfa. Grown under adequate conditions, alfalfa is capable of producing quality values of 400 g kg<sup>-1</sup> NDF, 300 g kg<sup>-1</sup> ADF (acid detergent fiber), and 200 g kg<sup>-1</sup> CP on a DM basis and will yield an average of 3 tons/acre on irrigated land in Montana (Fick et al., 1994; USDA, 2015). In 2015, Montana producers harvested 1,700,000 acres of alfalfa, which equated to 68% of the total hay produced (USDA, 2015). Total value of production or sales generated from alfalfa production was \$431,800,000 (USDA, 2015).

Improving the production potential and digestibility of alfalfa is critical for maintaining competitiveness against alternative crops used in animal production. Current technologies that have developed new traits and improved the availability of nutrients in the cell wall of alfalfa have been a recent focus in the agriculture industry.

## Lignin

Lignin, a major factor limiting the digestibility of cell wall polysaccharides by ruminants, interacts with other cell wall components such as hemicellulose, cellulose, and pectin to provide structural integrity to the plant cells, as well as resistance to degradation, turgor pressure, and water permeability (Albrecht et al., 1987; Hatfield et al., 1999). During maturation, lignin binds with cellulose and hemicellulose and provides strength to plants by filling the space between cellulose, hemicellulose, and pectin (Undersander et al., 2009). A study in Iowa found that as alfalfa matured from the vegetative stage to the pod stage, the proportion of lignin in the cell wall material of the stem increased by 30% (Albrecht et al., 1987). The binding of lignin to other cell wall polysaccharides allows water to move up the plant stem without leaking. However, this process also reduces digestion of cellulose in the rumen due to the relative indigestibility of lignin by both microbial and mammalian enzymes (Undersander et al., 2009). Lignification creates a barrier between rumen bacteria and the potentially digestible material in the cell wall if the cells have not been physically ruptured by mastication (Jung et al., 2012).

Lignin consists primarily of the monolignols coniferyl alcohol and sinapyl alcohol, with minor amounts of p-coumaryl alcohol (Vanholme et al., 2010). When these monolignols are incorporated into the lignin polymer they are referred to as guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) units (Vanholme et al., 2010). These units are randomly interlinked by carbon-carbon and ether linkages and vary in methylation

(Buxton and Russell, 1988). Guaiacyl units are mono-methylated, S units are dimethylated, and H units are non-methylated (Guo et al., 2001).

Lignification in the cell wall negatively impacts enzymatic breakdown of structural polysaccharides and impairs lignocellulosic conversion of feed for livestock utilization (Grabber et al., 2009). Grabber et al. (2009) demonstrated the effects of reduced ferulate-lignin cross linking and shifts in lignin composition on cell wall fermentation characteristics. The results of this study showed that increasing lignification in cell walls of maize from 0.5-124 mg g<sup>-1</sup> reduced hemicellulose fermentation rate by 37%, delayed lag time by 37%, reduced gas production by 18%, and delayed the lag time for cellulose digestion from 0.6 to 7.9 hours. Non-lignified cell walls containing ~5mg g<sup>-1</sup> of Klason lignin began fermentation less than two hours after inoculation (Grabber et al., 2009). The results of this study coincide with Hummel et al. (2006) and Grabber et al. (2009) who found that relative gas production rate decreases as NDF and lignin content increases. The inhibitory effect of lignin on cell wall fermentation in this study was due in part to ferulate-lignin cross-linking. Manipulating the monolignol composition influenced lignin formation but had no direct impact on cell wall fermentation because shifts in monolignol composition played a secondary role in enzymatic degradation in this situation.

As forage matures, lignin content and composition change. During maturation, total lignin content in the plant increases due to secondary wall lignification and thickening (Jung and Deetz, 1993) and lignin composition progresses toward a higher degree of methylation and a higher S to G ratio (Buxton and Russell, 1988; Jung and

Deetz, 1993; Wilson, 1994). Interestingly, secondary cell walls are more degradable than primary cell walls, suggesting that deposition of S units are less detrimental to total cell wall polysaccharide degradability than G units (Jung and Deetz, 1993). Syringyl and G units form covalent linkages to other lignin units through carbons of their propane sidechains, and through the C-4 phenolic hydroxyl (Jung and Deetz, 1993). Additionally, G units can form bonds through the C-5 position of the aromatic ring structure (Jung and Deetz, 1993). Syringyl units are unable to form a bond at the C-5 position due to the presence of a methyl group at this position. Consequently, S units exhibit less branching than G units, allowing microbes to penetrate and impact the secondary cell wall more efficiently than the primary cell wall and middle lamella (Buxton and Russell, 1988; Jung and Deetz, 1993; Guo et al., 2001).

To date, research presenting the effects of modified lignin content and composition vary based on forage species. One study found that reducing the S to G ratio 10-fold in tobacco, with no reduction in total lignin content, led to a 5.6% increase of *in vitro* digestibility (Vailhe et al., 1996). In comparison, increasing the S to G ratio and reducing total lignin content in alfalfa also improved digestibility (Guo et al., 2001). A brown mid-rib (BMR) mutant in maize, which causes reductions in both the S to G ratio and lignin content (Vignols et al., 1995) improved digestibility and resulted in increased DMI, milk yield, and solids-corrected milk in dairy cows compared to dairy cows consuming non-BMR maize (Oba and Allen, 1999) Although the results of these studies are conflicting, research does suggest that a combined effort of reducing the lignin

content and altering lignin composition is an effective strategy for improving digestibility of forage (Baucher et al., 1999; Guo et al., 2001; Nakashima et al., 2008).

### Reduced-Lignin Forage and its Effect on Animal Performance

Feed cost is a major expense in cattle operations and producers must utilize cost-effective management tools to reduce these expenses. Often times, producers who use forage-based systems supplement their cattle to correct for nutrient deficiencies, conserve forage, improve forage utilization, improve animal performance, and increase economic return (Kunkle et al., 2000). The challenge for these producers is determining appropriate supplementation to improve animal performance and forage utilization, while maintaining economic viability.

Forage-based production systems can be more expensive than grain-based production systems from a digestibility standpoint (Fluharty, 2015). For example, if corn is \$4.40 per bushel in Montana (USDA, 2016), it is worth \$0.079 per 0.5 kilogram. If hay is \$131 per ton in Montana (USDA, 2016), it is worth \$0.066 per 0.5 kilogram. The digestibility of corn and long stemmed hay is around 95% and 40%, respectively (Fluharty, 2015). If producers evaluate these two types of feed based on price per kilogram and digestibility, they will find that feeding long stemmed hay is going to be twice as expensive as feeding corn. After accounting for digestibility, the corn will be worth \$0.083 per 0.5 kilogram ( $\$0.079/.95$ ) and the hay will be worth \$0.165 per 0.5 kilogram ( $\$0.066/.40$ ). Therefore, producers must consider management options, such as feeding more digestible varieties of forage, to reduce the costs of feeding forages.

Minor modifications in digestibility of forages can make feeding forages more cost effective. Hatfield et al. (1999) reported that even a 10% increase in cell wall digestion would result in an additional \$380 million in milk and meat sales in the U.S dairy industry. To put this claim into perspective, let's increase the digestibility of the hay in the previous example from 40-55%. Increased digestibility in this example lowers the price of hay from \$0.165 per 0.5 kilogram to \$0.12 per 0.5 kilogram ( $\$0.066/.55$ ), equating to a 27% decrease in price. These examples suggest that purchasing or growing more digestible hay will allow cattle producers to have a higher return on investment.

One of the newest technologies currently receiving attention in the agriculture industry for improved digestibility is a variety of alfalfa called HarvXtra™. HarvXtra™ was developed in 2002 by Forage Genetics international (FGI), the Samuel Roberts Noble Foundation (Noble Foundation), and the U.S. Dairy Research Center, and is projected to improve feed efficiency and animal performance. HarvXtra™ was created by scientists from the Noble Foundation using gene suppression technology to down-regulate caffeoyl CoA 3-O-methyltransferase (CCOMT), a specific lignin biosynthesis pathway enzyme (McCaslin et al., 2014). Caffeoyl CoA 3-O-methyltransferase suppression reduced total lignin content and G units, however, S units remained unchanged (Guo et al., 2001; McCaslin et al., 2014). Several genes were tested during this trial, but down-regulating CCOMT was the only one that resulted in increased NDFD without affecting forage yield, persistence, multiple pest resistance, or lodging (McCaslin et al., 2014).

Forage Genetics International scientists found that HarvXtra™ alfalfa had a 15-20% decrease in lignin content and a 10-15% increase in NDFD and relative forage quality (RFQ) when compared to related null lines of alfalfa (McCaslin et al., 2014). These scientists also found that as HarvXtra™ became more mature, the quality declined at a slower rate than the quality of conventional alfalfa. The FGI trait development team tested this observation by delaying the harvest of HarvXtra™ alfalfa by seven days. After the seven-day delay, HarvXtra™ had about the same NDFD as related null lines that were harvested a week earlier (McCaslin et al., 2014). This discovery is expected to allow farmers who plant HarvXtra™ to produce high quality hay with a broader harvest window and a more flexible harvest management schedule.

Prior to the development of HarvXtra™, scientists cloned genes in each step of the lignin biosynthetic pathway to determine the impact of lignin content and composition on cell wall digestibility. The results of these studies concluded that, depending on the gene, species, and level of gene redundancy, reducing the expression of specific enzymes in the monolignol pathway had the potential to reduce the amount of lignin in the cell wall and alter lignin composition (Franke et al., 2000; Nakashima et al., 2008; Vanholme et al., 2010). Previous research found that reducing lignin content and/or manipulating lignin composition improved digestibility, allowing for potential improvements in animal performance and maximized profit for the farmer and rancher (Guo et al., 2001). Unfortunately, none of the studies evaluating modified alfalfa tested this claim on actual animals in feeding trials. Therefore, published data recording the

effects of feeding reduced-lignin alfalfa to animals are lacking, However, progress reports are available.

A progress report by Mertens (2009) found that down-regulating caffeic acid 3-O-methyltransferase (COMT) in alfalfa significantly improved fiber digestion, and that daily gains for lambs in the reduced-lignin treatment were 10% higher. Results of BMR forages are comparable to reduced lignin alfalfa because the BMR mutant produces plants with lower lignin content and higher IVDMD. In vitro and in situ NDF digestion trials using BMR mutant forages found that BMR forages have lower lignin content and greater NDF digestion than their nulls (Rook et al., 1977; Keith et al., 1979; Cherney et al., 1991). Studies using BMR varieties report increased DMI and improved milk production and weight gain (Rook et al., 1977; Aydin et al., 1999). Rook et al. (1977) found that total individual ruminal VFA was higher, pH was lower, and digestible energy intake was 27% greater for cows being fed BMR corn silage when compared to its null. Weller and Phipps (1985) found that the BMR gene significantly increased the digestibility of organic matter (OM), cell wall constituents (CWC), ADF, cellulose and hemicellulose in maize silage fed to sheep and lactating cows. Daily live-weight gain was significantly higher for calves fed BMR silage (0.92 kg/d) compared to the normal non-BMR diet (0.83 kg/d).

The results of the BMR research and the feeding-trial progress reports are prerequisites for implementing more research on the effects of feeding reduced-lignin alfalfa to animals. Published data regarding the effects of feeding reduced-lignin alfalfa to beef cattle will provide supporting evidence as to the value of reduced-lignin alfalfa in

livestock management systems.

### Hay Storage

Hay is commonly used as a feed source for animals confined to pens, when pasture forage quality fails to meet animal nutrient requirements, and during the winter months, when animals cannot physically access forage. Under these circumstances, producers rely on hay as the primary feed source to maintain the energy and nutrient requirements of their animals. Losses occur during hay production and storage due to environmental factors that cannot be controlled by the producer. Although producers cannot control environmental variability, they do have control over harvest date, baling method, stacking method, and storage method. Management decisions regarding these factors are based on demands of the intended market, and profoundly impact the extent of quality and DM losses in hay.

Losses associated with hay production commence at harvest and continue after hay has been stored. Feeding values of DM, along with quality parameters, can be determined after hay has been stored. Factors influencing DM and quality losses during hay production and storage include moisture, bale density, environmental conditions, and method of storage (Verma and Nelson, 1983). Declining quality associated with hay production and storage has a major impact on animal performance (Verma and Nelson, 1983). From a financial perspective, minimizing these losses is critical, especially when considering producer profitability.

### Moisture and Bale Density at Harvest and During Storage

Baling forage at the appropriate moisture content and at a high density are the first steps in maintaining quality and reducing DM losses in large round bales (LRB). The recommended moisture content before baling LRB and small square bales (SSB) is 18% and 20%, respectively (Lemus, 2009). Initial moisture content at baling influences IVDMD and NDF concentration after hay has been stored (Collins et al., 1987; Martinson et al., 2011). For every 1 g/kg increase in initial moisture content, IVDMD declines by 0.5 g/kg and NDF increases by 0.4 g/kg (Collins et al., 1987).

Forage should be baled as densely as possible, especially in the outer layers. The amount of moisture penetration and bale deterioration are inversely related to the tightness of the outer 2-6 inches of the bale (Huhnke, 2003). Recommended minimum moisture at harvest and for storage declines as bale density and volume increase (Muck and Shinnors, 2001). Baling and storing at recommended moisture content is especially important for high-density bales because they are unable to dissipate heat and are susceptible to quality and DM losses due to heating and microbial action.

During the first two to three weeks after baling, hay baled at recommended moisture contents (between 15-20%) will undergo a temperature change caused by plant respiration and microbial activity in an event referred to as “sweating” (Gay et al., 2003; Lemus, 2009). The extent and duration of sweating is influenced by moisture content at baling and lasts for up to 10 days (moisture content between 15-20%) and up to 40 days (moisture content at or above 30%) (Lemus, 2009). After the initial sweating period the temperature of hay baled at a recommended moisture content will fall to approximately

16° C (Gay et al., 2003; Lemus, 2009). At this time plant respiration and metabolic activity have concluded and bales are at “equilibrium” (Lemus, 2009). Reaching equilibrium happens at the expense of DM losses. For every 1% drop in original field baling moisture there will be a 1% loss in DM (Lemus, 2009). For example, if forage baled at 18% moisture reaches 12% moisture after three weeks, there will be a corresponding DM loss of 6%. Hay will not suffer significant DM or quality losses if temperature increase does not exceed 54° C during the sweat.

Hay baled at appropriate moisture will maintain quality due to minimal changes in leaf-to-stem ratio. Lowering the amount of high quality leaves in relation to low quality stems generally reduces the quality of hay (Buxton, 1996; Ball et al., 2001; Undersander, 2016). To elaborate, consider the quality of traditional dairy quality alfalfa hay. Dairy quality alfalfa hay is assumed to be composed of 45% leaves and 55% stems, with quality values of at least 20% CP and less than 40% NDF (Undersander, 2016). The leaves of alfalfa contain 2-4 times the CP concentration, twice the amount of nonstructural carbohydrates, and less than 1/3 of the fiber than that of the stems (236 g/kg NDF for leaves and 559 g/kg for stems; (Collins et al., 1987; Undersander, 2016). For every 5% loss of leaf material, it is estimated that there is a 1.2% loss in protein and 2.2% increase in NDF (Undersander, 2016). Preserving leaf content of alfalfa is critical in reducing DM losses and maintaining quality constituents for utilization by the animal. Minimizing losses in leaf to stem ratio by baling hay at appropriate moisture content is important for maintaining low NDF, and high CP and DM content (Collins et al., 1987).

Baling at moisture contents above recommended levels (> 20%) can promote heat damage, and lead to quality losses and reduced palatability due to bacterial degradation and mold (Collins et al., 1987; Lemus, 2009; Martinson et al., 2011). Heat damage is caused by microbes respiring non-structural carbohydrates and producing carbon dioxide, water, and heat (Martinson et al., 2011). Overheating can cause N to bind to fiber, resulting in acid detergent insoluble nitrogen (ADIN). Acid detergent insoluble nitrogen is completely indigestible and unavailable to the animal (Machacek and Kononoff, 2009). Quantitative values of ADIN are found by measuring the N remaining in the ADF portion of a feed sample. Russel and Buxton (1985) found that after 17 months of storage, ADIN in the total N value was more for high- versus low-moisture treatments (167 g/kg vs. 129 g/kg, respectively). Collins et al. (1987) found that after three months of storage, the proportion of ADIN in the total N increased as moisture content of the bales increased.

#### The Effect of Environment on Harvest and Storage

The environment is unpredictable and can impact hay quality and DM losses during harvest and storage. Humidity, weather, and solar radiation impact field drying rate, and can inhibit forages from reaching recommended moisture levels before baling and during storage (Rotz and Chen, 1985).

Humidity is a major factor limiting field drying rate and bale drying during storage. Hay production in humid climates is difficult because of slower field drying rates. Achieving desired moisture content before baling is almost impossible in humid locations in North America and Europe (Muck and Shinnars, 2001). Producers in these locations rely on mechanical factors (conditioning or swathing), machine treatments

(tedding or raking), and in some cases chemical treatments (anhydrous ammonia) to speed up field drying rates (Muck and Shinnars, 2001). Bale drying prior to storage is important in humid climates, especially for bales that have been baled at a high density, because the ability of moisture to dissipate from bales becomes more difficult in humid climates (Buckmaster et al., 1986; Muck and Shinnars, 2001). In arid regions, the risk of inclement weather during harvest is low and losses due to rainfall and humidity are minimal. Producers in these regions only bale hay after dew has accumulated and softened plant tissues enough to minimize leaf loss (Muck and Shinnars, 2001). Obvious and apparent differences for hay production between humid and arid climates demonstrate why producers must consider humidity in their production strategy.

Weather is a huge factor related to DM and forage quality losses associated with hay production and storage. Timing and amount of rain damage during field curing can cause DM losses and reduce IVDMD and total non-structural carbohydrates (TNC). This occurs through leaching, plant respiration, and leaf shatter (Ball et al., 2001). Leaching is the process by which water-soluble plant material is lost to the environment. Unfortunately, no distinction can be made between leaching and respiration; however, research suggests that the extent of leaching for different plant constituents varies (Collins, 1983). The severity of rain damage increases as forage becomes dryer and is particularly unsatisfactory when forage is ready to bale. Collins (1983) found that wetting field cured hay after it reached 85% DM reduced the yield of N, IVDMD, and TNC by 45.2, 63.9, and 85.6%, respectively.

The depth of weathering depends on environmental conditions, hay type, bale

shape, bale density, and storage technique (Huhnke, 2003). Hay stored uncovered outside is prone to weather deterioration and is susceptible to undesirable changes in moisture, DM, and quality. Changes in moisture content are significant in the outer 20 cm layer of the bale (Anderson et al., 1981; Belyea, 1985; Huhnke, 1988). Research by Huhnke (1988) found that total moisture content for alfalfa round bales stored uncovered in a single row for 8 months was over 20% in the top (20 cm depth), sides (15 cm depth), and bottom (10 cm depth) layers. Total bale moisture content for uncovered bales was substantially larger than that of covered bales, which was only 3%. Initial to final moisture accumulation in hay stored for 8 months ranked in order from highest to lowest is: outer 10 cm, total bale, and covered (Huhnke, 1988). Huhnke (1988) found that initial to final moisture content in the outer 10 cm of uncovered alfalfa bales stored in Oklahoma increased by 120% compared to total bale moisture, which only increased by 50% over the course of the storage period.

#### Dry Matter Losses and Changes in Palatability

Round bales are susceptible to significant DM losses when they are stored outside without cover. In this situation, DM losses occur even if hay has been baled and stored at appropriate moisture. Huhnke (1988) found that hay stored outside on the ground in Oklahoma lost more DM than hay stored inside on pallets after 8 months of storage (13.1% and 1.9%, respectively). These results coincide with Anderson et al. (1981), who found DM losses of 15% for outdoor stored and 2% for indoor stored alfalfa round bales in Pennsylvania, and Belyea et al. (1985), who found DM losses of 14% for outdoor stored and 3% for indoor stored alfalfa hay bales in Missouri. The results of these studies

only account for losses following storage and do not account for cumulative DM losses associated with hay processing, storage, and feeding.

The way in which hay is stored greatly impacts hay composition and palatability. Hay exposed to the environment is lower in quality and becomes weathered and unpalatable, reducing animal intake. A study in Louisiana reported 22.6% animal refusal loss for round bales stored outside, on the ground, and uncovered (Verma and Nelson, 1983). Belyea et al. (1985) found that heifers consuming LRB stored outside and uncovered in Missouri gained significantly less (0.39-0.46 kg/d) than heifers consuming hay stored inside (0.62-0.69 kg/d). Losses due to handling, DM, and animal refusal in Louisiana totaled 65.2% for ryegrass bales stored uncovered for 7 months compared to only 3.5% for bales stored inside a barn (Verma and Nelson, 1983). Similar research by Belyea (1985) found that total DM losses (accounting for feeding plus storage) in Missouri equated to 40% for outdoor stored bales, compared to only 14.8% for inside stored bales. These studies suggest that environment and weather have detrimental effects on DM and animal performance, especially for hay that is stored outside and uncovered.

#### Quality Losses

Environment and weather are also factors influencing undesirable changes in bale quality due to outdoor hay storage. Uncovered hay is prone to weathering and leaching of non-structural carbohydrates. Leaching negatively impacts forage quality, resulting in less-digestible forage because of an increased ratio of structural to non-structural carbohydrates (Lechtenberg et al., 1974). Research indicates that bales kept off the ground with at least the upper half covered or bales stored inside lose less DM and

exhibit less quality change during storage (Chung and Verma, 1991). Chung and Verma (1991) found that guinea grass hay stored inside a barn or outdoors on pallets with plastic covers had a decreased change in quality, and retained a higher level of digestibility compared to bales that were stored outside and uncovered. Huhnke (1988) also found that final IVDMD values were significantly higher for covered vs. uncovered bales (71.9 vs. 66.2% IVDMD, respectively). These results correspond with the results of Laflamme (1989) who found significant changes in digestibility between covered and uncovered bales stored in Canada, with covered bales having higher IVDMD than uncovered bales (53.6% and 50%, respectively). Chung and Verma (1991) found a 4.7% increase in NDF, an 8.29% increase in ADF, and a 6.27% decrease in IVDMD for bales stored outside on a gravel bed in Hawaii. In comparison, changes in NDF, ADF, and IVDMD values for bales stored inside a barn in this study did not exceed 2%. These results correspond to the results of Huhnke (1988), Brasche and Russell (1988), and Verma and Nelson (1983), who found changes in total and outer layer final NDF, ADF, and N content to be higher and IVDMD to be lower for uncovered versus covered bales. Due to a majority of forage quality losses occurring in the outer 20 cm layers of bales, which accounts for 40-50% of bale volume (Anderson et al., 1981), this research indicates that increases in NDF, ADF, and N contents during storage are most likely a result of DM losses caused by weathering.

Changes in protein content vary based on how hay is stored. Changes in available protein (AP) during storage occur due to heat generated inside bales during storage, causing AP to bind to structural carbohydrates and become unavailable for use by the

animal (Verma and Nelson, 1983; Chung and Verma, 1991). For example, initial values of CP and AP for ryegrass bales stored outside in Louisiana were 8.9% and 6.7%, respectively (Verma and Nelson, 1983). CP and AP values for bales stored inside in this study were 10% and 8.4%, respectively. After 7 months of storage CP content for outside stored bales increased significantly to 12%, however, AP was reduced to 6.5%. For the indoor stored bales, CP content increased to 12%, and AP increased to 9.4%. In this study, heating was the most likely the cause of changes in protein content for outdoor stored bales, which was explained by lower AP content in these bales. In contrast, Chung and Verma (1991) found that inside- and outside-stored bales increased in CP content, as well as AP content, which indicated that bales were not subjected to heating. In Chung and Verma's study, increases in AP ranged from 8.5% for covered bales to 24% for uncovered bales, and was most likely due to DM losses associated with weathering. These results coincide with the results of Huhnke (1988) and Brasche and Russell (1988), who found that covered bales had more AP than uncovered bales. The results of these studies indicate that minimizing DM and quality losses associated with outdoor storage is a huge concern in maintaining palatable, high quality forages for consuming animals.

### Hay Storage Methods

Large round bale production is highly productive and requires fewer inputs compared to other hay processing methods. Labor requirements for LRB production are minimal, only requiring one person to operate production from start to finish. Storage and stacking method vary between producers and depend on available resources, space, and

geography. A Kansas Cooperative Extension survey found that of the 72% of respondents who used LRB to package hay, 60% of them stored their bales uncovered outside (Taylor et al., 1995). Large round bale shape, which is inconvenient for indoor storage, along with costs associated using hay tarps and constructing buildings, are the main reasons producers choose to store their LRB outside.

Research studying the effects of different outdoor hay storage methods on DM and quality losses is available in regions throughout the United States, however, published research regarding Montana hay storage is lacking. Hay storage research in Montana is necessary to generate data and allow Extension agents to appropriately recommend hay storage methods to individual producers. Recommending storage systems that reduce DM losses and extend hay quality are optimal for reducing hay production costs, and have the potential to lower total operating costs of cattle production substantially.

Knowledge of climate and geography can help minimize DM and quality losses for outdoor stored hay and contribute to maximal animal response. The literature reports varying differences in DM losses based on environment, location, and hay storage method. Reports for DM losses range from a low of 0.8% DM losses in South Dakota (Chisholm et al., 1980) to a high of 40% DM losses in Canada (Atwal et al., 1984). Determining whether bales should be stored inside or outside in an individual, end to end, or stacked formation requires consideration of the environment in which hay will be stored. This section is dedicated to discussing hay storage methods.

### Storage Method

The previous section provided a detailed description of the factors associated with DM and quality changes for bales that have been stored either inside under cover, or outside, with no cover. This section is dedicated to discussing DM and quality losses associated with common outdoor hay storage methods in Montana, the economics behind covering hay, and why there is a need for hay storage research specific to Montana environment and climate.

Three common stacking methods in Montana are: 1) end-to-end, 2) pyramid, and 3) mushroom. These stacking methods will be discussed in detail in this section.

End-to-end Storage. End-to-end storage involves tightly stacking bales end-to-end to utilize storage area and protect the ends of the bales from weathering. Orienting end-to-end bale rows in a north-south direction allows sunlight to reach both sides of the bale rows and leaving at least 3 feet between rows increases air circulation and sunlight penetration (Huhnke, 2003). Research regarding this storage method is one of the most common found in the literature. Losses associated with this method have been reported in various climatic and geographic locations. Researchers in South Dakota found that bales stored in an end-to-end formation were the best method of storage compared to bales stored individually and bales stored in a pyramid arrangement (Chisholm et al., 1980). In this study, end-to-end stored bales only lost 0.8% DM after 1 year of storage compared to 10.3% for bales stored as a pyramid. These bales were also highest in CP (5.65%) and IVDMD (39%) when compared to bales stored in the pyramid formation (CP: 5.41%; IVDMD: 32.2%) after storage. Although Chisholm et al. (1980) found bales stored end-

to-end to be the best storage method in South Dakota, their research did not compare end-to-end storage to indoor stored bales. Researchers in Canada compared end-to-end stored bales to indoor stored bales and found that bales stored uncovered in end-to-end rows lost 40% total DM after one year of storage due to oxidation, weathering, and molding compared to only 6% for indoor stored bales (Atwal et al., 1984). Bales in this study were higher in ADF and lower in digestible energy and DMI compared to hay stored under cover. Researchers in Hawaii found that end-to-end bales stored uncovered lost 27.9% DM, compared to only 3.5% for barn stored bales after 6 months of storage (Chung and Verma, 1991). End-to-end stored bales in this study increased in CP (21%), AP (22.5%), ADF (5.91%), and NDF (0.7%), and decreased in IVDDM (4.87). End-to-end stored quality values in this study were much different than the observed values for bales stored in a barn. Bales stored in a barn increased in CP (10.9%), AP (8.5%), ADF (1.94%), and IVDDM (0.10) and decreased in NDF (0.90). The results of this study depict the extreme difference in DM and forage quality losses for indoor compared to outdoor stored bales in the end-to-end formation. Dry matter and quality changes are different between the previously mentioned studies and are indicative of varying environmental conditions.

Pyramid Storage. Stacking bales as a pyramid requires much less storage area than other stacking methods. However, leaving pyramids uncovered results in substantial DM and quality losses. Researchers in South Dakota found that hay stored in a pyramid formation held the most moisture (initial moisture = 13.3% vs. final moisture = 18.5%) compared to end-to-end stored bales (initial moisture = 12.8% vs. final moisture = 12.7%) and individually stored bales (initial moisture = 13.8% vs. final moisture =

11.8%) after one year of storage (Chisholm et al., 1980). The pyramid formation in this study lost more DM and IVDMD (DM loss = 10.3%; initial IVDMD = 38% vs. final IVDMD = 32.2%) than bales stored end-to-end (DM loss = 0.8%; initial IVDMD = 39.1% vs final IVDMD = 39%). Researchers in Canada reported 30% DM losses due to oxidation, weathering, and molding for the pyramid stack, which was covered with a 6-mil black plastic tarp (Atwal et al., 1984). The tarp in this study was lost due to high velocity winds after one month of storage and had to be replaced two different times. Unfortunately, replacing the tarp during the winter was reported as an “impossible task” and it is assumed that for most of the storage period, the pyramid stack remained uncovered. Although there were issues with the tarp in this study, the results indicated that hay stored as a pyramid formation was higher in ADF and lower in digestible energy and DMI than barn stored hay after storage. Researchers in Australia found similar results to that of Atwal et al. (1984) but found that bales stored as a pyramid formation lost 29.7% DM, 26.4% CP, and 38.5% digestible dry matter (DDM) after 8 months of storage (Wickes and Cochrane, 1982).

Mushroom Storage. Stacking hay in a mushroom formation has recently become a popular hay storage technique. This formation involves placing a bottom bale upright (so the flat end of the bale is in contact with the ground) and stacking a second bale sideways, on top of the bottom bale. Hay spoilage at the bottom of the bale is higher for this method, but less hay is exposed to the ground when compared to end-to-end stacking (Taylor et al., 1995). Taylor et al. (1995) suggested that DM and quality losses for the mushroom are comparable to bales stored end-to-end. Taylor et al. (1995) also suggested

that stacking hay in a mushroom formation requires well-formed and highly-dense bales to maintain sufficient DM and quality. Unfortunately, the use of the mushroom stack as suggested by Taylor et al. (1995) is not supported by published data. Literature pertaining to quality and DM losses associated with the mushroom stack is lacking. Research encompassing varying climatic and environmental conditions in Montana is necessary to validate the claims made by Taylor et al. (1995).

Covered Storage. Storing hay inside or covered by plastic tarps reduces storage losses substantially. Research by Anderson et al. (1981) found that the interior quality of outdoor-stored bales is comparable to the quality of indoor-stored bales, suggesting that storing hay inside is the ideal storage method. This research coincides with the research of Atwal et al. (1984), Brasche and Russell (1988), Chung and Verma (1991), and Verma and Nelson (1983).

Providing cover for hay requires monetary input and can be inconvenient for producers who need to store large quantities of hay. Construction of a building can be as low as \$1.50 per square foot for a roof-only structure and up to \$6.00 for a turn-key enclosed building (Huhnke, 2003). Annual costs associated with buildings are: depreciation (D), interest on investment (I), repairs (R), taxes (T), and insurance (I). The annual cost of a building is calculated by multiplying the initial investment by the sum of these annual percentage costs (D+I+R+I+T+I). Total storage cost is the sum of the storage system and the cost of projected storage losses. Huhnke (2003) explains that as the price per ton of hay increases, the loss in dollar value of hay based on percentage of storage loss, will increase. For example, hay valued at \$100/ton will be reduced to

\$75/ton after a 25% storage loss, compared to hay valued at \$150/ ton, which would be reduced to a value of \$112.5/ton with a 25% loss. In this example, the higher-valued hay lost approximately \$37/ ton, while the lower-valued hay lost only \$25/ ton. This reduction in price could be devastating to a producer.

Although DM losses are significantly reduced for barn stored hay (DM loss being roughly 2%), justifying the cost of building a hay storage structure or purchasing hay tarps depends on whether protection will produce sufficient savings to cover all costs associated with a covered hay storage system (Huhnke, 2003). The price of hay covers can range from \$0.06/30 square centimeters for a 6-mil black polyethylene or plastic to over \$0.75/30 square centimeters for a tarp (Huhnke, 2003). These covers are relatively low cost but have several shortcomings. The lifetime of a plastic cover is typically one year, so purchasing a plastic cover could be an annual expense. Each cover requires fasteners to secure the plastic and extra labor to put the covers on and take them off, along with frequent inspections and continual maintenance to replace fasteners and repair tears when necessary. Additionally, the material for covers does not allow moisture to escape and subjects hay to potential DM and quality loss if any excess moisture is in the bales at time of covering. Although tarps have some major shortcomings, research suggests that improving bale storage with tarps results in enough savings to cover costs associated with purchasing the tarps. Collins et al. (1997) evaluated the costs associated with several hay storage methods (Table 1) and found that improved bale storage resulted in sufficient savings to cover costs associated with the storage system employed (except for conventional shed and \$40 per ton grass hay). Hay valued at \$40/ton stored in a

conventional shed was only \$0.27 per ton short of covering all costs for constructing the shed.

From a financial perspective, minimizing DM and forage quality losses during hay storage is critical, especially when considering producer profitability. The research cited in the previous paragraphs suggests that losses are minimized when bales are stored under a barn, however, the capacity to store hay under a barn is limited by space and financial barriers. Hay storage literature is limited to the Eastern half of the United States and addresses hay storage methods that are different from Montana hay storage conditions. Research mimicking Montana storage methods and conditions is necessary to determine how to minimize DM and quality losses in this geographic location and provide practical information for Montana producers to initiate changes in their hay storage methods.

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Table 1. Total annual costs for each hay storage system, including dry matter (DM) losses, on a per ton basis								
Storage System	DM Loss*	System Cost	Grass Hay			Alfalfa Hay		
			Hay Value	DM Loss	DM Savings over Stack on Ground	Hay Value	DM Loss	DM Savings over Stack on Ground
	%	.....\$/ton.....						
Conventional Shed	5	10.27	40.00	2.00	10.00	85.00	4.25	21.25
Pole structure with plastic roof on pad	7	6.42	40.00	2.80	9.20	85.00	5.95	19.55
Reusable tarp on pad	7	5.81	40.00	2.80	9.20	85.00	5.95	19.55
Bale sleeve on ground	8	6.36	40.00	3.20	8.80	85.00	6.80	18.70
Plastic wrap on ground	5	3.15	40.00	2.00	10.00	85.00	4.25	21.25
Elevated stack on pad	10	1.90	40.00	4.00	8.00	85.00	8.50	17.00
Net wrap on ground	23	3.15	40.00	9.20	2.80	85.00	19.55	5.95
Stack on ground	30	1.58	40.00	12.00	0.00	85.00	25.50	0.00

\* The weight of the weathered layer is considered part of the total DM loss.

CHAPTER THREE

THE EFFECTS OF FEEDING REDUCED-LIGNIN ALFALFA ON GROWING BEEF  
CATTLE PERFORMANCE

Contribution of Authors and Co-Authors

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**ABSTRACT**

Gene suppression techniques down-regulating caffeoyl CoA 3-O-methyltransferase (CCOMT), a specific lignin biosynthesis pathway enzyme, have permitted the development of an alfalfa variety with an altered lignin content and composition known as HarvXtra (HX-4114). Published research describing the effects of feeding reduced-lignin alfalfa on ruminant animals is lacking; however, preliminary data suggests that dairy cattle and sheep consuming reduced-lignin alfalfa varieties perform better compared to animals receiving conventional alfalfa varieties. Therefore, a study was designed to evaluate the effects of feeding a reduced-lignin alfalfa variety (HX-4114) versus a conventional alfalfa variety (WL336HQRR) on growing beef cattle performance. Twenty-four Crossbred Angus heifers were selected to participate in this study and their performance was evaluated based on changes in BW, ADG, DMI, and G:F. *In situ* digestibility was tested at 6, 12, 24, 30, 48, 96, and 240 h using four ruminally cannulated Hereford cows. Hay samples were collected and used to determine forage quality and leaf to stem ratio. There were no differences ( $P \geq 0.05$ ) in forage quality between treatments, except for DM ( $P = 0.01$ ). Means did not differ by treatment for percent leaf ( $P = 0.06$ ), but differed by treatment in leaf-to-stem ratio ( $P = 0.04$ ). There were no treatment or treatment by day interactions ( $P \geq 0.05$ ) for BW, ADG, DMI, or G:F. There were no treatment or treatment by time interactions ( $P \geq 0.05$ ) for *in situ* digestibility. Overall, the results of this study suggest no difference in forage quality between reduced-lignin and conventional alfalfa. Additionally, animal performance did not differ for growing crossbred Angus cattle consuming reduced-lignin or conventional alfalfa. Research

exploring forage quality and animal performance for reduced-lignin alfalfa post-seeding is warranted to determine the value of this new technology in the U.S beef industry.

**Key words:** alfalfa, beef, cattle, HarvXtra, performance, reduced-lignin

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## INTRODUCTION

Improving the production potential and digestibility of alfalfa is critical for maintaining competitiveness against alternative crops used in animal production. Current technologies that have developed new traits and improved the availability of nutrients in the cell wall of alfalfa have been a current focus in the agriculture industry.

Innovative technology using gene suppression techniques to down-regulate caffeoyl CoA 3-O-methyltransferase (CCOMT), a specific lignin biosynthesis pathway enzyme, has permitted the development of a more digestible variety of alfalfa known as HarvXtra (HX-4114; McCaslin et al., 2014). Compared to related null lines of alfalfa, HarvXtra is expected to exhibit a 15-20% reduction in lignin content and a 10-15% increase in neutral detergent fiber digestibility (NDFD) and relative forage quality (RFQ; McCaslin et al., 2014).

Published research describing the effects of feeding reduced-lignin alfalfa on ruminant animals is lacking; however, one progress report suggests that feeding reduced-lignin alfalfa to lambs significantly improves fiber digestion and increases ADG by 10%

for lambs consuming reduced-lignin alfalfa varieties compared to lambs consuming conventional alfalfa varieties (Mertens, 2009).

Comparable research testing the effects of feeding other reduced-lignin forages to animals suggests that reducing the lignin content improves animal performance. The brown midrib (BMR) mutant found in a variety of forages produces plants with lower lignin content and higher IVDMD. *In vitro* and *in situ* NDF digestion trials using BMR mutant forages found that BMR forages had lower lignin content and higher NDF digestibility than their nulls (Rook et al., 1977; Keith et al., 1979; Cherney et al., 1991). Studies using BMR varieties report increased DMI, improved milk production, and better weight gain compared to animals consuming conventional forages (Rook et al., 1977; Aydin et al., 1999).

The objective of this study was to determine the differences in forage quality between reduced-lignin and conventional alfalfa and test these differences using animal performance measurements such as BW, ADG, DMI, and G:F. We hypothesized that reduced-lignin alfalfa would have less lignin and higher RFQ than conventional alfalfa and that animals consuming reduced-lignin alfalfa would weigh more on the final day of sampling and have higher ADG, DMI, and G:F compared to animals consuming conventional varieties of alfalfa.

## MATERIALS AND METHODS

All protocols for this study were reviewed and approved by the Montana State University Agricultural Animal Care and Use Committee (#2016-AA15).

### *Forage Establishment and Harvest*

A variety of reduced-lignin alfalfa (HX-4114) and a variety of conventional alfalfa (WL336HQRR) were planted south of Townsend, MT, on May 19, 2016. Soil samples were collected prior to planting and laboratory results for nutrient analysis were used to determine and apply appropriate fertilization. Approximately 3.6 kg pure live seed/ ha were drilled using a Great Plains 3S-3000HD double disc drill at a 1.27 cm depth, spaced 15 cm apart, into a firm, weed-free seedbed. Both varieties were planted in the same field, each on 8 ha. The field was irrigated with a center-pivot already in place at the research site. First and second harvests were taken on July 29, 2016 and October 10, 2016, respectively, at approximately 10% bloom. Production for the second harvest, which was used to feed the animals in this study, totaled 2,951 kg/ha for the conventional alfalfa and 2,908 kg/ha for the reduced-lignin alfalfa.

### *Feeding Trial*

Twenty-four crossbred Angus heifers (initial BW =  $270 \pm 21$  kg) were utilized in a completely randomized design in an 84-day trial beginning on November 14, 2016 and ending on February 7, 2017. Heifers were stratified by BW to one of two alfalfa varieties

(3 heifers/pen). There was a total of 8 pens in this study (4 pens per treatment). Each pen was equipped with a GrowSafe System (GrowSafe Systems Ltd.) to determine individual heifer intake. Heifers were fed diets consisting of 100% alfalfa at 3.40% BW on a DM basis once daily and allowed *ad libitum* access to water and Easylix 12-12-12 mineral pressed blocks.

Heifers were acclimated to their respective treatments 10 d prior to the start of the trial. No data were recorded during this time. Heifers were weighed immediately prior to the start of the trial on d -1 and 0, and every 27 and 28 d thereafter. Unshrunk weights were taken for two consecutive d, and individual BW were averaged to obtain individual BW by period. Sampling occurred on days -1, 0, 27, 28, 55, 56, 83, and 84.

### ***Forage Analysis***

Forage quality was determined and analyzed using core samples taken with a Penn State electric drill mounted hay probe (Nasco Corporation, Modesto, CA). Samples were taken weekly and two weeks were combined for each treatment, totaling six composited samples per treatment (n = 6). Samples were dried at 60°C and then ground in a Wiley mill to pass a 2-mm screen and sent to a commercial lab (Cumberland Valley Analytical Services, Hagerstown, MD) to be analyzed for DM (modified Goering and Van Soest, 1970), NDF (Van Soest et al., 1991), ADF (modified AOAC, 2000), CP (AOAC, 2000 and FP528 analyzer, LECO Corporation, St. Joseph, MI), lignin (modified Goering and Van Soest, 1970), net energy gain (NEg), relative feed value (RFV), relative forage quality

(RFQ), TDN, and neutral detergent fiber digestibility at 48 hours (NDFD48; Goering and Van Soest, 1970).

Core samples were taken (same method as above) to determine and analyze percent leaf and leaf-to-stem ratio. The leaves and stems of five core samples from each treatment (n = 5) were hand separated and weighed. Weights of the separated leaves and stems were used for the analysis.

### ***In situ Digestibility***

Four ruminally cannulated Hereford cows (BW = 595 ± 35 kg) were fed grass hay *ad libitum* and alfalfa hay at 1% BW once daily. One Dacron bags (10 cm x 20 cm; pore size = 50 µm), containing 5 g of forage for each of the two forage treatments (reduced-lignin and conventional), one grass hay standard, and one blank bag, were placed into the rumen of each cow and removed after 6, 12, 24, 30, 48, 96, and 240 h. After removal from the rumen, bags were rinsed in cold water until the water ran clear. Excess water was squeezed gently from each bag. Bags were then dried overnight in a forced-air oven at 60°C. Residue remaining in each bag was used for NDF analysis (Van Soest et al., 1991). This entire procedure was replicated two days following the removal of bags at 240 h. Replicated digestion values were averaged for each cow for each time point and digestibility was determined by calculating the dry weight difference before and after digestion for each cow.

### ***Statistical Analysis***

All data in this study were analyzed using SAS (version 9.4; SAS Inst. Inc., Cary, NC). Forage quality data was analyzed using the PROC GLM procedure of SAS. Individual core samples were treated as the experimental unit and treatment and day were set as fixed effects. Leaf-to-stem data and percent leaf data were analyzed using the PROC GLM procedure of SAS with core sample as the experimental unit. Feeding trial data were analyzed as a completely random design using the PROC MIXED procedure of SAS, with heifer as the experimental unit. Treatment and day were set as fixed effects, with heifer as a random effect. *In situ* data were analyzed using the PROC MIXED procedure of SAS with Dacron bag as the experimental unit. All means in this study were separated using the LSMEANS procedure and considered significant at  $P \leq 0.05$ .

## **RESULTS AND DISCUSSION**

### ***Forage Analysis***

Forage quality parameters are reported in Table 1. There was a difference between treatments in DM ( $P = 0.01$ ), with reduced-lignin alfalfa having 92.23% DM and conventional alfalfa having 92.87% DM. There were no significant differences ( $P \geq 0.11$ ) between treatments for CP, ADF, NDF, lignin, NEg, RFV, RFQ, TDN, or NDFD48. The reduced-lignin alfalfa in this study did not meet the expected 10-15% reduction in lignin content and 10-15% increase in NDFD and RFQ. When compared to the conventional

alfalfa treatment in this study, the lignin content for the reduced-lignin alfalfa was only reduced by 5.5% and RFQ and NDFD48 were only increased by 3% and 1%, respectively. One possible explanation for these results is the late timing of harvest. Alfalfa harvested later in the year is associated with lower yields and lower quality (Ottman et al., 2014). Alfalfa produced in the fall typically has smaller leaves, lower leaf percentage, and carbohydrates that are being rapidly metabolized due to higher temperatures (Ottman et al., 2014). These factors explain why fiber (NDF and ADF) values are higher and CP is lower in alfalfa during the fall. Both varieties of alfalfa in this study were rated as “premium quality” and the lignin content was considered low; however, we cannot be certain that late harvest date was not confounding when considering reduction in lignin content in increases in RFQ and NDFD. Another more reasonable explanation to describe the results of our study may be due to harvesting and feeding animals reduced-lignin alfalfa during the seeding year. Preliminary, unpublished research testing the forage quality (lignin, RFQ, and NDFD) of reduced-lignin alfalfa has found that quality is not improved during the seeding year. These results correspond with the results of our study. Research testing forage quality of reduced-lignin alfalfa post-seeding year is the next step in determining the efficacy of feeding reduced-lignin alfalfa to animals.

Percent leaf and leaf-to-stem ratio are reported in Table 2. Means did not differ by treatment for percent leaf ( $P = 0.06$ ) but did differ for leaf-to-stem ratio ( $P = 0.04$ ), with reduced-lignin alfalfa having a higher leaf-to-stem ratio than conventional alfalfa. Based on these results, we would assume that the reduced-lignin treatment would be higher in forage quality than the conventional treatment due to leaves being higher in quality than

stems. However, there were no differences in forage quality between treatments, which makes this phenomenon difficult to explain. Follow-up research is needed to address this finding.

### ***Feeding Trial***

Heifers in this study were fed at 3.40% BW (DM basis), permitting each heifer full access to feed. This protocol was implemented to ensure adequate intake, to warrant a practical feeding application, and to maximize use of limited resources.

Main effects of alfalfa treatment on BW, ADG, DMI, and G:F during the 84-day study period are presented in Tables 3 and 4. No treatment by day interactions were observed for any of the animal performance parameters ( $P \geq 0.05$ ). Animal BW did not differ ( $P = 0.35$ ) between treatments at d 0, 28, 56, or 84. ADG, DMI, and G:F did not differ ( $P = 0.32, 0.82, \text{ and } 0.40$ , respectively) between treatments for d 0-28, 29-54, or 55-84.

One noticeable observation during this study was the lower ADG and higher DMI during d 57-84 compared to the ADG and DMI for d 0-28 and d 29-56. Average daily gain and DMI values during this time may have been the result of less efficient use of feed associated with larger animals as they mature or cold stress associated with extreme temperatures. The heifers in this study were exposed to temperatures outside their thermoneutral zone for extended periods of time during d 57-84. The thermoneutral zone for healthy beef cattle is between 0° and 25° C. Cattle exposed to temperatures within this range do not have to expend energy to maintain normal body temperature. The low and

high temperatures for the month of January were  $-33^{\circ}$  and  $7.22^{\circ}$  C, respectively, with an average of  $-12^{\circ}$  C (Underground, 2016). Houseal and Olson (1995) concluded that animals exposed to lower critical temperatures ( $-13^{\circ}$  to  $-47^{\circ}$  C depending on size, sex, age plane of nutrition, and previous acclimatization) for prolonged periods of time have the potential to become cold stressed and must increase metabolic heat production to maintain homeostasis. Birkelo et al. (1991) found that cold increased requirements for weight maintenance or gain and that acute cold stress resulted in lower performance of feedlot animals during the colder parts of the year. It is quite possible that the animals in this study were cold stressed during the month of January and the beginning part of February due to prolonged cold temperatures, resulting in lower ADG.

### ***In Situ Digestibility***

*In situ* digestibility for each treatment is reported in Table 5. There were no treatment or treatment by time interactions ( $P = 0.26$  and  $0.77$ , respectively) for *in situ* digestibility (Figure 1). These results parallel the forage quality and feeding trial results discussed in the previous section.

## **IMPLICATIONS**

Differences between treatments in this study were not significant, however, the BW data are indicative of potential improved animal performance for animals being fed reduced-lignin alfalfa for prolonged periods. This study was preliminary and tested the

effects of feeding 100% reduced-lignin alfalfa harvested during the seeding year. More research is necessary to explore the effects of feeding reduced-lignin alfalfa to growing crossbred Angus heifers post-seeding year. Research is also needed to evaluate the effects of feeding reduced-lignin alfalfa in a restricted feed setting, along with similar research using cattle of various ages. Additional research is needed to further explore the quality and value of feeding reduced-lignin alfalfa on beef cattle performance and its potential impact on the U.S beef industry.

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Table 1. Forage quality parameters for reduced-lignin and conventional alfalfa grown in Montana

Item	Treatment		SEM	P-value
	Reduced-lignin	Conventional		
DM (%)	92.23	92.87	0.13	0.01
CP (%)	20.51	20.70	0.32	0.69
ADF (%)	24.17	25.61	0.54	0.11
NDF (%)	30.61	31.17	0.72	0.60
Lignin (%)	5.69	6.02	0.19	0.25
NEG (mcal/lb)	0.40	0.39	0.01	0.31
RFV <sup>1</sup>	213.71	207.43	5.82	0.47
RFQ <sup>2</sup>	218	212	6.31	0.53
TDN (%)	64.67	63.96	0.33	0.18
NDFD48 <sup>3</sup> (%)	47.44	46.49	0.72	0.39

<sup>1</sup>RFV: relative feed value; <sup>2</sup>RFQ: relative forage quality; <sup>3</sup>NDFD48: neutral detergent fiber digestibility at 48 hours

Table 2. Percent leaf and leaf-to-stem ratio of reduced-lignin and conventional alfalfa hay

<b>Item</b>	<b>Treatment</b>		<b><i>P</i>-value</b>
	<b>Reduced-lignin</b>	<b>Conventional</b>	
<b>% Leaf</b>	0.62	0.53	0.06
<b>Leaf: stem</b>	1.67 <sup>a</sup>	1.17 <sup>b</sup>	0.04

<sup>ab</sup> Within a row, means without a common superscript differ ( $P \leq 0.05$ )

Table 3. LS means and standard errors for BW across dates and treatments for crossbred Angus heifers being fed two varieties of alfalfa

<b>BW (kg)</b>				
<b>Day</b>	<b>R</b>	<b>C</b>	<b>SEM</b>	<b>P-value</b>
<b>0</b>	270	270	11.82	<i>0.96</i>
<b>28</b>	302	300	11.82	<i>0.68</i>
<b>56</b>	338	334	11.82	<i>0.48</i>
<b>84</b>	367	363	11.82	<i>0.43</i>
<b>Avg</b>	319	317	4.18	<i>0.93</i>

R: reduced-lignin alfalfa; C: conventional alfalfa

Table 4. LS means and standard errors for ADG, DMI, and G:F across dates and treatments for crossbred Angus heifers being fed two varieties of alfalfa

Day	Trait											
	ADG (kg)				DMI (kg)				G:F			
	R	C	SEM	<i>P</i> -value	R	C	SEM	<i>P</i> -value	R	C	SEM	<i>P</i> -value
<b>0-28</b>	1.14	1.06	0.20	<i>0.34</i>	8.64	8.66	1.66	<i>0.97</i>	0.13	0.12	0.01	<i>0.24</i>
<b>28-56</b>	1.29	1.24	0.20	<i>0.54</i>	10.47	10.04	1.66	<i>0.57</i>	0.13	0.12	0.01	<i>0.88</i>
<b>56-84</b>	1.04	1.02	0.20	<i>0.87</i>	11.26	11.19	1.66	<i>0.92</i>	0.10	0.10	0.01	<i>0.73</i>
<b>Avg</b>	1.16	1.10	0.08	<i>0.85</i>	10.12	9.96	1.07	<i>0.69</i>	0.12	0.11	0.005	<i>0.71</i>

R: reduced-lignin alfalfa; C: conventional alfalfa

Table 5. LS means and standard errors for *In situ* digestibility of reduced-lignin and conventional alfalfa hay grown in Montana

Time	Digestibility (%)		SEM	P-value
	Reduced-lignin	Conventional		
6	29.5	23.4	4.32	0.16
12	38.3	41.4	4.17	0.45
24	49.3	46.1	4.17	0.44
30	53.4	50.5	4.17	0.49
48	52.6	54.2	4.17	0.70
96	58.2	55.6	4.17	0.53
240	64.9	62.3	4.32	0.54

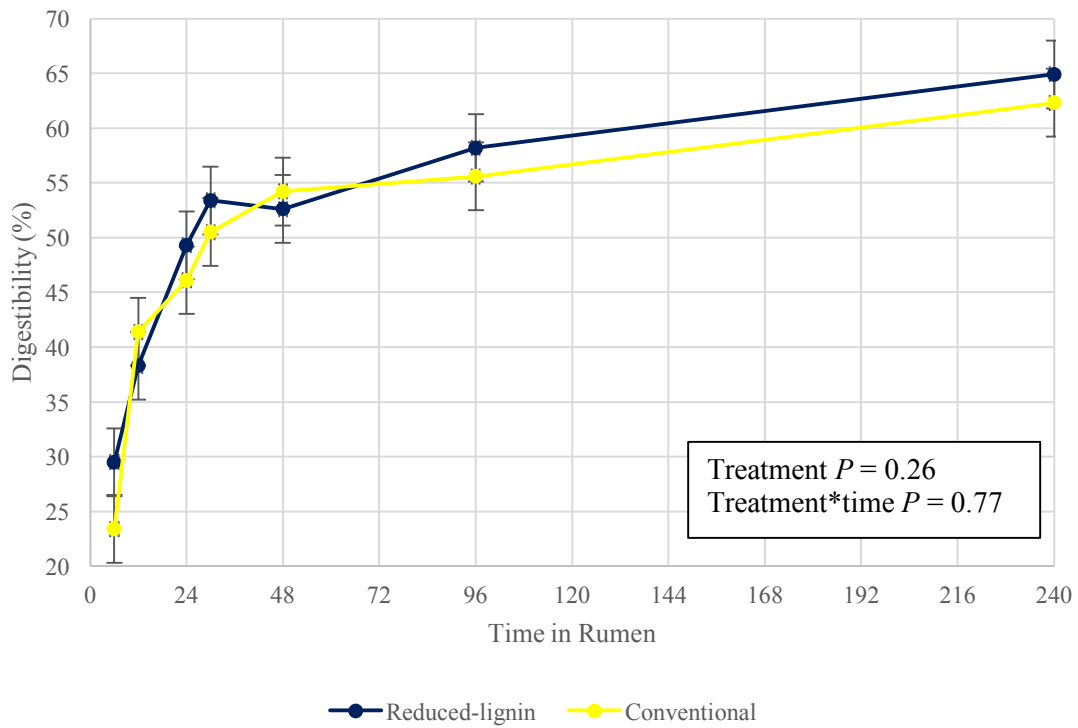


Figure 1. *In situ* digestibility at 6, 12, 24, 30, 48, 96, and 240 hours for reduced-lignin and conventional alfalfa hay grown in Montana

CHAPTER FOUR

DRY MATTER AND FORAGE QUALITY LOSSES ASSOCIATED WITH STORING  
LARGE ROUND BALES OUTSIDE AT VARYING GEOGRAPHIC LOCATIONS IN  
THE STATE OF MONTANA

Contribution of Authors and Co-Authors

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Contributions: Main author and lead scientist responsible for data collection, data analysis and interpretation, and drafting of this thesis.

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Contributions: Aided in experimental design and revisions.

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**ABSTRACT**

Hay storage method is highly variable and differs by geographic location, however, it is known that hay stored in Montana is commonly left uncovered and exposed to the elements. Research testing dry matter (DM) and forage quality losses associated with different outdoor hay storage methods and differences in quality based on bale placement within hay stacks in Montana is lacking. Therefore, a study was designed with a specific objective to quantify DM and forage quality losses associated with three different methods of outdoor round bale hay storage at two different sites in Montana, the Bozeman Agricultural Research and Teaching Farm (BART) and the Northern Agricultural Research Center (NARC). The goal of this research was to determine which hay storage method maintained the most DM and forage quality in varying Montana conditions. Large round bales consisting of 100% grass hay, baled at similar moisture levels, and wrapped in plastic net wrapping were placed into one of four storage systems at both BART and NARC. The four storage systems were: single-stack (SS), pyramid (PYR), mushroom (MSH), and inside stored bales (INSIDE). Results, which were considered significant if  $P \leq 0.10$ , indicated that DM and forage quality losses differed based on geographic location. Percent change for initial compared to final forage quality did not differ at either of the research locations for any of the quality parameters, except for NDF ( $P = 0.03$ ) and TDN ( $P = 0.08$ ) in the MSH at NARC. Results of this study suggest that bale placement is more important for changes in forage quality than treatment itself. Differences in forage quality and bale weight change were observed for

inner versus outer bales stored as a SS and as a PYR at BART. Differences in forage quality were observed for top versus bottom bales stored as a MSH at NARC.

**Key words:** large round bales, hay storage, DM loss, quality loss, Montana

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## INTRODUCTION

Hay is commonly used as a feed source for animals confined to pens, when forage quality fails to meet animal nutrient requirements, and during the winter months, when animals cannot physically access forage. Under these circumstances, producers rely on hay as the primary feed source to maintain the energy and nutrient requirements of their animals. Adequate animal nutrition and performance are reliant on the quality of the forage consumed.

Previous literature confirms that method of hay storage and the time in which the hay is stored can have tremendous impacts on DM and quality losses (Atwal et al., 1984; Huhnke, 1988; Chung and Verma, 1991; Huhnke, 2003) and that declining quality associated with hay production and storage have major impacts on animal performance (Verma and Nelson, 1983). Researchers in South Dakota found that the single-stack (SS) formation was the best method of hay storage compared to individual stored bales and bales stored as a pyramid (PYR) formation (Chisholm et al., 1980). In this study, SS stored bales only lost 0.8% DM after 1 year of storage compared to 10.3% for bales

stored as a pyramid. Single-stack stored bales were also highest in CP (5.65%) and IVDMD (39%) when compared to bales stored as a PYR formation (CP: 5.41%; IVDMD: 32.2%) after storage. Although Chisholm et al. (1980) found bales stored as a SS to be the best storage method in South Dakota, their research did not compare SS storage to indoor stored bales. Researchers in Canada compared SS stored bales to indoor stored bales and found that bales stored uncovered as a SS lost 40% total DM after one year of storage due to oxidation, weathering, and molding compared to only 6% for indoor stored bales (Atwal et al., 1984). Bales in this study were significantly higher in ADF and significantly lower in digestible energy and DMI compared to hay stored under cover.

Bales stored as a PYR in South Dakota lost more DM and IVDMD (DM loss = 10.3%; initial IVDMD = 38% vs. final IVDMD = 32.2%) than bales stored as a SS (DM loss = 0.8%; initial IVDMD = 39.1% vs final IVDMD = 39%; Chisholm et al., 1980). Researchers in Canada reported 30% DM losses due to oxidation, weathering, and molding in bales stored as a PYR formation (Atwal et al., 1984). Bales in this study were significantly higher in ADF and significantly lower in digestible energy and DMI than barn stored hay after storage.

Stacking hay in a mushroom formation has recently become a popular hay storage technique. Unfortunately, literature pertaining to quality and DM losses associated with the mushroom stack is lacking. Research encompassing varying climatic and environmental conditions in Montana is necessary to determine the efficacy of this formation in Montana.

From a financial perspective, minimizing DM and forage quality losses during hay storage is critical, especially when considering producer profitability. Research regarding DM and quality losses associated with hay storage has been limited to the Eastern half of the United States and addresses covered hay storage methods without considering losses based on bale placement during storage. The literature suggests that hay storage method is highly variable and differs by geographic location, however, it is known that hay stored in Montana is commonly left uncovered and exposed to the elements. Our research will be one of the first in the Western United States to test DM and quality losses of three different outdoor hay storage methods and differences in quality based on bale placement within these hay stacks. This applied research is meant to provide practical information for Montana producers to initiate changes in their hay storage methods.

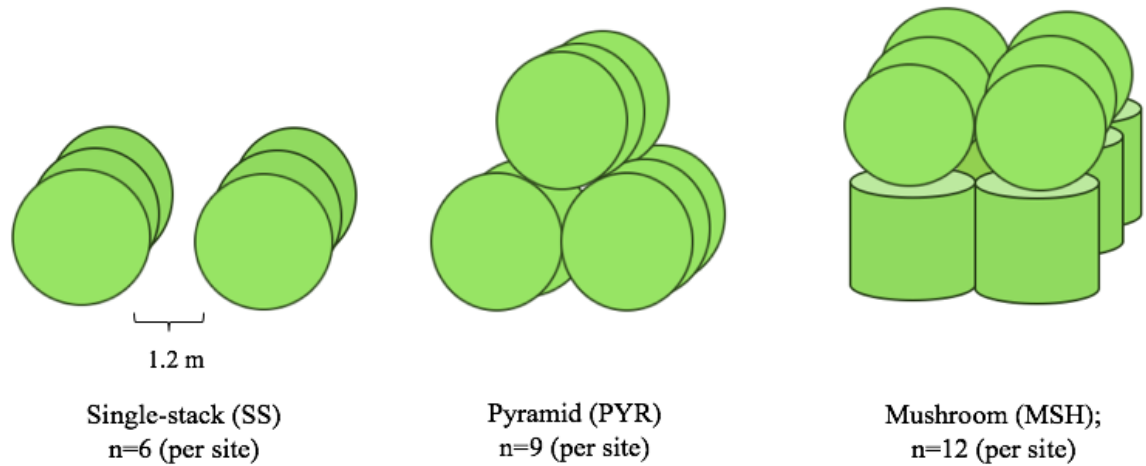
The objective of this research was to quantify DM and forage quality losses associated with three different methods of outdoor round bale hay storage at two different sites in Montana. The goal of this research was to determine which hay storage method maintained the most DM and forage quality in varying Montana conditions.

## **MATERIALS AND METHODS**

### ***Study Sites and Treatments***

Research was conducted at two locations in Montana: 1) the Bozeman Agricultural Research and Teaching Farm (BART Farm, Bozeman, MT) and 2) the

Northern Agricultural Research Center (NARC, Havre, MT). Bales at both sites consisted of 100% grass hay, baled at similar moisture levels (12-15%), and were wrapped using plastic net wrapping. Bales at BART were baled using a Vermeer 605M baler set at 454 kg. Prior to storage bales at BART were weighed 411 kg on average and measured 1.7 m in diameter and 2 m in length. Bales at NARC were baled using a Case IH RB565 set at 816 kg. Prior to storage bales at NARC weighed 644 kg on average and measured 1.7 m in diameter and 1.5 m in length. Bales at each location were weighed and immediately placed in their respective treatments 11 days after baling. Bales were assigned to the following three treatments at each location: 1) Single-stack (SS; n = 6) with two rows spaced 1.2 m apart, three bales per row, laid end-to-end 2) Pyramid (PYR; n = 9) with two bales on the bottom and one bale on top, laid three in a row 3) Mushroom (MSH; n = 12) with two rows of 6 bales, spaced 1.2 m apart, three in a row on the bottom, stacked on their ends, with one bale situated on top of each bottom bale (see Figure 1). Bales in each treatment were placed directly on the soil to mimic common Montana storage methods. The effects of weathering on each treatment were evaluated based on two control bales, stored inside, elevated off the ground, at each location. Precipitation was tracked using the U.S. Climate Data Service (U.S. Climate Data, 2017). Precipitation for 7 months of storage at NARC was 220 mm, which was almost twice the normal 30-year average for precipitation during this time (120 mm). Precipitation for 9 months of storage at BART was 230 mm, which was close to the normal average for precipitation during this time (200 mm).

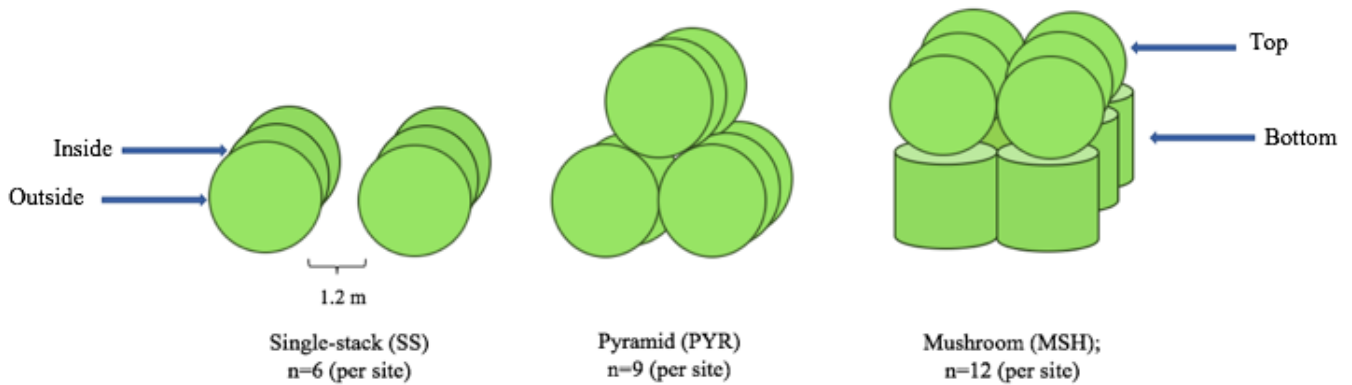
**Figure 1.** Large round bale storage treatments***Dry Matter Loss Evaluation***

Bale DM losses were quantified using the difference between initial bale weights, taken 11 days after baling, and final bale weights, taken on the last day of sampling in March. Bales were given a 10-day lag period in between baling and storing to reach their equilibrium temperature and moisture content. This 10-day process is described in detail by Gay et al. (2003) and Lemus (2009).

***Forage Quality Loss Evaluation***

Forage quality losses were determined using core samples taken with a Penn State electric drill mounted hay probe (Nasco Corporation, Modesto, CA). Comparisons were made based on bale placement within each formation (Figure 2). For the SS formation, bales situated in the inner part of the stack (SS.I) were compared to bales situated on the outside of the stack (SS.O). For PYR formation, bales situated in the inner part of the

stack (PYR.I) were compared to bales situated on the outside of the stack (PYR.O). For the MSH formation, the bales situated on the top of the stack (MSH.T) were compared to the bales situated on the bottom of the stack (MSH.B). Samples for each of these treatments were extracted from both a 15 and 30 cm depth, combined and averaged, and totaled four samples per treatment (two for 15 cm and two for 30 cm; n = 4 per treatment) per month during the entirety of the study. Samples from bales stored inside the hay shed (INSIDE) were extracted at both a 15 and 30 cm depth once during the first month of storage, and once during the last month of storage, due to minimal expected quality differences in these bales. All core samples in this study were stored in a cool, dry room at the BART nutrition center until completion of the study. At the completion of the study, all samples were ground in a Wiley mill to pass a 2-mm screen and sent to a commercial lab (Cumberland Valley Analytical Services, Hagerstown, MD) to be analyzed for DM (modified Goering and Van Soest, 1970), CP (AOAC, 2000 and FP528 analyzer, LECO Corporation, St. Joseph, MI), ADICP (AOAC, 2000 and FP528 analyzer, LECO Corporation, St. Joseph, MI), NDF (Van Soest et al., 1991), ADF (modified AOAC, 2000), relative feed value (RFV), relative forage quality (RFQ), TDN, and neutral detergent fiber digestibility at 48 hours (NDFD48; Goering and Van Soest, 1970).

**Figure 2.** Bale placement for large round bale storage

### *Statistical Analysis*

Data at each location were analyzed separately using SAS (version 9.4; SAS Inst. Inc., Cary, NC). Weight data were analyzed using the PROC GLM procedure, with bale as the experimental unit and treatment and bale placement as fixed effects. Initial and final quality data were analyzed using the PROC MIXED procedure using core sample as the experimental unit and treatment and date as fixed effects. Percent change comparing initial and final samples was analyzed using the PROC MIXED procedure with core sample as the experimental unit and treatment as a fixed effect. Quality data were analyzed over time using the PROC MIXED procedure with core sample as the experimental unit and treatment, date, and depth as fixed effects. Bale placement for each treatment over time was analyzed using the PROC MIXED procedure with core sample as the experimental unit and date and bale placement as fixed effects. All means in this study were separated using the LSMEANS procedure and considered significant at  $P \leq 0.10$ .

## RESULTS

### *Dry Matter Loss*

Dry matter losses for bales stored at both BART and NARC are reported in Table 1. Bale weight change at BART was lowest for bales stored INSIDE. These bales were stored under a hay shed and only weighed 3.8 kg more by the end of the study. These results correspond with the results of Atwal et al. (1984); Belyea et al. (1985); Huhnke (1988); Chung and Verma (1991); and Collins et al. (1997), who reported the lowest losses occurring during storage for INSIDE stored bales.

Bales stored at BART on the bottom of the mushroom formation (MSH.B) gained the most weight by the end of the study (27.6 kg). Weight gain for PYR.I was comparable to MSH.B, and gained 21.4 kg by the end of the study. Bales stored on the bottom of the MSH formation are positioned in a way so that the thatch layer is not in contact with the soil surface. Instead, the bale is positioned on its end. The thatched layer limits the amount of water that can enter and move toward the inside of the bale (Taylor et al., 1995). Our results suggest that bottom bales stored in the MSH formation retained more moisture due to bottom bales lacking a thatch, allowing excess moisture to penetrate the bale. Unpublished research discussed by Taylor et al. (1995) suggests that although less hay is exposed to the ground in bottom bales stored in the MSH formation, hay spoilage at the bottom of the bale is higher for this method. For the PYR formation, bales stored on the inner part of the stack most likely gained the most weight by the end of the study because they were surrounded by more bales and were unable to evaporate

moisture as effectively as the outside bales. Our results correspond with the results of Chisholm et al. (1980), who found that bales stored in the PYR formation held the most moisture and lost the most DM after one year of storage compared to bales stored individually on their side and in the SS formation.

Mushroom top, PYR.O, and SS.O did not differ from INSIDE stored bales and gained 5, 12, and 9 kg, respectively. These results suggest that bales stored on the top in the MSH formation and on the outside in the PYR and SS formation can evaporate enough moisture, as well as increase wind exposure, after precipitation events to maintain similar weight gains as INSIDE stored bales.

Interestingly, the previously mentioned studies reported seeing DM losses after storage, whereas all bales in all treatments in our study exhibited DM gains following storage. Differences in DM losses/gains in our study and other studies are most likely due to variation in the type of hay used for storage, moisture at baling, density of bales, type of wrapping, timing of storage, variation in storage location, and more importantly, environment. Published research regarding different outdoor hay storage methods in the state of Montana is lacking and a follow up study is necessary to verify these results.

Results for DM losses at NARC are reported in Table 1. Bales stored INSIDE gained 44 kg. MSH.B bales gained the least weight (7 kg) and INSIDE, MSH.T, PYR.I, PYR.O, SS.I, and SS.O did not differ from one another. These results are highly unlikely and are most likely due to sampling issues. Scale availability was limited and we were unable to use the same scale at the beginning and end of the study for bales stored at NARC. These results represent the importance of using the same equipment throughout

the entire study period. Due to the high chance that these results are inaccurate, these results will not be discussed any further.

### ***Forage Quality Loss***

Forage quality losses were examined at both a 15 and 30 cm depth for this study, however, differences in quality between treatments at these depths were non-existent. Based on previous research testing differences in quality at varying depths (Anderson et al., 1981; Harrigan and Rotz, 1994) and on the results of our research, it is concluded that sampling depth for the inner layer must exceed 30 cm to see quality differences between depths.

Forage quality losses for bales stored at BART are reported in Table 2. Treatment averages from the beginning of the study compared to the end differed ( $P \leq 0.09$ ) in DM CP, ADF, NDF, TDN, and RFQ. Although treatment differences for initial compared to final quality values were observed, percent change over time did not differ by treatment. These results suggest that overall quality change did not differ between treatments at BART. These results do not correspond with previous research that found the SS to have higher CP and digestibility than the PYR (Chisholm et al., 1980) and the SS and PYR to have higher ADF and lower digestible energy than inner stored bales (Atwal et al., 1984).

Although percent change in quality did not differ by treatment at BART, quality differences were observed in the SS and PYR formations for inner compared to outer stored bales. A treatment by date interaction ( $P = 0.0434$ ) was observed for bale

placement in the SS formation at BART due to an increase in DM for SS.O (92.3-93%) and a decrease in DM for SS.I (93.7-93.1%) from the start of the study to the end of the study (Figure 5). Treatment by date interactions for inner compared to outer bales were observed in the PYR stack at BART for ADF ( $P = 0.0878$ ; Figure 6), NDF ( $P = 0.0039$ ; Figure 7), and TDN ( $P = 0.0283$ ; Figure 8). Bales stored on the outside of the stack had higher ADF and NDF values and lower TDN than bales stored on the inner part of the stack by the completion of the study (ADF: 42.9 vs. 41.1%; NDF: 61.8 vs. 55%; TDN: 54.9 vs. 56%).

Forage quality losses for bales stored at NARC are reported in Table 3. Treatment averages from the beginning of the study compared to the end differed for CP ( $P = 0.0657$ ), ADF ( $P = 0.0003$ ), NDFD48 ( $P = 0.0398$ ), and RFQ ( $P = 0.0037$ ). Although treatment differences for initial compared to final quality values were observed in bales stored at NARC, percent change over time did not differ by treatment. These results suggest that overall quality did not differ between treatments for bales stored at NARC for those quality parameters.

Treatment by date interactions and treatment effects for percent change in quality for bales stored at NARC (Table 4; Figures 3 and 4) were observed for NDF ( $P = 0.0314$  and  $0.0327$ , respectively) and TDN ( $P = 0.0980$  and  $0.0751$ , respectively). Percent change for NDF and TDN was highest in bales stored in the MSH formation and was not different for INSIDE, PYR, or SS stored bales. Bales in the MSH had the highest NDF (61.5%) and lowest TDN (55.9%) compared to other treatments. Comparatively, bales on the INSIDE treatment had 58.7% NDF and 57.9% TDN.

Similar to bales stored at BART, major differences were observed for bales placed in different locations throughout the SS and MSH formations at NARC. A treatment by date interaction for CP was observed for inner and outer bales stored as a SS at NARC ( $P = 0.0006$ ; Figure 9). The large difference observed in CP between SS.I and SS.O bales occurred during the month of January (15.3% vs 12.6%, respectively) and was most likely due to sampling variability, as we do not expect there was an actual increase in CP for only a single month.

Some of the most apparent differences in quality based on bale placement in this study were observed in bales stored in the MSH formation at NARC. Treatment by date differences based on bale placement were observed in the MSH formation for DM ( $P = 0.0489$ ; Figure 10), ADICP ( $P = 0.0320$ ; Figure 11), ADF ( $P = 0.0232$ ; Figure 12), NDF ( $P = 0.0016$ ; Figure 13), NDFD48 ( $P = 0.0207$ ; Figure 14), TDN ( $P = 0.0015$ ; Figure 15), RFQ ( $P = 0.0314$ ; Figure 16). Mushroom top bales exhibited higher DM than MSH.B bales throughout the study and at the completion of the study (94.2 vs. 93.4% upon completion, respectively). Mushroom bottom bales were higher in ADICP, ADF, and NDF values than MSH.T bales (ADICP: 4 vs. 1.1%; ADF: 47.9 vs. 39.2%; NDF: 69.7 vs. 59.7%). Total digestible nutrients and RFQ were lower for MSH.B bales than MSH.T bales (TDN: 51 vs. 56.9% and RFQ: 83 vs. 140), however, NDFD48 did not differ due to bale placement upon completion of the study (40.2 vs. 39.4%).

## DISCUSSION

Overall quality of bales after storage varies based on geographic location, which may affect hay storage recommendations. Weight change and forage quality losses differed based on location in this study and the results did not correspond with the results of previous research testing hay storage in different parts of the United States. These differences are most likely due to environmental factors, such as precipitation, humidity, wind speed and direction, and sun exposure (Rotz and Chen, 1985).

Precipitation in Bozeman at BART during storage was close to the yearly average and overall hay quality did not differ by treatment. Bale placement within a storage system, rather than the storage system itself, seemed to be the most important factor when considering forage quality losses for hay stored in Bozeman. This was demonstrated by differences in DM for SS.I compared to SS.O and differences in ADF, NDF, and TDN for PYR.I and PYR.O at BART. The increase in initial to final DM observed for SS.O bales combined with lower weight gain during storage suggests that SS.O bales either hold less moisture during storage or are able to evaporate moisture more effectively than SS.I bales. Differences in ADF, NDF, and TDN observed for PYR.I and PYR.O may be due to the fact that PYR.O bales were more exposed to the elements than PYR.I bales. Bales exposed to elements of their environment are more susceptible to microbial respiration and losses of nonstructural carbohydrates (Harrigan and Rotz, 1994). The increase in fiber concentrations for the PYR.O bales in this study indicates increased respiration by microorganisms and increased leaching of soluble plant constituents by precipitation during storage for the outer bales (Harrigan and Rotz, 1994).

Precipitation in Havre at NARC during storage was almost twice the yearly average for precipitation at this site. Percent change for initial compared to final quality values did not differ by treatment during storage, except for NDF and TDN values in the MSH formation. High NDF and low TDN observed for the MSH formation at NARC was most likely due to differences based on bale placement. Mushroom bottom bales had the lowest DM, highest ADICP, ADF, and NDF and lowest TDN and RFQ. These results suggest that MSH.T bales either retain less water during storage or can evaporate water more effectively than MSH.B bales and that although quality values were lower for MSH.B bales, bale placement in the MSH formation did not affect digestibility. More research is warranted to determine the effect of bale placement on other animal related factors, such as intake. Based on these results, we conclude that storing bales in the MSH formation is not ideal for NARC conditions.

It is interesting that the MSH formation performed so poorly at NARC and was not different than other treatments at BART, as well as the fact that and that bale placement was only important for the SS and PYR formations at BART, while no differences were observed at NARC for these formations. These differences are difficult to explain but may be due to differences in initial bale weights and densities, along with differences in precipitation at each location. Upon completion of this study, it is apparent that indoor stored bales and bales stored as a SS formation maintain the most consistent DM and forge quality in Montana conditions. A follow up study designed using bales of similar weights and densities would be valuable to find more consistent results at each location.

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Table 1. Weight change (DM basis) for bales stored inside a barn (INSIDE), on the bottom of the mushroom formation (MSH.B), on the top of the mushroom formation (MSH.T), on the inside of the pyramid formation (PYR.I), on the outside of the pyramid formation (PYR.O), on the inside of the single-stack formation (SS.I), or on the outside of the single-stack formation (SS.O) in Bozeman, MT (BART) and Havre, MT (NARC)

<b>Bale Placement</b>	<b>Weight Change (kg)</b>	
	<b>BART</b>	<b>NARC</b>
<b>INSIDE</b>	3.8 <sup>d</sup>	43.9 <sup>a</sup>
<b>MSH.B</b>	27.6 <sup>a</sup>	7.3 <sup>b</sup>
<b>MSH.T</b>	5.0 <sup>d</sup>	21.2 <sup>ab</sup>
<b>PYR.I</b>	21.3 <sup>ab</sup>	25.3 <sup>ab</sup>
<b>PYR.O</b>	12.2 <sup>bcd</sup>	14.6 <sup>ab</sup>
<b>SS.I</b>	14.1 <sup>bc</sup>	31.8 <sup>ab</sup>
<b>SS.O</b>	9.1 <sup>cd</sup>	13.2 <sup>ab</sup>

<sup>a,b,c,d</sup> Within a column, means without a common superscript differ ( $P \leq 0.10$ )

Table 2. Changes in quality for large round bales stored inside a hay barn (INSIDE), as a mushroom formation (MSH), as a pyramid formation (PYR), or as a single-stack formation (SS) in Bozeman, Montana for nine months

Quality	Date	Storage Method				P values	
		INSIDE	MSH	PYR	SS	Treatment Effect	% Change
DM (%)	Start	93.6	92.6	92.3	93.0		
	Finish	93.2	92.8	92.9	93.0		
	Average	93.4 <sup>a</sup>	92.7 <sup>b</sup>	92.6 <sup>b</sup>	93.0 <sup>ab</sup>	0.0344	
	% Change	-0.5	0.2	0.7	0.0		0.2966
CP (%)	Start	10.1	11.1	11.7	11.1		
	Finish	10.5	11.6	11.9	10.8		
	Average	10.3 <sup>b</sup>	11.4 <sup>ab</sup>	11.8 <sup>a</sup>	10.9 <sup>ab</sup>	0.0886	
	% Change	4.1	5.2	1.5	-2.1		0.7706
ADICP <sup>1</sup> (%)	Start	1.2	1.6	1.5	1.4		
	Finish	1.4	1.6	1.5	1.4		
	Average	1.3	1.6	1.5	1.4	0.1069	
	% Change	23.8	2.6	-0.6	-1.4		0.3428
ADF (%)	Start	43	43.5	41.7	42.9		
	Finish	41.7	43.9	42.9	42.5		
	Average	42.4 <sup>b</sup>	43.7 <sup>a</sup>	41.8 <sup>ab</sup>	42.7 <sup>ab</sup>	0.0591	
	% Change	-3.0	1.1	0.8	-0.8		0.8341
NDF (%)	Start	63.1	64.5	60.9	61.4		
	Finish	60.5	62.5	58.4	61.0		
	Average	61.8 <sup>ab</sup>	63.5 <sup>a</sup>	59.6 <sup>b</sup>	61.2 <sup>ab</sup>	0.0611	
	% Change	-4.1	-2.8	-4.2	-0.7		0.8861
NDFD48 <sup>2</sup> (%)	Start	38.1	39.0	36.9	36.3		
	Finish	37.3	37.7	35.8	37.8		
	Average	37.7	38.4	36.4	37.1	0.1394	
	% Change	-2.0	-3.3	-3.1	4.1		0.2279
TDN (%)	Start	54.7	54.5	55.2	55.4		
	Finish	55.5	53.7	55.4	55.1		
	Average	55.1 <sup>ab</sup>	54.1 <sup>b</sup>	55.3 <sup>a</sup>	55.2 <sup>a</sup>	0.078	
	% Change	1.5	-1.4	0.5	-0.4		0.7621
RFQ <sup>3</sup>	Start	123.0	119.5	126.3	120.8		
	Finish	129.0	116.8	135.3	127.3		
	Average	126.0 <sup>ab</sup>	118.1 <sup>b</sup>	130.5 <sup>a</sup>	124.0 <sup>ab</sup>	0.0677	
	% Change	5.1	-1.1	8.9	6.2		0.7783

<sup>a,b</sup> Within a row, means without a common superscript differ ( $P \leq 0.10$ )

<sup>1</sup> ADICP: acid detergent insoluble crude protein; <sup>2</sup> NDFD48: 48-hour neutral detergent fiber digestibility; <sup>3</sup> RFQ: relative forage quality

Table 3. Changes in quality for large round bales stored inside a hay barn (INSIDE), as a mushroom formation (MSH), as a pyramid formation (PYR), or as a single-stack formation (SS) in Havre, Montana for seven months

Quality	Date	Storage Method				P values	
		INSIDE	MSH	PYR	SS	Treatment Effect	% Change
DM (%)	Start	93.1	93	93.1	93.1	0.1421	0.2457
	Finish	94.2	93.8	94.4	93.9		
	Average	93.7	93.4	93.7	93.5		
	% Change	1.18	0.83	1.5	0.9		
CP (%)	Start	12.7	12.1	11.4	12.1	0.0657	0.4824
	Finish	11.6	12.1	11.4	12.2		
	Average	12.2 <sup>ab</sup>	12.1 <sup>a</sup>	11.4 <sup>b</sup>	12.1 <sup>a</sup>		
	% Change	-8.7	0.8	0.3	0.3		
ADICP <sup>1</sup> (%)	Start	1.0	1.0	1.0	0.8	0.1369	0.1895
	Finish	0.9	2.5	1.1	1.0		
	Average	1.0	1.8	1.0	0.9		
	% Change	-6.2	148.1	8.1	27.5		
ADF (%)	Start	38.7	38.8	39.2	34.1	0.0003	0.2783
	Finish	38.1	43.5	40.3	36.0		
	Average	38.4 <sup>a</sup>	41.2 <sup>a</sup>	39.7 <sup>a</sup>	35.0 <sup>b</sup>		
	% Change	-1.5	12.1	3.0	5.5		
NDFD48 <sup>2</sup> (%)	Start	41.4	38.9	39.8	35.9	0.0398	0.2304
	Finish	37.8	39.8	37.0	36.7		
	Average	39.6 <sup>a</sup>	39.4 <sup>a</sup>	38.4 <sup>ab</sup>	36.3 <sup>b</sup>		
	% Change	-8.7	2.4	-6.6	2.5		
RFQ <sup>3</sup>	Start	152.5	146.5	143.3	163.8	0.0037	0.1523
	Finish	147.0	111.5	136.0	156.8		
	Average	149.8 <sup>ab</sup>	129.0 <sup>c</sup>	139.6 <sup>bc</sup>	160.3 <sup>a</sup>		
	% Change	-3.6	-24.0	-5.1	-4.2		

<sup>a,b,c</sup> Within a row, means without a common superscript differ ( $P \leq 0.10$ )

<sup>1</sup> ADICP: acid detergent insoluble crude protein; <sup>2</sup> NDFD48: 48-hour neutral detergent fiber digestibility; <sup>3</sup> RFQ: relative forage quality

Table 4. Changes in quality for large round bales stored inside a hay barn (INSIDE), as a mushroom formation (MSH), as a pyramid formation (PYR), or as a single-stack formation (SS) in Havre, Montana for seven months

Quality	Date	Storage Method				P values	
		INSIDE	MSH	PYR	SS	Treatment *date	% Change
NDF (%)	Start	60.0 <sup>b</sup>	58.4 <sup>bc</sup>	59.6 <sup>b</sup>	53.9 <sup>d</sup>	<i>0.0314</i>	<i>0.0327</i>
	Finish	57.3 <sup>bcd</sup>	64.7 <sup>a</sup>	59.7 <sup>b</sup>	54.9 <sup>cd</sup>		
	Average	58.7	61.5	59.6	54.4		
	% Change	-4.5 <sup>a</sup>	10.8 <sup>b</sup>	0.4 <sup>a</sup>	2.0 <sup>a</sup>		
TDN (%)	Start	57.9 <sup>abc</sup>	57.8 <sup>bc</sup>	57.0 <sup>c</sup>	60.1 <sup>a</sup>	<i>0.098</i>	<i>0.0751</i>
	Finish	57.9 <sup>abc</sup>	54.0 <sup>d</sup>	56.3 <sup>c</sup>	59.5 <sup>ab</sup>		
	Average	57.9	55.9	57.0	59.8		
	% Change	0.0 <sup>a</sup>	-6.6 <sup>b</sup>	-1.3 <sup>a</sup>	-1.1 <sup>a</sup>		

<sup>a,b,c</sup> Within a row, means without a common superscript differ ( $P \leq 0.10$ )

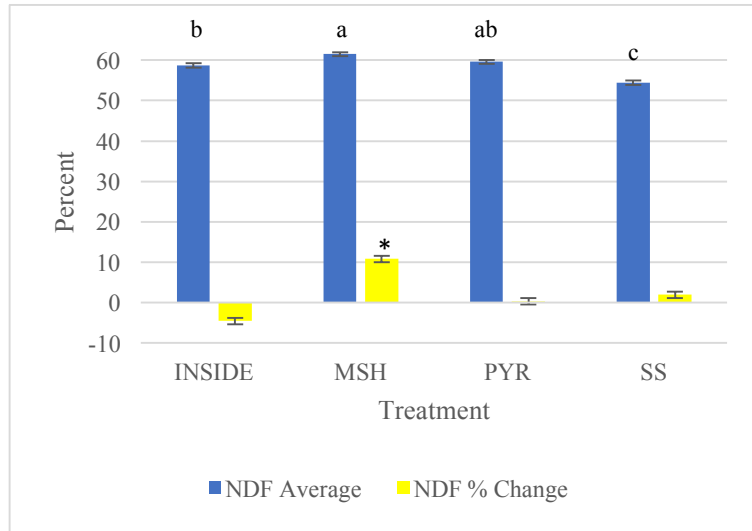


Figure 3. Treatment-average NDF and percent change over time for bales stored in Havre, MT at the Northern Agricultural Research Center (NARC)

<sup>a,b,c</sup> Means without a common superscript differ ( $P \leq 0.10$ )

\* Mean differs

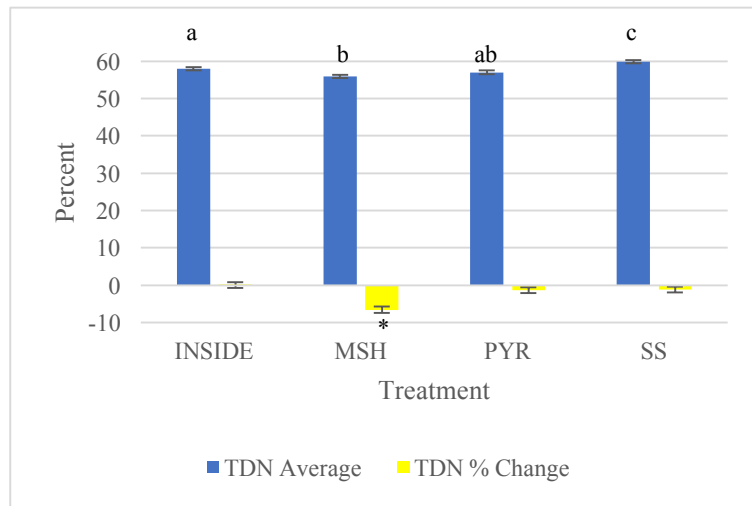


Figure 4. Treatment-average TDN and percent change over time for bales stored at the Northern Agricultural Research Center (NARC)

<sup>a,b,c</sup> Means without a common superscript differ ( $P \leq 0.10$ )

\* Mean differs

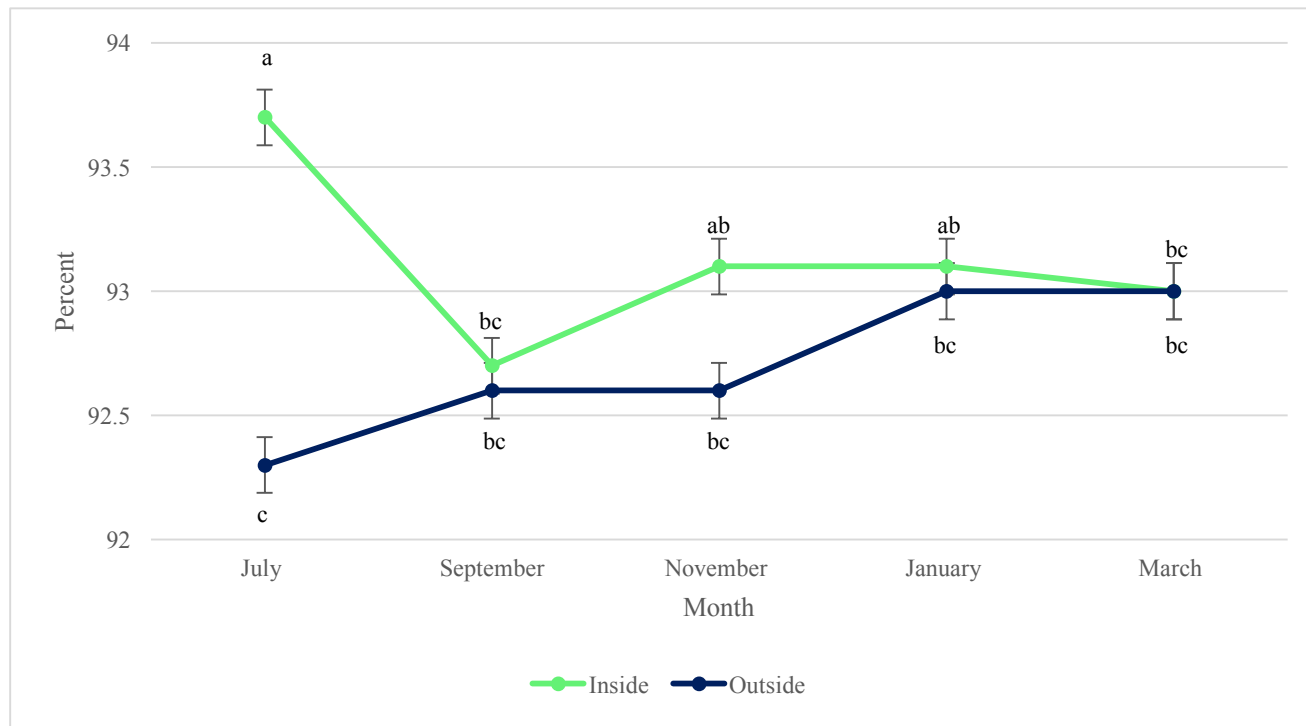


Figure 5. Treatment-average DM over time for inner and outer bales stored in the single-stack formation in Bozeman, Montana at the Bozeman Agriculture Research and Teaching Farm (BART) for nine months  
<sup>a,b,c</sup> Means without a common superscript differ ( $P \leq 0.10$ )

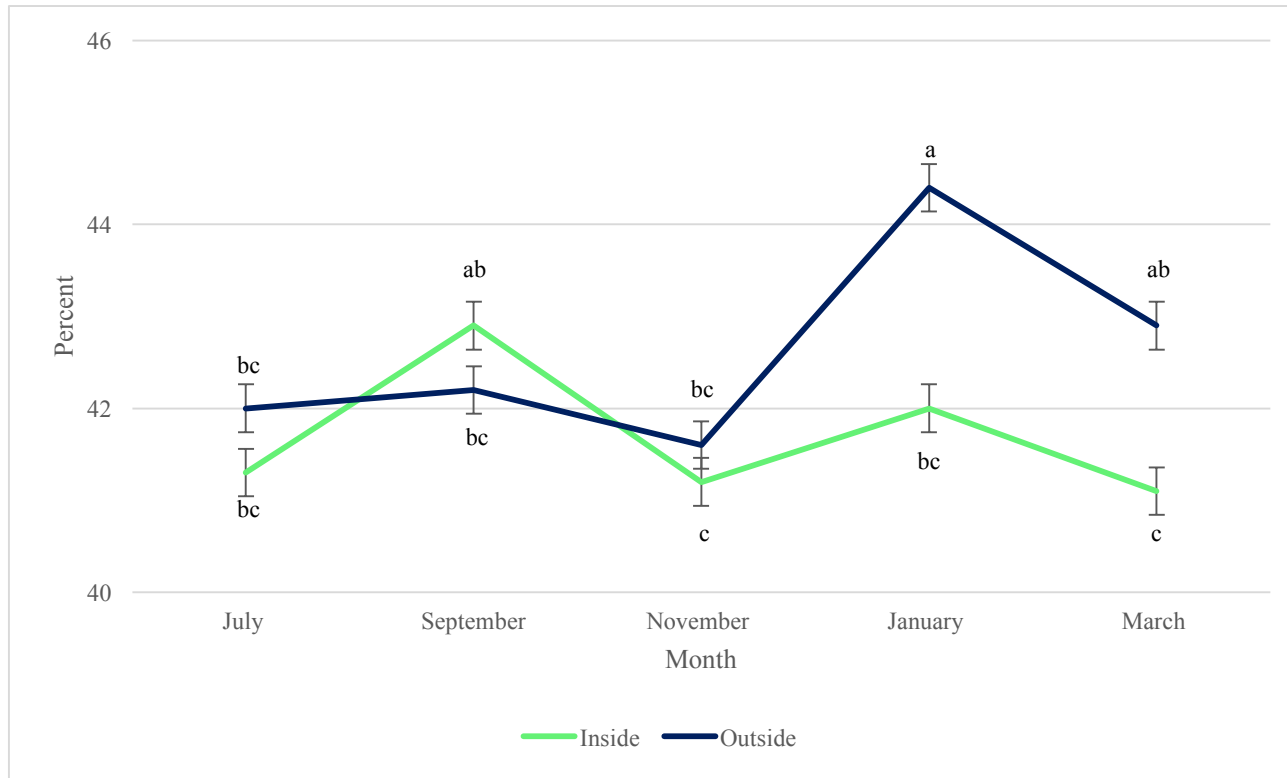


Figure 6. Treatment-average ADF over time for inner and outer bales stored in the pyramid formation in Bozeman, Montana at the Bozeman Agriculture Research and Teaching Farm (BART) for nine months  
<sup>a,b,c</sup> Means without a common superscript differ ( $P \leq 0.10$ )

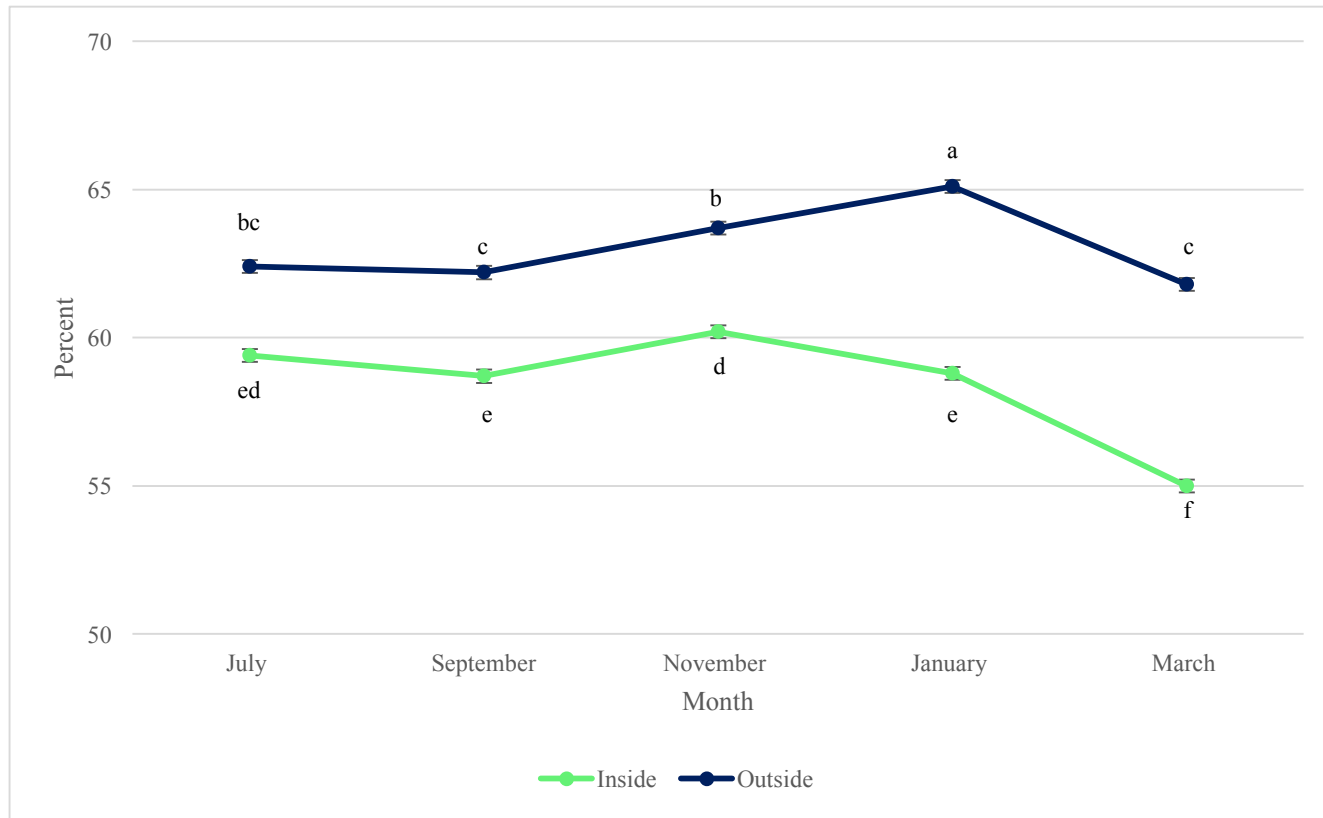


Figure 7. Treatment-average NDF over time for inner and outer bales stored in the pyramid formation in Bozeman, Montana at the Bozeman Agriculture Research and Teaching Farm (BART) for nine months  
<sup>a,b,c,d,e,f</sup> Means without a common superscript differ ( $P \leq 0.10$ )

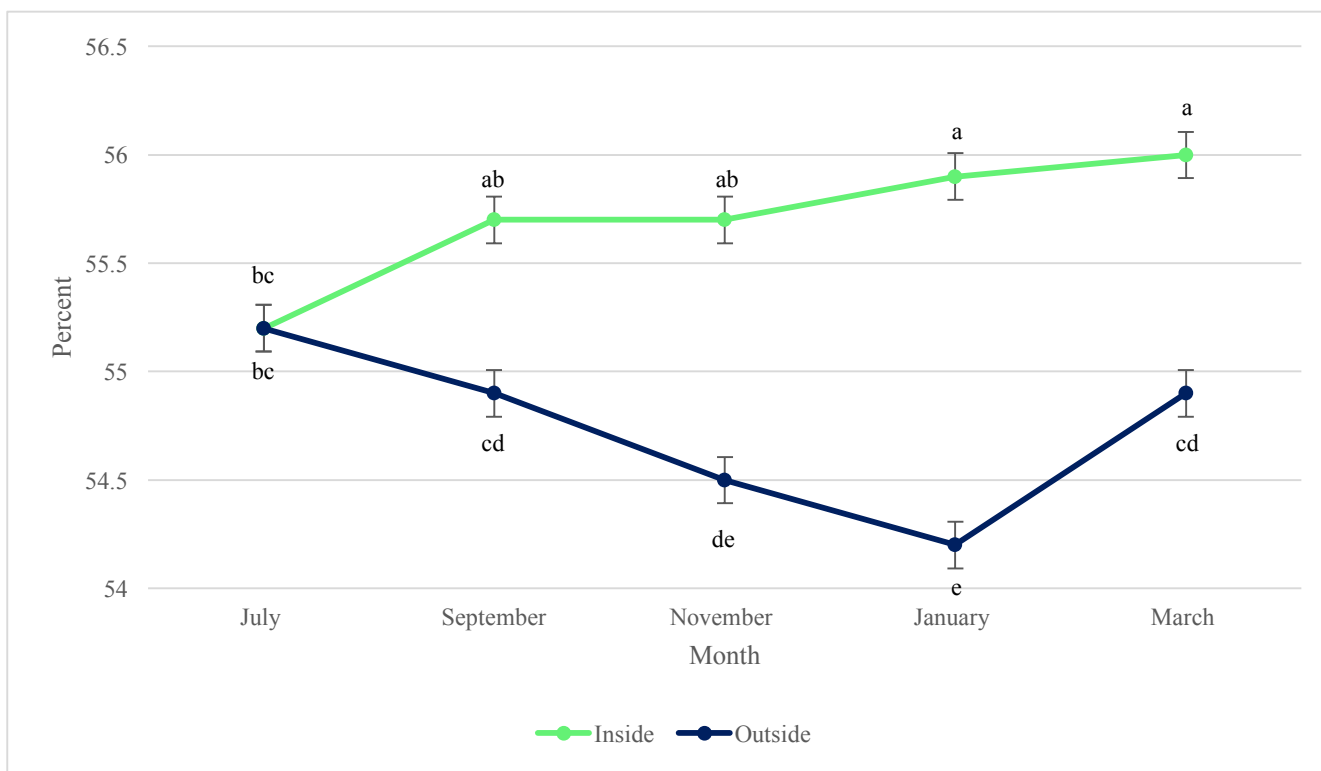


Figure 8. Treatment-average TDN over time for inner and outer bales stored in the pyramid formation in Bozeman, Montana at the Bozeman Agriculture Research and Teaching Farm (BART) for nine months  
<sup>a,b,c,d,e</sup> Means without a common superscript differ ( $P \leq 0.10$ )

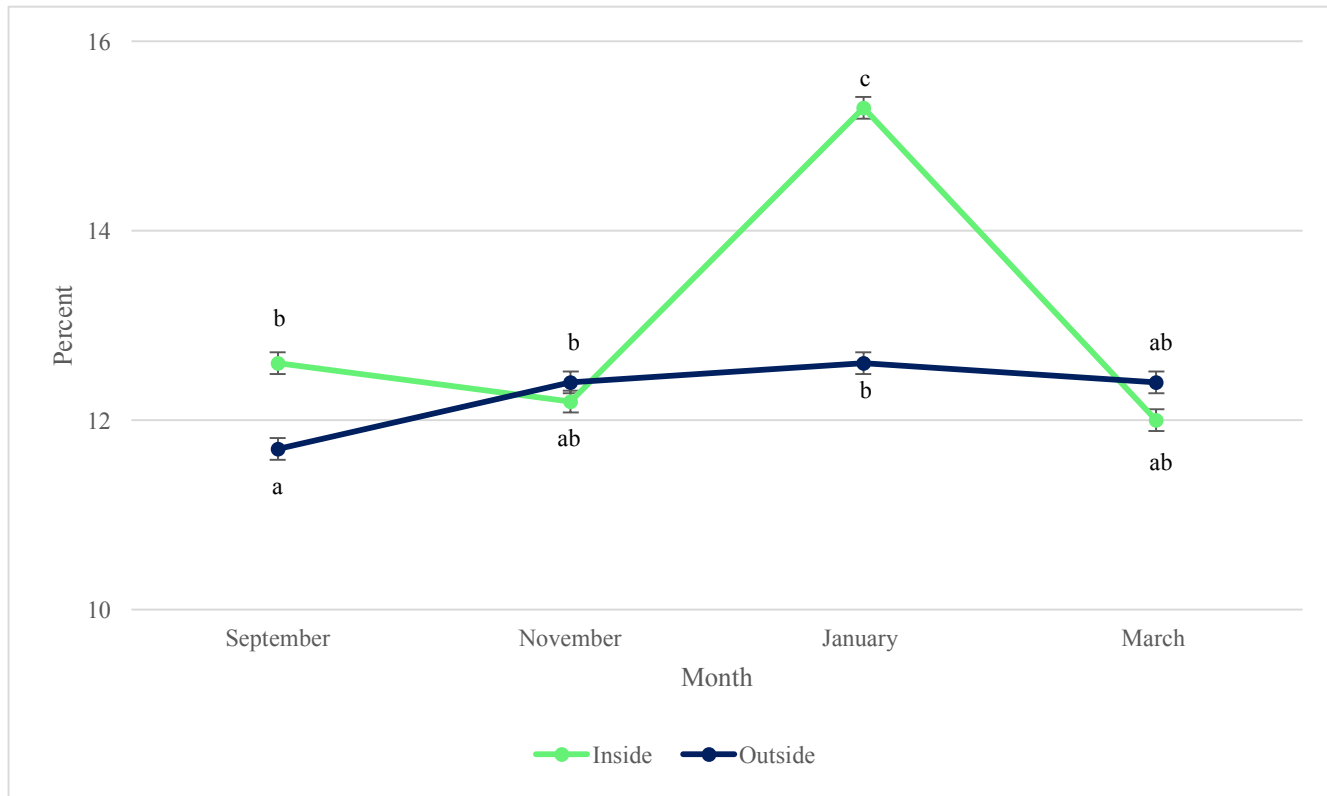


Figure 9. Treatment-average CP over time for inner and outer bales stored in the single-stack formation in Havre, Montana at the Northern Agricultural Research Center (NARC) for seven months  
<sup>a,b,c</sup> Means without a common superscript differ ( $P \leq 0.10$ )

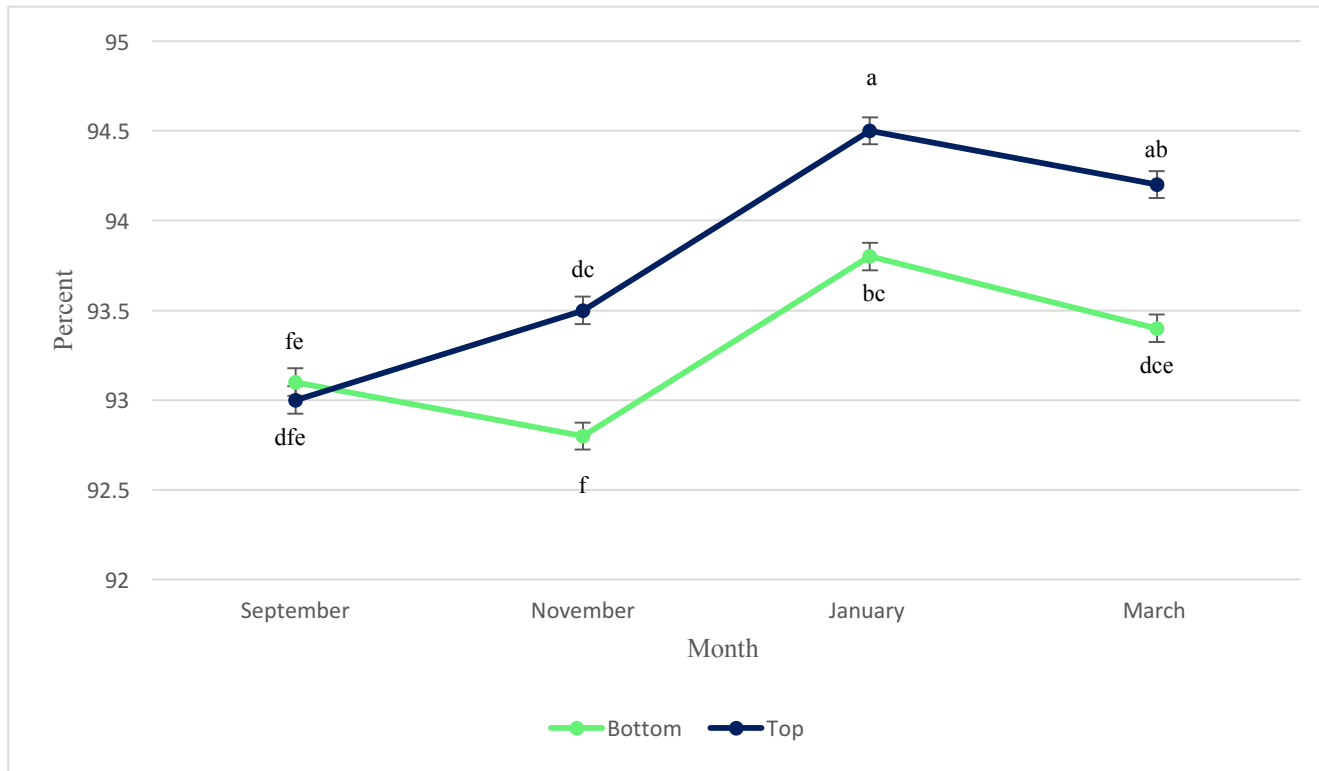


Figure 10. Treatment-average DM over time for bottom and top bales stored in the mushroom formation in Havre, Montana at the Northern Agricultural Research Center (NARC) for seven months  
<sup>a,b,c,d,e,f</sup> Means without a common superscript differ ( $P \leq 0.10$ )

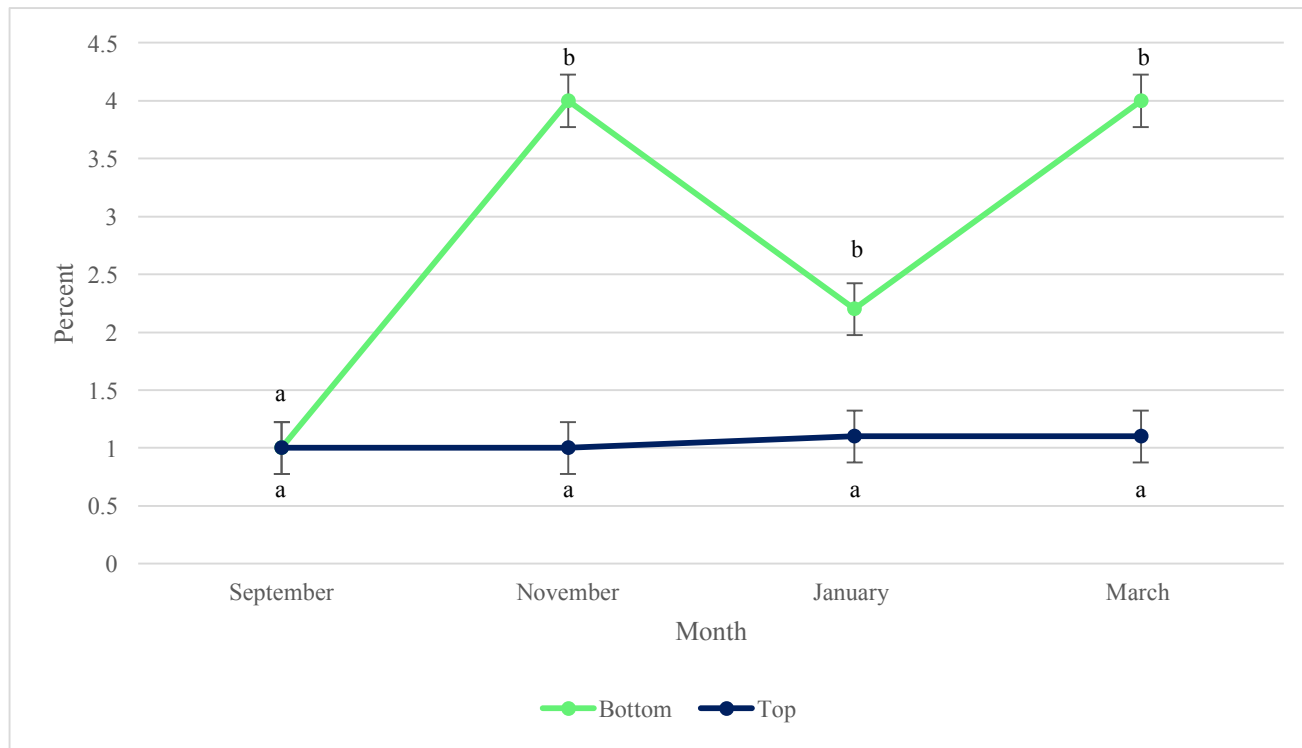


Figure 11. Treatment-average acid detergent insoluble crude protein (ADICP) over time for bottom and top bales stored in the mushroom formation in Havre, Montana at the Northern Agricultural Research Center (NARC) for seven months<sup>a,b</sup> Means without a common superscript differ ( $P \leq 0.10$ )

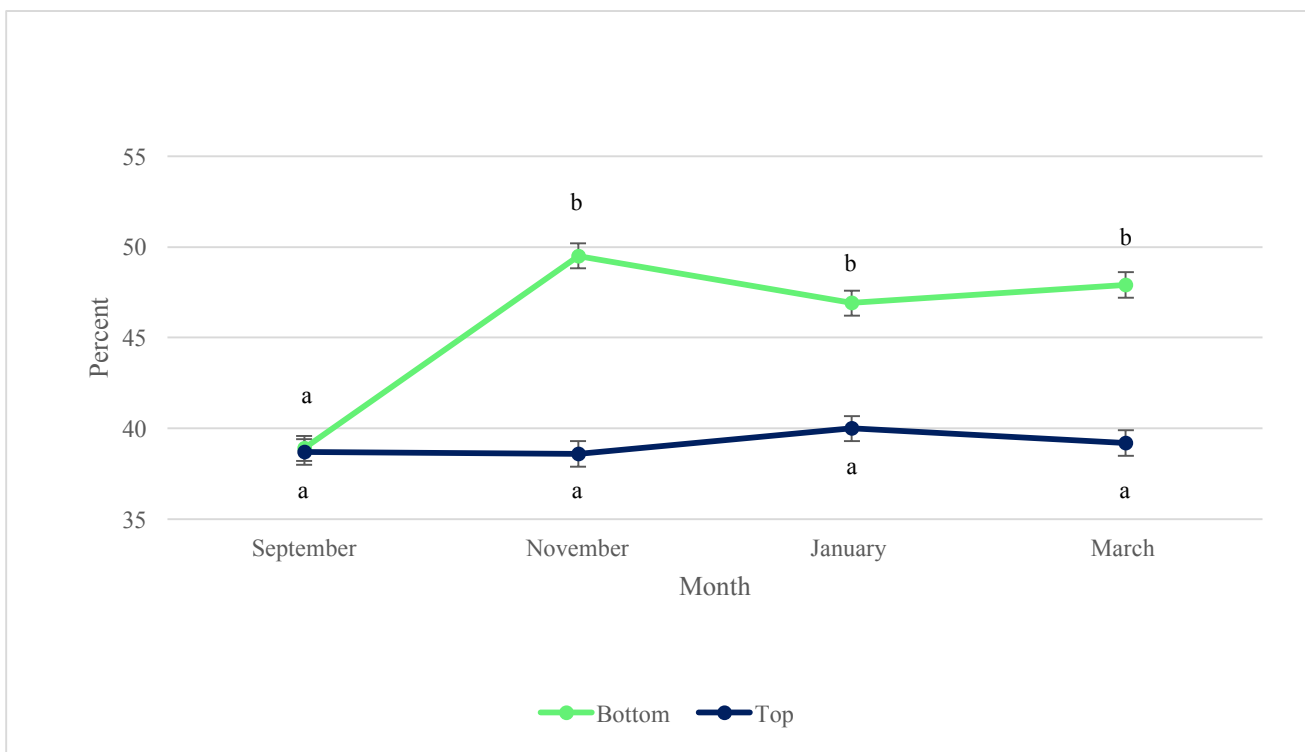


Figure 12. Treatment-average ADF over time for bottom and top bales stored in the mushroom formation in Havre, Montana at the Northern Agricultural Research Center (NARC) for seven months  
<sup>a,b</sup> Means without a common superscript differ ( $P \leq 0.10$ )

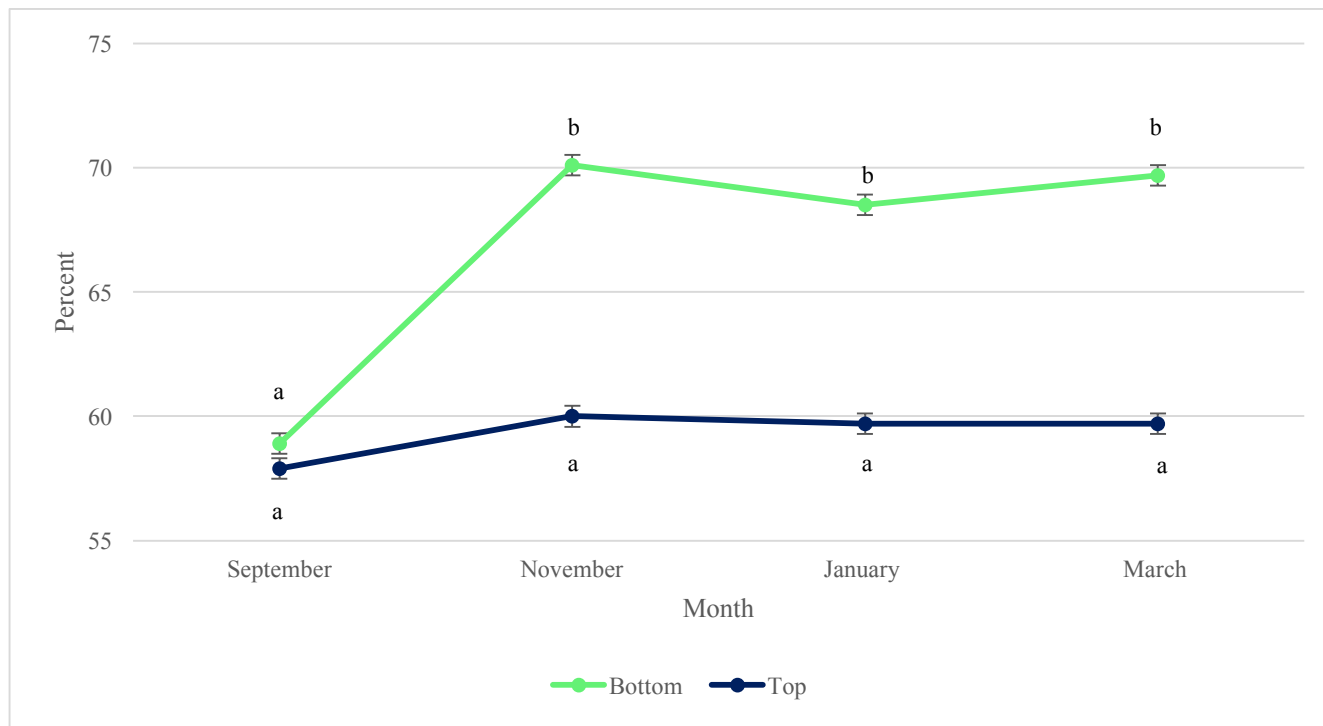


Figure 13. Treatment-average NDF over time for bottom and top bales stored in the mushroom formation in Havre, Montana at the Northern Agricultural Research Center (NARC) for seven months  
<sup>a,b</sup> Means without a common superscript differ ( $P \leq 0.10$ )

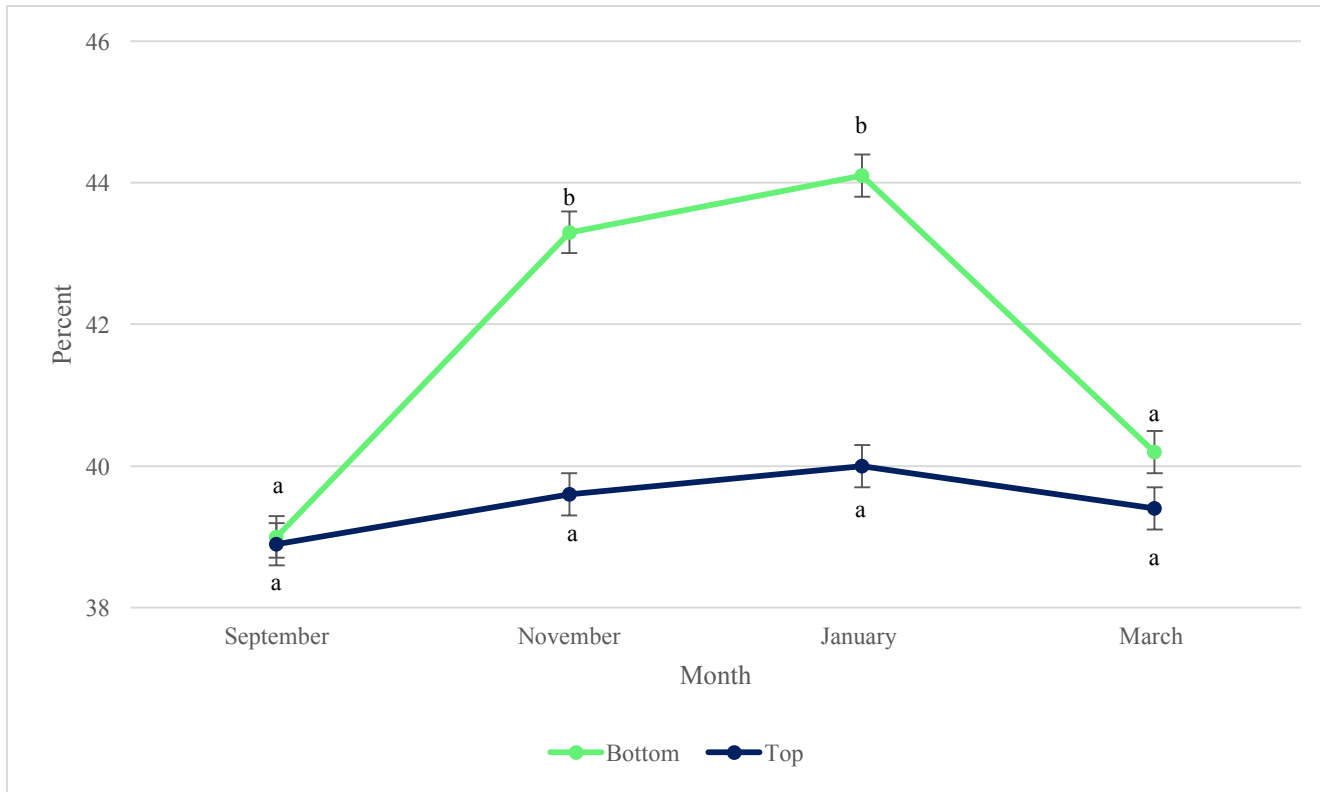


Figure 14. Treatment-average 48-hour neutral detergent fiber digestibility (NDFD48) over time for bottom and top bales stored in the mushroom formation in Havre, Montana at the Northern Agricultural Research Center (NARC) for seven months

<sup>a,b</sup> Means without a common superscript differ ( $P \leq 0.10$ )

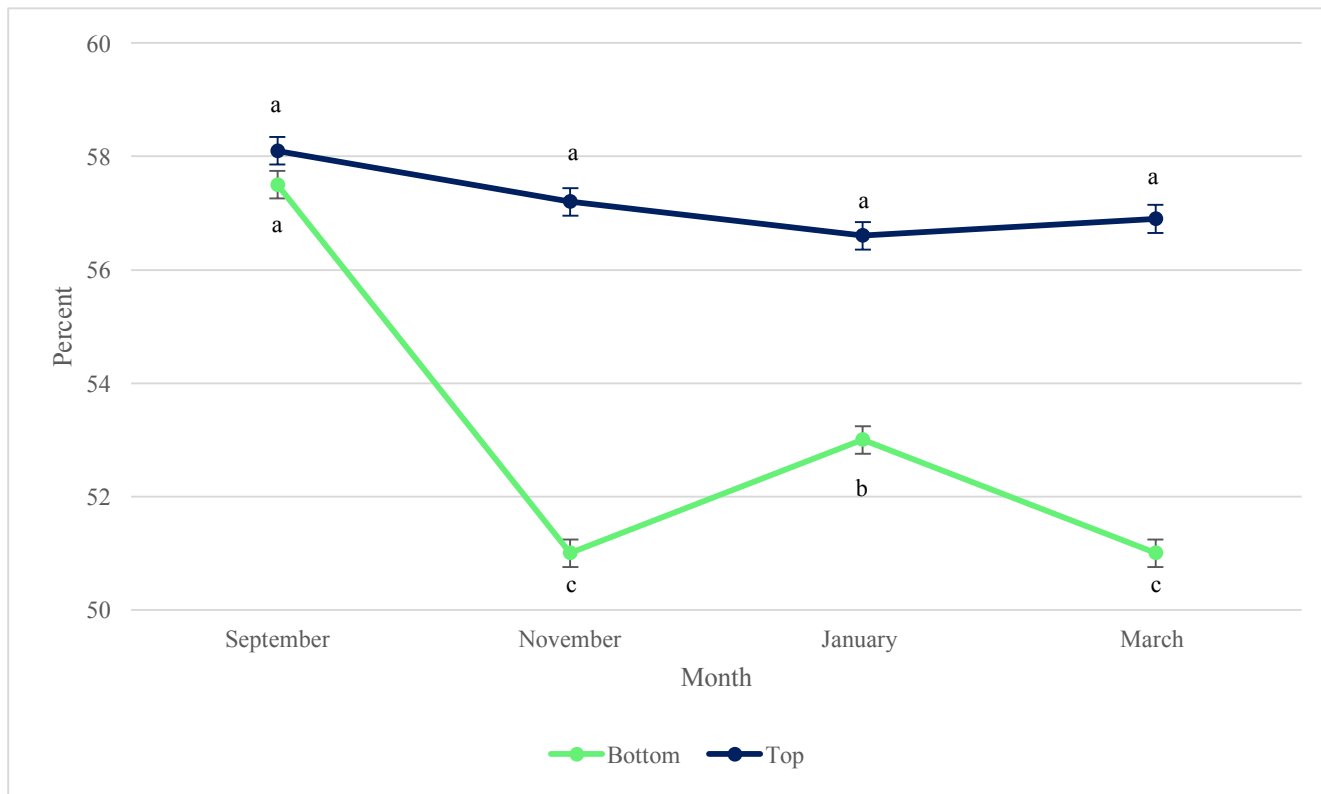


Figure 15. Treatment-average TDN over time for bottom and top bales stored in the mushroom formation in Havre, Montana at the Northern Agricultural Research Center (NARC) for seven months  
<sup>a,b,c</sup> Means without a common superscript differ ( $P \leq 0.10$ )

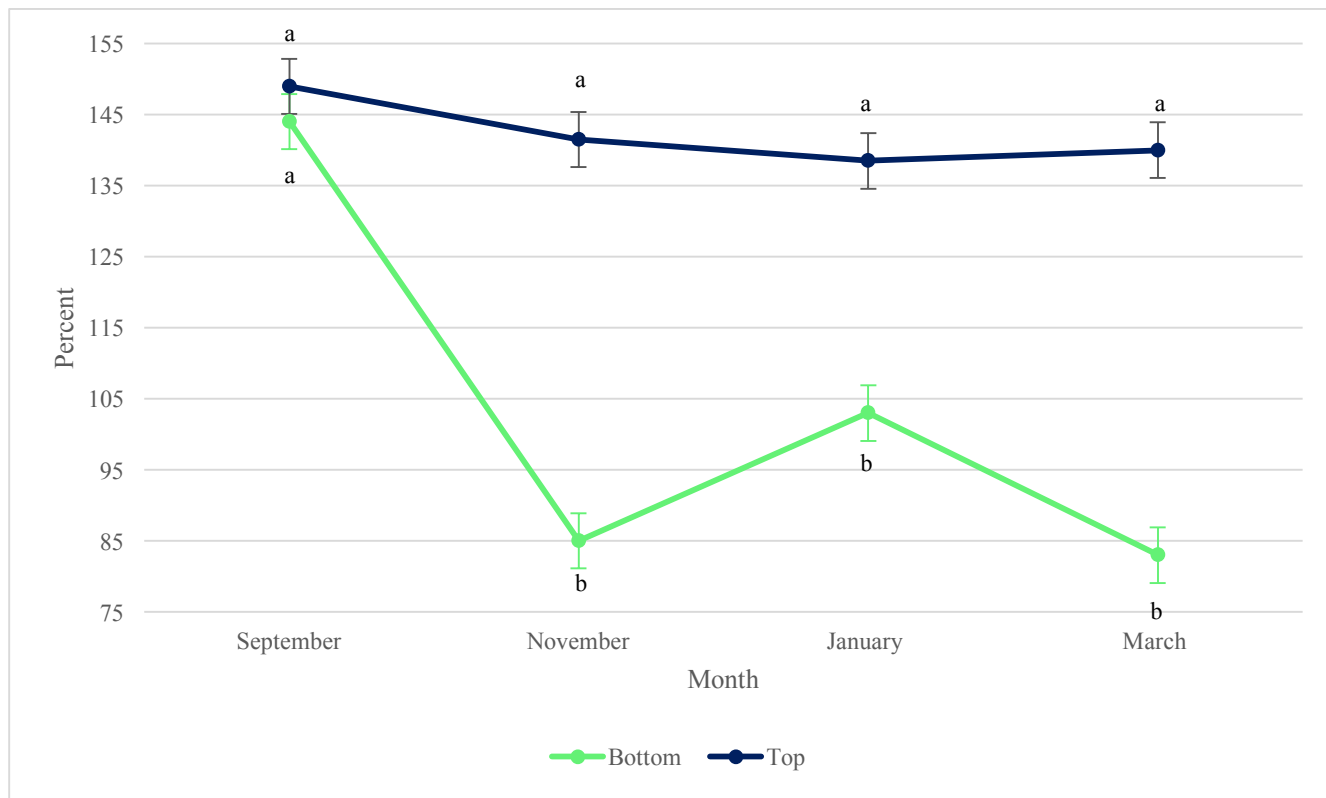


Figure 16. Treatment-average relative forage quality (RFQ) over time for bottom and top bales stored in the mushroom formation in Havre, Montana at the Northern Agricultural Research Center (NARC) for seven months  
<sup>a,b</sup> Means without a common superscript differ ( $P \leq 0.10$ )

## CHAPTER FIVE

## CONCLUSIONS

The results of utilizing genetically modified alfalfa and forage management techniques to improve forage quality and animal performance were not as expected. In the first study, the objective was to determine the differences in forage quality between reduced-lignin and conventional alfalfa and test these differences using animal performance measurements such as: BW, ADG, DMI, and G:F. There were no differences ( $P \geq 0.05$ ) in forage quality between treatments, except for DM ( $P = 0.01$ ). Means did not differ by treatment for percent leaf ( $P = 0.06$ ) but did differ for leaf-to-stem ratio ( $P = 0.04$ ). There were no treatment or treatment by day interactions ( $P \geq 0.05$ ) for BW, ADG, DMI, or G:F. There were no treatment or treatment by time interactions ( $P \geq 0.05$ ) for *in situ* digestibility. Overall, the results of this study suggested no difference in forage quality between reduced-lignin and conventional alfalfa. Additionally, animal performance did not differ for crossbred Angus cattle consuming reduced-lignin or conventional alfalfa. Research exploring forage quality and animal performance for reduced-lignin alfalfa post-seeding is warranted to determine the value of this new technology in the U.S beef industry.

In the second study, the objective was to quantify DM and forage quality losses associated with three different methods of outdoor round bale hay storage at two different sites in Montana. The goal of this research was to determine which hay storage method maintained the most DM and forage quality in varying Montana conditions. Percent

change for initial compared to final forage quality did not differ at either of the research locations for any of the quality parameters, except for NDF ( $P = 0.03$ ) and TDN ( $P = 0.08$ ) in the MSH at the NARC. The results of this study suggest that bale placement is more important for changes in forage quality than treatment itself. Differences in forage quality and bale weight change were observed for inside versus outside bales stored as a SS and as a PYR at the BART farm. Differences in forage quality were observed for top versus bottom bales stored as a MSH at the NARC. This study suggested that DM and forage quality losses differ by location and that bale placement, rather than hay storage formation, is more important for changes in DM and quality for bales stored in Montana over the winter months.

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