APPLICATION OF FIBROLYTIC ENZYMES AND BACTERIAL INOCULANTS TO SORGHUM SILAGE AND SMALL-GRAIN HAY

A Dissertation

by

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ABSTRACT

Fibrolytic enzymes and microbial inoculants have potential to improve the value of feedstuff and feedstock. An experiment was conducted to determine the nutritive value, ensiling characteristics, and *in situ* disappearance kinetics of sorghum (S*orghum bicolor* L.) silages pretreated with fibrolytic enzyme (xylanase plus cellulase: **XC**) or microbial [Promote ASB (*Lactobacillus buchneri* and *L. plantarum*); **PRO**] inoculants. The greatest yield was for cultivar PS 747 and the least for MMR 381/73 (MMR). Neutral detergent fiber (**NDF**) concentration was least for XC treated silage, and acid detergent fiber (**ADF**) concentration was least for XC and PRO treated silage. *In vitro* true digestibility (**IVTD**) was greatest for PRO treated Dairy Master BMR (DBMR), whereas, acid detergent lignin was least for PRO treated DBMR. Aerobic stability was not improved by PRO, however, aerobic stability of XC treated MMR was 63 h greater than the control. Generally, the *in situ* disappearance kinetics were improved with the application of XC and PRO, and XC had the greatest effect on silage with greater NDF and ADF concentrations. A second experiment was conducted to determine if the same application rates of either inoculant would reduce the fiber fraction of two cultivars each of wheat (*Triticum aestivum* L.) or oat (*Avena sativa* L.) hays. Forage was harvested twice during the tillering stage (**H1**) and (**H2**) and a third after grain harvest (**H3**). The IVTD was greater for oat than wheat due to a lesser fiber fraction. Forage from H2 had lesser NDF and ADF and greater CP and IVTD concentrations. *In situ* DM, NDF, ADF, and ERD were greater for wheat and oat at tillering than stover and NDF and ERD were

greater for Harrison than Fannin at tillering. Treatment of oat or wheat hays with XC or PRO enhanced *in situ* disappearance kinetics. Both XC and PRO may be used to reduce the fiber fractions of sorghum silage and small-grain hay. Additionally, it appears the inoculant PRO can be used to improve fermentation characteristics of sorghum silage.

DEDICATION

I would like to dedicate this dissertation to my parents, John and Ella Thomas, for the sacrifices they have made to raise their children in a loving home. My father worked endlessly to provide for his family and still found time to teach his children how to work hard and have fun when the work was completed. My mother was always near when her children and husband needed her love and support. My mother taught me what it means to be a lady, whether I was covered in mud in the cow pens or in a beautiful prom dress she handmade. My father taught me my way around the woods and river. Both my parents instilled a love of agriculture in my heart which encouraged my career endeavors. Without my parents' faith in God and love for one another I would not have achieved this goal of receiving a Ph.D. in agronomy.

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NOMENCLATURE

A	Percentage of substrate washed out of the bag at 0 hour
ADF	Acid detergent fiber
ADG	Average daily gain
ADIN	Acid detergent insoluble nitrogen
ADL	Acid detergent lignin
B	Insoluble, potentially digestible fraction
Bee	Beeville, Texas
BMR	Brown midrib sorghum
BW	Body weight
C	Fraction not digested after 96 hours of incubation
CP	Crude protein
CFU	Colony forming units
CS	College Station, Texas
d	Day(s)
DBMR	Sorghum cultivar dairy master BMR
DM	Dry matter
ERD	Effective rumen degradability (extent of digestion)
g	Gram(s)
h	hour(s)

IVDMD	*In vitro* dry matter digestibility
IVTD	*In vitro* true digestibility
L	Discrete lag time in hours
LA	Lactic acid
LAB	Lactic acid bacteria
k_d	Fractional rate of digestion of B
k_p	Ruminal passage rate
min	minute(s)
MMR	Sorghum cultivar MMR 381/73
NH_3-N	Ammonia-nitrogen
NDF	Neutral detergent fiber
NDIN	Neutral detergent insoluble nitrogen
PPS	Photoperiod sensitive
PRO	Promote ASB bacterial inoculant
PS	Sorghum cultivar PS 747
$R(t)$	Total indigested residue at any time
RUP	Rumen-undegradable protein
Silo	Sorghum cultivar diary silo 700
t	Time incubated in the rumen in hours
TDN	Total digestible nutrients
VFA	Volatile fatty acids
WSC	Water soluble carbohydrate

XC	Xylanase plus cellulase

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CHAPTER I

INTRODUCTION

Forage production in the United States is a major source of feedstuff for livestock consumption and lignocellulosic feedstock for ethanol production. Throughout Texas and much of the southwestern United Stages sorghum *(Sorghum bicolor* L.*)* silage and cool-season forage hay such as oat (A*vena sativa* L.) and wheat (*Triticum aestivum* L.), are stored for feeder cattle and dairy cattle feed. Due to a growing biofuel industry there is an increase demand for crops such as sorghum silage and cool-season grass stover that can be harvested for use as lignocellulosic feedstock (Jessup, 2009). Two major limitations reduce the value of forage crops used for feedstuff and feedstock production. Primarily, value is decreased due to the strong linkage of lignin to cellulose and hemicellulose which limits the amount of soluble carbohydrates available for conversion to energy for feed and fuel (Eggeman and Elander, 2005; Han et al., 2007; Sipos et al., 2009). Value of stored forage is also inhibited because of loss of dry matter (**DM**) which results in lost biomass and reduced nutritive value (Muck, 1988; Wiselogel et al., 1996). Storage losses occur due to continued respiration post-harvest, nutrient leaching, and loss of leaf material (Rotz and Muck, 1994).

Application of fibrolytic enzymes (xylanase:cellulase; **XC**) or bacterial (Promote ASB [*Lactobacillus buchneri and L. plantarum*]; **PRO**) inoculants to sorghum silage and oat and wheat hay may reduce fiber fractions and improve forage conservation. Elwakeel et al. (2007) found *in vitro* dry matter digestibility (**IVDMD**) of four different

fibrous dairy feedstuffs were improved by addition of fibrolytic enzyme mixtures, which were composed of β-glucanase, xylanase, and cellulase. Improvements in feed nutrient utilization may also decrease nutrient excretion, decreasing the potential negative environmental impact of confined animal feeding operations (Hersom, 2008). Lactic acid bacteria (**LAB**) inoculant promotes the production of lactic acid (**LA**), which causes the pH of silage to decrease more rapidly thereby inhibiting aerobic spoilage (Kung and Charley, 2010). Both enzyme and microbial inoculants could serve as a pretreatment for feedstuff and feedstock production to improve their value.

For a lignocellulosic feedstock plant to produce 114 million L of ethanol in a year, 907- 953 Mg of forage sorghum would be required per day, or 340,194 dry Mg $year^{-1}$(Trostle, 2012). Approximately 18,546 ha of land would be required to produce enough sorghum feedstock to supply a 114 million L capacity ethanol plant (Dahlberg et al., 2011; Trostle, 2012). Improving fiber degradation, energy efficiency, and storage of sorghum silage and cool-season grasses will decrease the amount of forage production needed to produce a liter of ethanol or a kilogram of milk or meat. Therefore, a successful pretreatment inoculant for feedstock is necessary for economically viable ethanol production (Aden et al., 2002; Eggeman and Elander, 2005).

The hypothesis for these experiments was treatment of sorghum silage and small-grain hay with fibrolytic enzyme (xylanase plus cellulase: XC) or microbial (Promote ASB [*Lactobacillus buchneri* and *L. plantarum*]; PRO) inoculants would improve nutritive value, ensiling characteristics, and *in situ* disappearance kinetics.

CHAPTER II

LITERATURE REVIEW

Forage Dry Matter Conservation

Where forages do not grow year round, there is a need for the conservation of forage DM. The preservation of forage enables livestock producers to store them for feeding to ruminant livestock in times of shortage of forage for grazing. Stored forage also allows for an increased forage component in total mixed rations fed to dairy or feedlot cattle. Conserved forage is more expensive than grazed forage, but less expensive than other supplements, which may reduce the cost of purchased feeds for confined animal operations (Ball et al., 2007).

In addition, there is a need to store feedstock from harvest until use at lignocellulosic biofuel processing centers. Biofuel production is a continuous process despite limitations of forage growing and harvest season, therefore, forage must be conserved following harvest until it can be used at the processing center (Wiselogel et al., 1996).

Forages can be stored as silage, hay, or haylage and the basis for determining method of storage is species dependent (Ball et al., 2007). Forage conservation methods are dependent on the curing conditions. Hay curing can take two to three days of drying time, whereas, silage and haylage are stored the same day they are cut (Rotz and Muck, 1994). Corn (Zea *maize* L.) and some types of sorghum have thicker stems which inhibit field drying. Therefore, they are most commonly utilized to make silage which is approximately 70% moisture (Kung, 2000). Cool-season annuals and warm-season

perennials, such as oat or Bermudagrass (C*ynodon dactylon* L. Pers.), are often harvested for hay which is approximately 15% moisture (Rotz and Muck, 1994). A third conservation method is haylage which is baled and wrapped with plastic when it reaches approximately 50% moisture (Ball et al., 2007). Since haylage is stored at greater moisture concentration than hay, it requires less drying time. In climates where there are few consecutive days of rain free weather haylage maybe a more efficient conservation method than hay (Ball, 2007).

Ensiling Process

Ensiling is a method used to preserve and store forage crops after harvesting until they can be fed to livestock or processed for biofuel. After cutting and removal from the field, the crop is placed in an airtight container where aerobic bacteria utilize the oxygen within 4 to 6 hours and produce carbon dioxide and heat.

In the oxygen-deprived environment anaerobic microbes convert water-soluble carbohydrates (**WSC**) to volatile fatty acids (**VFA**) (Kung, 2000). The anaerobic environment is essential for stopping plant respiration, preventing aerobic microbial growth, and stimulating growth of lactic acid bacteria (**LAB**), which reduces the pH (Rotz and Muck, 1994). This low pH inhibits plant enzyme activity and prevents the growth of undesirable anaerobic microorganisms (Rotz and Muck, 1994). The ensiling process has four distinctive phases each dominated by different plant, microbial, and chemical processes. These phases in order are pre-seal, active fermentation, stable phase, and feedout (Rotz and Muck, 1994) (Figure 2.1). Each of these ensiling phases has different factor determining the amount of DM loss.

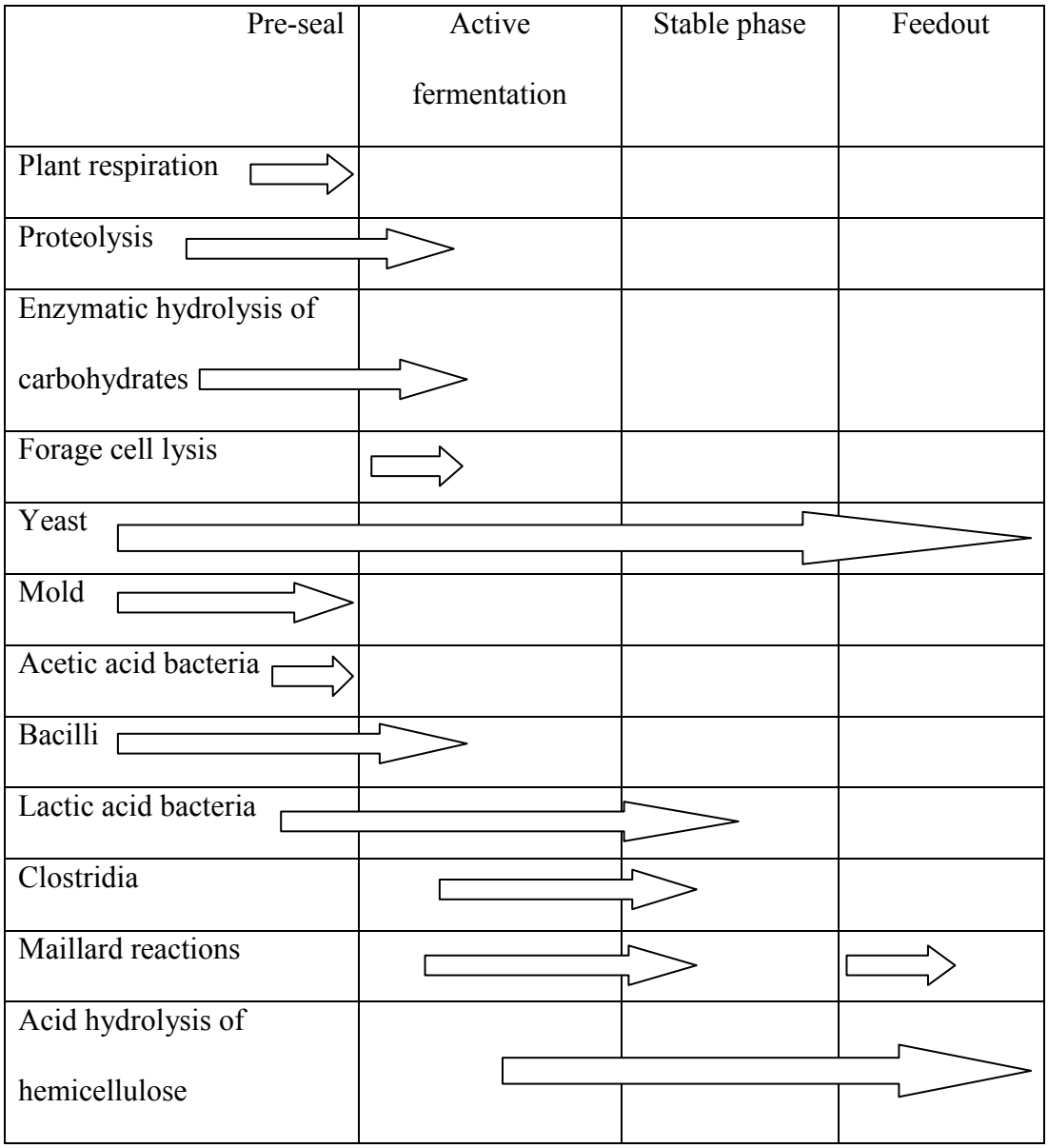

Figure 2.1 Four ensiling phases when various plant, microbial, and chemical processes are most active (Rotz and Muck, 1994).

During the pre-seal phase, plant material is still respiring, therefore, energy is metabolized to heat which raises the silage temperature (McDonald, 1981). This

respiration and temperature rise causes silage DM loss and affects other ensiling processes. If temperature becomes greater than 35°C a maillard reaction can occur causing amino acids and sugars to be polymerized increasing the acid detergent insoluble nitrogen (**ADIN**) content (Rotz and Muck, 1994).

Enzymes released due to cell lysis at harvest degrade protein to peptides and amino acids, and carbohydrates to sugars which provides substrate for aerobic microorganisms, yeasts, and molds during the pre-seal phase (Rotz and Muck, 1994). The growth of these microbes does not reduce silage quality unless approximately 10^8 colony forming units (CFU) of yeasts or 10^6 CFU of molds are reached (Pitt et al., 1991). If fermentation yeasts are great in number, their metabolism of sugars to ethanol cause DM loss (McDonald, 1981). The growth of certain molds can produce mycotoxins harmful to ruminant's health (Woolford, 1990).

The active fermentation phase and subsequent pH decrease are ideally activated by homofermentative LAB, which produce only lactic acid from the fermentation of glucose (Kung, 2000). Lactic acid is the most efficient VFA because it has a larger acid dissociation constant than other VFA (Kung, 2000). A common homofermentative LAB species, *Lactobacillus plantarum*, is known for its ability to grow at a low pH and to ensure a low final pH (Jones, 2012). Alternatively, heterofermentative LAB such as, *L. buchneri*, ferment glucose into lactic and acetic acids (Kung, 2000). The combination of lactic and acetic acid quickly decreases the pH of silage for preservation and stabilizes silage during aerobic exposure (Zhang et al., 2009).

Production of acetic acid is desirable because acid enhances aerobic stability by decreasing the growth rate of spoilage organisms, such as yeasts and molds (Danner et al., 2003). Aerobic stability, which is controlled by acetic acid is the time it takes silage to heat a couple of degrees after container opening and oxygen exposure (Jones, 2012). Fermentation of glucose to acetic acid instead of lactic acid is less efficient (Jones, 2012). The combination of lactic acid which decreases pH and acetic acid which inhibits yeasts and molds stabilizes silage during aerobic exposure (Jones, 2012).

During the production of acetic acid a third carbon is lost in the form of carbon dioxide which causes a loss of energy and production of a water molecule, thus the loss of forage DM (Jones, 2012). The ratio of lactic acid to acetic acid is used as an indicator of the effectiveness of fermentation and this ratio should not be less than 3:1 and a greater ratio is better (Kung et al., 2003). When lactate:acetate is less than 3:1 and homolactic acid bacterial numbers are low, a bacterial inoculant may be needed (Kung et al., 2003).

During the stable phase, anaerobic activity has ceased because of low pH and lack of substrate, however, if oxygen leaks into the silo microbial growth can occur. Oxygen infiltration during the stable phase causes loss of the most digestible components of the silage (Rotz and Muck, 1994). Overly wet silage causes DM losses during the active fermentation phase as effluent (highly digestible soluble carbohydrates and N fractions) is lost from the silo (Rotz and Muck, 1994). If the pH is not low enough (< 4.5) during the stable phase, anaerobic clostridia can produce butyric acid and

amines causing DM loss and reduce the palatability of silage (McDonald, 1981; Jones, 2012).

During the last phase, which is feedout, the silage face is penetrated with oxygen allowing aerobic microbial growth of spoilage microbes, such as yeasts and acetic acid bacteria, resulting in heating and DM loss (Courtin and Spoelstra, 1990; Woolford, 1990). If silage is not readily consumed after opening, bacilli and molds develop increasing DM losses and health concerns (Muck and Pitt, 1992).

Sorghum Silage for Livestock

Sorghum has two main classifications, grain and forage, and each has qualities relevant to specific uses. Grain sorghum is used for grain production in arid regions. Grain sorghum usually grows 0.91-1.5 m tall depending on the cultivar and climate conditions and is not usually grown for forage because it has a lesser DM yield than forage sorghum (Bolensen et al., 2003). Forage sorghums include sorgo or sweet sorghum, dual-purpose varieties, and hybrids. Forage sorghum usually grows 2.44- 3.96 m tall and is grown for silage production (Undersander et al., 1991). The average yield of forage sorghum silage produced in the Texas Panhandle was 43.71 Mg ha^{-1} at 68% moisture and a relative forage quality of 132 (suitable for dairy cows in the last 200 days of lactation, heifers, and stocker cattle; Undersander, 2003; Bean et al., 2009).

Sudangrass is grown for grazing, green chop, hay, or silage and grows 1.22-2.13 m tall (Undersander et al., 1991). The smaller stem of sudangrass gives it better drying characteristics than forage sorghum making it most feasible for hay production. When sudangrass is crossed with sorghum, the resulting hybrids are intermediate in size, yield

is generally less than forage sorghum but similar to or slightly greater than sudangrass making it a good crop for hay, haylage, green-chop and pasture (Undersander, 2012).

Among the forage types there are classifications such as photoperiod sensitive (**PPS**) and brown midrib (**BMR**). These traits have been selected for improved production and nutritive value. Varieties selected for the PPS trait are extremely photoperiod sensitive and do not initiate flowering until day length is less 12 hours and 20 minutes, which is late in the growing season (Morgan et al., 2002). This delay in flowering is critical because the transition from vegetative to reproductive growth in sorghum and sorghum-sudangrass hastens the decline in quality of the vegetative portion of the plant (McCollum et al., 2012). The PPS varieties yield well and utilize water efficiently, however, they have less digestibility than non-BMR and BMR varieties because of greater fiber concentration, which limits their broad application (McCollum et al., 2012). Murray et al. suggested yield traits be selected over composition traits for maximizing energy yield of sorghum biomass (2008). This suggestion from Murray et al. (2008) is similar to the conclusion by McCuistion et al. (2011) that PPS varieties fed more cattle than varieties with greater nutritive value.

In grazing trials PPS sorghum had lesser average daily gain (**ADG**) than BMR varieties (McCuistion et al., 2011). However, PPS supported more head of grazing cattle day^{-1} ha^{-1} than BMR varieties (McCuistion et al., 2011). Varieties selected for BMR traits have less acid detergent lignin (**ADL**) and may be 10 to 30% more digestible, however, yield is 15 to 20% less DM than conventional forage sorghum varieties and lodge more easily (Ball et al., 2007; Bean et al., 2009). Among BMR varieties there is a

great deal of variation in *in vitro* true digestibility (**IVTD**) and fiber digestibility (McCollum et al., 2012).

The production of sorghum silage is common in the southern United States because it is well adapted to the warm climate, produces a high yield, and is drought tolerant (Prostko et al., 1998). However, acceptance of sorghum silage for lactating dairy rations in the United States has been limited due to its greater acid detergent fiber (**ADF**) and ADL levels than corn grown for silage (Prostko et al., 1998). The greater fiber levels found in sorghum reduce forage digestibility and may compromise milk production (Prostko et al., 1998). In Nebraska, conventional and BMR sorghum silages had a pH of 4.0 and 4.1, crude protein (**CP**) of 7.3 and 7.8% of DM, neutral detergent fiber (**NDF**) of 48.2-58.1% of DM, and ADL of 2.3-2.9 % of DM, respectively (Oliver et al., 2004). The silage from BMR sorghum had greater nutritive value and ensiling characteristics than silage from the conventional hybrid, but lesser than corn silage (Oliver et al., 2004). Oliver et al. reported NDF intake of corn and conventional silage to be 9.0-10.4 kg/d, respectively and milk production was 33.8-31.0 kg/d, respectively (2004). The study by Oliver et al. and those of other research teams indicates silage from BMR sorghum supports milk production similar to corn silage (Lusk et al., 1984; Grant et al., 1995; Oliver, 2004). Bolsen et al. suggest grain sorghum silage can be substituted for corn silage in mid-lactation dairy cow diets (1989). Despite a slight reduction in milk yield, conventional forage sorghum silage is used in many tropical countries around the world because it may be planted later than corn, uses water more

efficiently, has greater yields and when exposed to drought still produces an acceptable silage yield (Sanderson et al., 1992).

Sorghum maturity is a critical factor in maximizing nutritive value, therefore, harvest should be performed at the correct stage of development (Table 2.1). Grain sorghum should be cut at late milk to late dough stage and forage sorghums should be 1.02 m tall or at late boot stage when forage is harvested (Ball et al., 2007). Achieving the greatest nutritive value and yield are competing production goals. When yield is maximized lodging increases, and management practices such as reduced seeding rate, proper application of nitrogen, and harvest of the crop as soon as it reaches the proper stage will minimize lodging (Bean et al., 2009). For proper fermentation, sorghum silage should be chopped to a length of approximately 0.95-1.27 cm and stored in an air-tight container and feeding should begin after three to five weeks of storage (Ball, et al., 2007).

Sorghum Silage as Lignocellulosic Feedstock

Currently, limitations in the production of lignocellulosic feedstock and its conversion into bioenergy are hindering progress and long-term sustainability of bioenergy manufacturing. Production of biofuel has been criticized since most of the biofuel is coming from first generation crops such as corn, sugar cane (*Saccharum officinarum* L.), soybean (*Glycine max* L.) and rapeseed (*Brassica napus* L.) which provide food and feed therefore their demand as fuel increases the costs of global food production (Jessup, 2009).

Table 2.1 Chemical composition of silage made from different types of sorghum and harvested at various maturity stages

		Yield, Mg/ha		Dry matter basis	
Crop	Maturity Stage	DM	35% DM	CP, %	CF[1], %
Grain	Dough	8.97	17	8	24
Forage	Dough	11.21	14	8	33
Forage	Early head	8.97	11	11	29
Sorghum	Dough	10.09	13	10	34
Sorghum	Early head	5.60	7	12	26

(S.J. Donohue et al. 2000)
[1] Crude Fiber

Thus, interest has switched to production of second generation, energy crops, which include lignocellulosic biofuels (Jessup, 2009). Lignocellulosic feedstock is plant material with a lesser water soluble carbohydrate concentration than grain sources. The fibrous components of lignocellulosic feedstock include a mixture of cellulose, hemicellulose, and lignin (Han et al., 2007). These structural polymers, including hemicellulose and lignin, surround the cellulose component of the plant cell wall and serve to protect the cells against enzymatic attack (Figure 2.2; Han et al., 2007). This protective barrier is a major impediment to bioconversion of lignocellulosic biomass because cellulose contains the fermentable sugars that are converted to ethanol (Han et al., 2007).

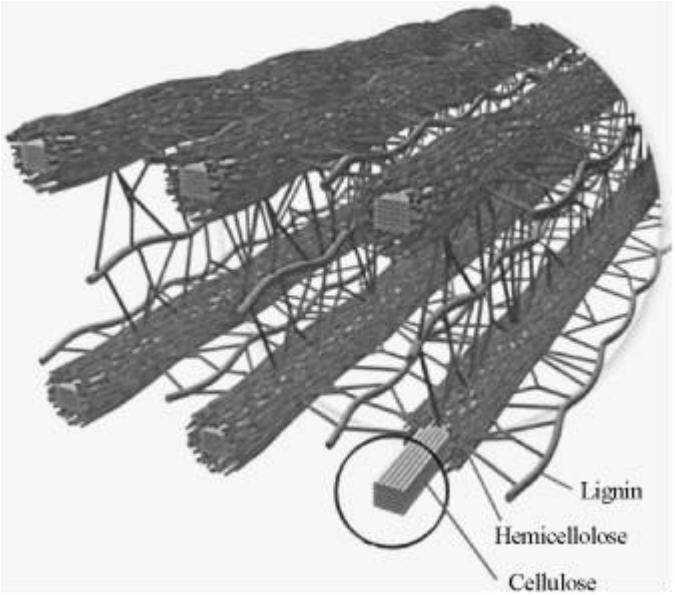

Figure 2.2 Cellulose strands surrounded by hemicellulose and lignin (DOE, 2007).

In the southern United States the production of lignocellulosic C_4 crops for biofuel is a promising opportunity. Production of these crops is common for use as livestock forage. Use of common forage species for bioenergy will support the United States federal mandate requiring production of 79.5 million liters of renewable fuel from non-corn sources to be blended with gasoline by 2022 (Environmental Protection Agency, 2012). Furthermore, lignocellulosic crops are predicted to yield five times more energy per land unit area than grain starch and sugar crops while producing only a quarter of the greenhouse gasses (Farrell et al., 2006; DOE, 2007; Somerville, 2007).

Perennial forage crops having been suggested as dedicated energy crops include switchgrass (*Panicum virgatum* L.), miscanthus (*Miscanthus* spp.), and energy cane (*Saccharum* spp.) (Jessup, 2009). Lignocellulosic perennial crops are preferable to

annuals because they have reduced greenhouse gas and carbon emissions than annual crops (NRCS, 2006; Adler et al. 2007; DOE, 2007). Carbon emissions are reduced because perennial crops are not reseeded each year which reduces fuel and decomposition of soil organic matter (Jessup, 2009). Perennial crops also require less fertilizer because nutrients are more readily recycled (Jessup, 2009). These perennial C_4 grasses have great amounts of photosynthetic activity and drought tolerance which encourages cultivation in temperate and tropical climates (Sree, 1999). Perennial energy crops can also be planted on degraded lands thus improving the soil organic matter and structure (Sartori et al., 2006).

Annual warm-season grasses, such as sorghum, also have potential for feedstock production because of their rapid growth and the management flexibility imparted by their growth cycle (Sipos, 2009). Crop residue of annual grain crops is a lignocellulosic feedstock option which would enable dual use of one crop (Gallagher et al., 2003). Feasibility of dual-use energy crops are important to the long term, sustainability of agriculture because it enables farmers to get the greatest return from inputs invested into growing a crop (Gallagher et al., 2003).

Sorghum is a superior annual warm-season grass choice for feedstock production in Texas because it is well adapted and can be grown successfully with limited water inputs (Dahlberg et al., 2011; Rooney et al., 2007). It has potential as a dual-purpose crop because sorghum stover and ratoon are commonly used to feed livestock (Powell, et al., 2006). Conversion of sorghum biomass to ethanol could produce an average of 6147 L ha^{-1} (Dahlberg et al., 2011). Besides ethanol produced from juice squeezed from

sorghum, bagasse is a by-product of sorghum feedstock (Sipos et al., 2009). Bagasse can be utilized as livestock feed, soil fertilizer, or combusted for energy (Sipos et al., 2009).

A second major constraint to production of bioenergy from lignocellulosic feedstock is providing a continuous source of feedstock to refineries. Viable storage solutions are critical to the success of the bioenergy industry since refineries need a continuous supply of feedstock to keep the refinery operating (Wiselogel et al., 1996; Rentizelas, et al., 2009). Field curing of sorghum is difficult due to the high moisture content and coarseness of leaves and stems, making the process costly in terms of weather risks, energy inputs, and harvest timelines (Williams and Shinners, 2012). Drying sorghum feedstock enhances resistance to enzymatic degradation since the curing of cellulose microfibrils results in irreversible shrinking of the pore space and reduces the accessible surface area (Esteghlalian, et al., 2001). Ensiling reduces field wilting time, weather risks, negative consequences of cell wall hornification, and reduces the water requirements for conversion at the biorefinery (Williams and Shinners, 2012). Therefore, ensiling practices can provide the biofuel industry with options for biomass storage and improvement (Williams and Shinners, 2012). Ensiling feedstock allows for pretreatment prior to storage, which can improve extraction of cellulose bound in hemicellulose and lignin (Han et al., 2007). Additionally, inoculation of corn stover silage with biological amendments can improve conservation (Ren et al., 2006). The lignin and hemicellulose bound to cellulose and storage constraints of feedstock are

two limitations to lignocellulosic bioenergy production. Pretreatment with fibrolytic enzymes or bacterial inoculants may be solutions to these problems.

Hay Production

Forages stored as hay are dried and field cured for future use as livestock feed or for use as feedstock for biofuels, including ethanol and combustion. When curing hay the objective is the rapid removal of excess moisture, while minimizing DM and nutrient losses (Hart and Burton, 1967). To produce hay the crop is dried in the field to a moisture content of less than 20% which requires 3 to 5 days when hay is harvested in thin, wide swaths (Rotz and Muck, 1994). Plant respiration is the complete oxidation of hexose sugar to carbon dioxide and water (Parkes and Greig, 1974). Cut forage continues to respire, losing carbohydrate until the moisture content drop to 30-40% (Greenhill, 1959).

Carbohydrates are the principal compound used in respiration, therefore, there is little loss of total nitrogen (**N**) and fiber (Rotz and Muck, 1994). The more rapidly the forage is dried, the less loss of DM and nutrient that occurs (Greenhill, 1959). The drying rate of grass hay is positively correlated with water content and inversely correlated with DM yield (Hart, 1967). Measured DM losses due to plant respiration are difficult to measure during field curing and vary widely. In alfalfa (*Medicago sativa* L.*)* DM losses due to respiration varied between -8 and 19% of the initial crop DM (Rotz and Abrams, 1988). Across species, loss is influenced by drying conditions with the greatest loss in material dried in a warm, humid environment (Rotz and Muck, 1994).

Rain damage can significantly increase DM loss since it reenacts the respiration process (Rotz and Muck, 1994). Dry matter losses from hay yielding 2 Mg ha^{-1} were 3% in dry weather and 9.6% when 5 cm of rain fell; losses from hay yielding 10 Mg ha^{-1} were 4.6 and 11.1%, respectively (Hart and Burton, 1967). The nutritive value of hay can differ greatly depending on the forage species, maturity, and drying time (Rotz and Muck, 1994).

Mechanical operations cause additional loss of nutrients adversely affecting forage quality. This is due to leaf shatter which reduces DM yield and nutritive value since leaves have a greater nutritive value than stem (Rotz and Muck, 1994). In both grass and legume hays leaf shatter can double as forage matures from late, vegetative to a full bloom stage of development which may be due to reduced moisture and/or weakened attachment of leaves causing greater leaf shatter (Rotz and Muck, 1994).

Losses during storage and feeding can also be great if proper measures are not taken to protect the forage. Storing hay under a barn is the most effective means of preventing DM loss due to precipitation (Ball et al., 2007). Reported DM losses of round bales stored outside range from 3 to 40% with the greatest impacts being weather, length of storage, and storage method (Rotz & Muck, 1994). Dry matter losses also affect feedstock plants because of DM lost due to weathering (including leaching, ultraviolet degradation, and erosion) and biochemical reactions produced by microbial life (Cusi, 1979; Moser, 1980; Jirjis & Theander, 1990). Loss of DM from weathering and biochemical reactions causes a negative economic affect (Wiselogel et al., 1996).

Cool-Season Small-Grain Hay for Livestock

Cool-season, small grains include oat, wheat, and ryegrass (*Lolium* L.). These grasses are primarily harvested for hay during boot and soft dough maturity stages as this maturity stage balances yield and nutritive value (Figure 2.2; Collar et al., 2006). Yield of small-grain hay range from 4.48 to 8.96 Mg ha^{-1} and baling should take place at 15-20% moisture (Smith et al., 2009). Early harvest reduces yield and provides a greater nutritive value, whereas, later harvest increases yield but sacrifices nutritive value (Figure 2.3; Ball et al., 2007). Stage of maturity is the predominant factor influencing palatability, crude protein concentration, and amount of digestible energy (Ball et al., 2007).

Due to the relatively greater nutritive value of cool-season hay compared to other forages it can be fed to livestock with greater nutrient requirements, such as young calves, replacement heifers, and lactating beef cows (National Research Council, 2000). Oat and wheat are commonly grown in the southern United States because there are sufficient growing degree days to produce 120 to 150 d of pastures that could be grazed, harvested for conserved forage, or harvested for grain making it a dual-purpose crop (Holman et al., 2010).

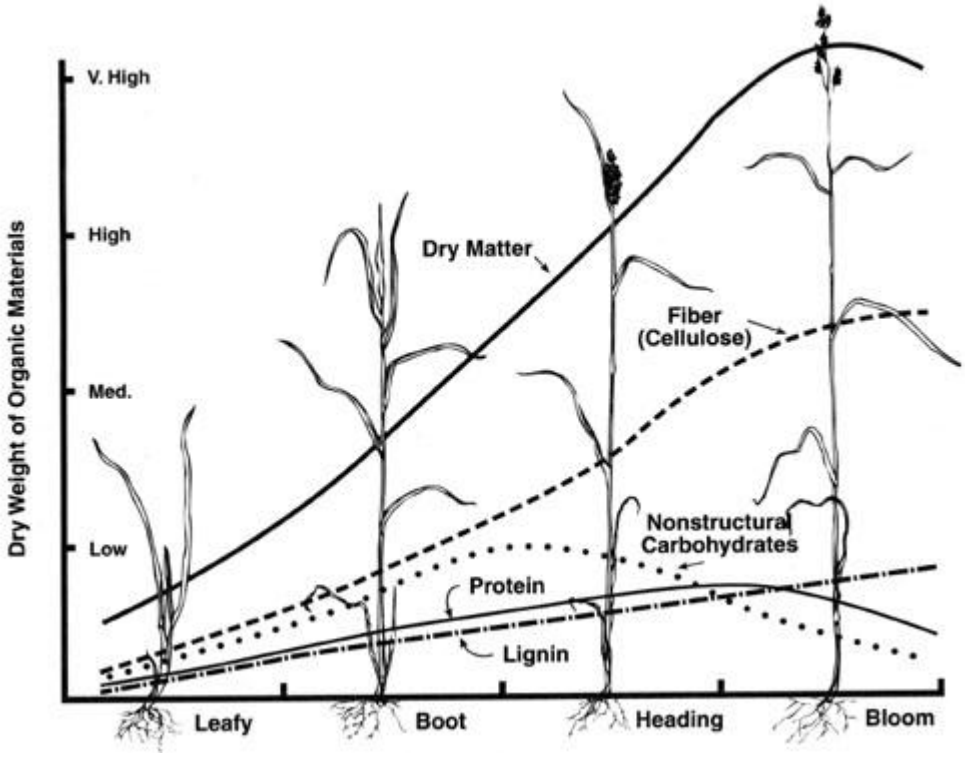

Figure 2.3 The effect of maturity stage on nutritive value and yield of small-grain grown for forage (Virginia Tech, 2009).

Oats usually produces 6,000 kg DM ha^{-1}, through a growing season and the majority of the yield occurs in March (Mackowiak et al., 2011), whereas, wheat yield is similar to oat but the majority of the yield occurs earlier in the growing season (Holman et al., 2010). Steers grazing oat had an ADG of 1.33 kg hd^{-1} d^{-1} (Pereira, 2009), whereas, steers grazing wheat had an ADG of 0.89 kg h^{-1} d^{-1} (Pinchak et al., 1996).

When feeding small-grain forage to ruminant livestock it is important to consider the energy to nitrogen ratio (carbon:nitrogen; **C:N**) because these livestock will have increased ruminal ammonia production resulting in increased N excretion when there is

not sufficient energy for ruminal microbes to use the N for growth (Poppi and McLennan, 1995). This loss of N from the rumen is costly due to significant energetic expenditure associated with urea synthesis and excretion (Vendramini et al., 2006) and the loss of N to the environment.

Nutrient Synchrony Constraints of Small-Grain Hay

The performance and production efficiency of forage-fed ruminants can be improved by enhancing the synchrony of C:N. Nutrient synchrony is the parallel occurrence of nutrients consumed or present in the diet and rumen, whereas, nutrient asynchrony is a deficiency of energy or N which decreases digestibility of the diet and microbial efficiency (Hersom, 2008). Ruminants are unique in that they are able to recycle N, which is not the case with carbohydrates and ruminal microbes are also limited in their ability to store carbohydrate (Hersom, 2008). Therefore, a consistent supply of energy in the diet is critical to promoting greater capture of N in the rumen which improves efficiency (Lardy et al., 2004; Hersom, 2008). A continuous 1:1 supply of C:N is the most beneficial diet to promote healthy ruminal fermentation patterns and overall nutrient metabolism (Huston et al., 1999; Hersom, 2008).

Nutrient synchrony should provide an increase in ruminal metabolism, including enhanced microbial efficiency and growth, compared to asynchronous diets (Herrera-Saldana et al., 1990; Kim et al., 1999a; Richardson et al., 2003). Improving the efficiency of energy and nitrogen metabolism should reduce costs of animal production and decrease excess N excretion from livestock (Hersom, 2008). Not all research has shown a positive response of microbial efficiency and yield when synchronous diets

were fed (Kim et al., 1999b; Richardson et al., 2003). This could be due to the type or amount of energy fed in these studies which was predominately soluble and likely negatively affected ruminal microorganisms (Kim et al., 1999a).

When nutrient synchrony is successful, increased nutrient efficiency and microbial yield will result in enhanced digestibility and/or intake which then results in improved animal performance (Herrera-Saldana et al., 1990; Hersom, 2008). Steers fed bermudagrass (total digestible nutrients) (**TDN:CP** > 7) and supplemented with corn exhibited lesser ADG than those fed bermudagrass alone (Garcés-Yépez et al., 1997). This is because the bermudagrass had excess energy compared to N and the rapid degradation rate of carbohydrate in the rumen was in excess of the slowly degradable protein of bermudagrass (Moore et al., 1999). The addition of rumen degradable protein (**RDP**) synchronizes the C:N because rapidly released N matches the rapid energy release from corn (Bodine et al., 2001; Bodine and Purvis, 2003). Adding grain-based supplements to the forage diet of ruminant ruminal livestock is not always desirable because grain-based supplements may shift the rumen microorganism population reducing forage digestibility (El-Shazly et al., 1961).

The timing of feeding also impacts nutrient synchrony. A study by Richardson et al. (2003) compared diets fed to lambs which were formulated based on the rumen ratio of nitrogen to organic matter (**N:OM**) hourly release rate and fed at several times of the day to represent synchronous (0.86 and 0.85 synchrony index for barley-based and sugar beet pulp based diets, respectively), intermediate (0.76 for each diet), or asynchronous (0.63 and 0.61, respectively; Figure 2.4).

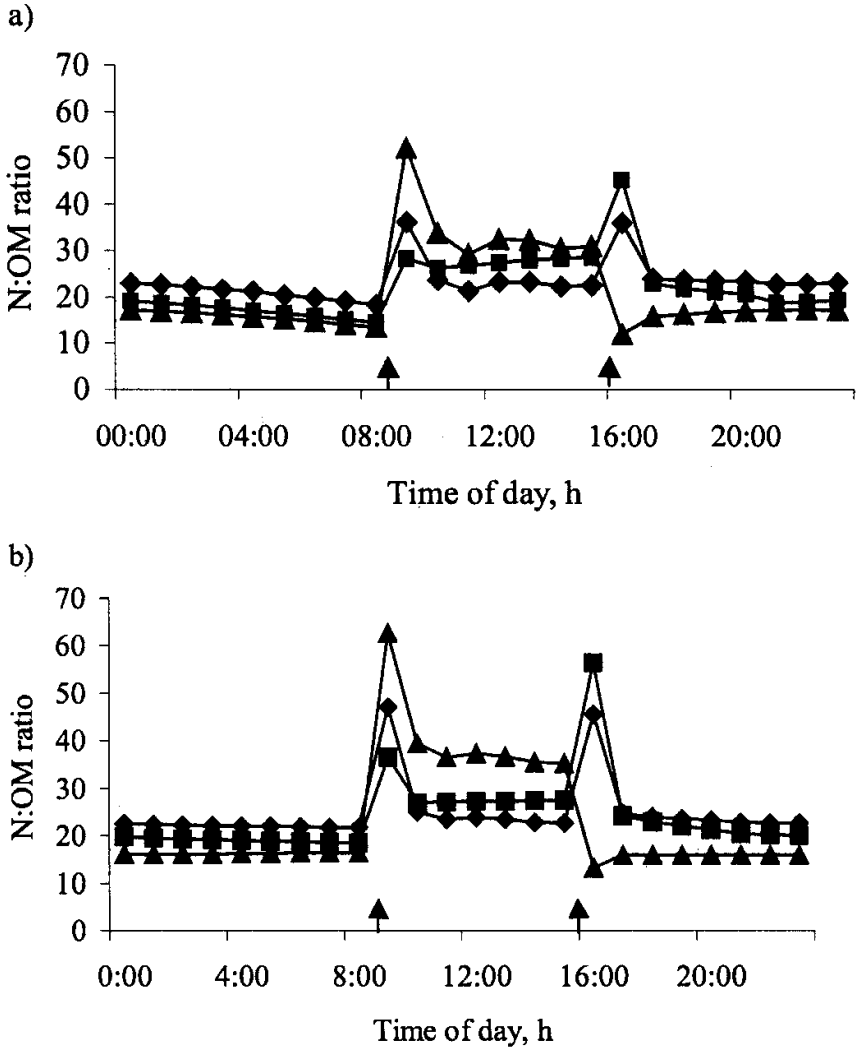

Figure 2.4 Predicted ratio of N:OM in the rumen of lambs (fed diets based on either a. barley or b. unmolassed sugar beet pulp and offered in three patterns within a day to be synchronous (♦), intermediate (■), or asynchronous (▲) arrow indicates feeding time) (Richardson et al., 2003).

The diet included the same ingredients in varying amounts except for the dietary energy source. The growth rate of lambs and nitrogen retention were not impacted by nutrient synchrony, however, there was greater lipid content in the meat of lambs fed synchronous diets indicating enhanced energy efficiency (Richardson et al., 2003).

High quality forages, such as small-grains, may not support dietary nutrient synchrony because of excess N and a relative deficiency of energy (Hersom, 2008). Typically the total digestible nutrients to crude protein ratio (**TDN:CP**) is 3.75 in wheat (Holman, 2010). Before a C:N assessment can be made, consumed forage chemical composition must be determined (Hersom, 2008). This may be a difficult task in grazing lands since there are differences in the chemical composition of forage harvested by hand and that consumed by grazing livestock (Coleman and Barth, 1973; Fisher et al., 1991; Dubbs et al., 2003; Hersom, 2008). Differences also exist in protein concentration, protein form, and degradability of standing forage, hay, or forage silage (Hersom, 2008). Preserved forage, such as hay and silage, typically have a greater CP concentration compared with fresh or standing forage (Messman et al., 1994; Farmer et al., 2001; Volden et al., 2002). Therefore, there may be a greater need for nutrient synchrony of preserved forages than fresh or standing forage.

Digestible energy intake has been increased due to improvements in ruminal fiber digestion from the application of fibrolytic enzymes (Beauchemin et al., 2003). Therefore, application of fibrolytic enzymes to cool-season, small-grain grasses may increase the amount of soluble carbohydrates available during digestion which would enhance the synchronization of C:N.

Enzyme Treatment

The application of enzymes may be desirable when forages species or cultivars have a greater fiber concentration, were harvested at increased maturity, or grown or harvested in unfavorable climatic conditions. Fibrolytic enzymes are applied to plant material to break the bonds between hemicellulose and lignin. These bonds must be broken to hydrolyze the cell wall (Beauchemin, 2003). If the bond linking lignin to hemicellulose and lignin is not broken, then the carbohydrates within the cellulose and hemicellulose are unavailable for fermentation or extraction (Figure 2.2; Aden et al., 2002; Eggeman and Elander, 2005). Commercial fibrolytic enzymes are created by microbial fermentation and produced by a batch fermentation process, beginning with a seed culture and growth media (Cowan, 1994). Interest in applying fibrolytic enzymes to ruminant feed and lignocellulosic feedstock has increased due to the favorable results *in vitro* and *in vivo* (Dean et al., 2005; Chen et al., 1994; Sipos et al., 2009).

Not all studies evaluating fibrolytic enzymes demonstrate consistent improvement in animal performance, and where improvements were seen the enzyme may not have been the cause (Dean et al., 2005). Inconsistencies may be related to enzyme type, concentration and activity, application method, substrate to which enzymes were applied or animal differences (Dean et al., 2005). Other factors affecting improvement could have been feed temperature and pH, presence of cofactors and inhibitors, and enzyme and substrate concentration (Dean et al., 2005). There appears to be endless possibilities for the use of these enzymes that will improve the value of livestock feeds and lignocellulosic feedstock. Identification of appropriate enzymes and

applications has the potential to increase efficiency and sustainability of the conversion of lignocellulosic material into food and fuel.

Cellulases

Cellulases hydrolyze cellulose, which is a major structural polysaccharide in plants (Figure 2.2). The cellulases of microorganisms are enzyme complexes that cleave β-bonds between glucose molecules within cellulose. Beta linkages are those found in structural carbohydrates and require microbial enzymes for the breakage of bonds unlike α-bonds that can be broken by enzymes found in non-ruminant stomach (Chesson and Forsberg, 1988). Cellulases consists of endoglucanases (1,4-b-D-glucan glucanohydrolase, EC 3.2.1.4), exoglucanases (1,4-b-D-glucan cellobiohydrolase, EC 3.2.1.91), and b-glucosidases (b-D-glucoside glucohydrolase, EC 3.2.2.21) (Figure 2.5 and 2.6 (Desai, 1982; Van Soest, 1994). Endoglucanases hydrolyze cellulose chains at random to produce cellulose oligomers of varying degree of polymerization (Bhat and Hazlewood, 2001). Recently the enzyme (β -1,4-Endoglucanase) which cleaves the internal bonds of cellulose chains was identified (Kumar et al., 2008). Exoglucanases hydrolyze the cellulose chain from the nonreducing end, producing cellobiose(Bhat and Hazlewood, 2001). Exoglucanases and β -glucosidases break cellulose into glucose monomers (Kumar et al., 2008). There is a wide diversity of microorganisms in the rumen and the ruminal microbial population shifts depending on the amounts of nonstructural versus structural cellulose in the diet (Hoover, 1986). This shift of ruminal microbial population impacts the cellulase production and type (Bhat and Hazlewood, 2001).

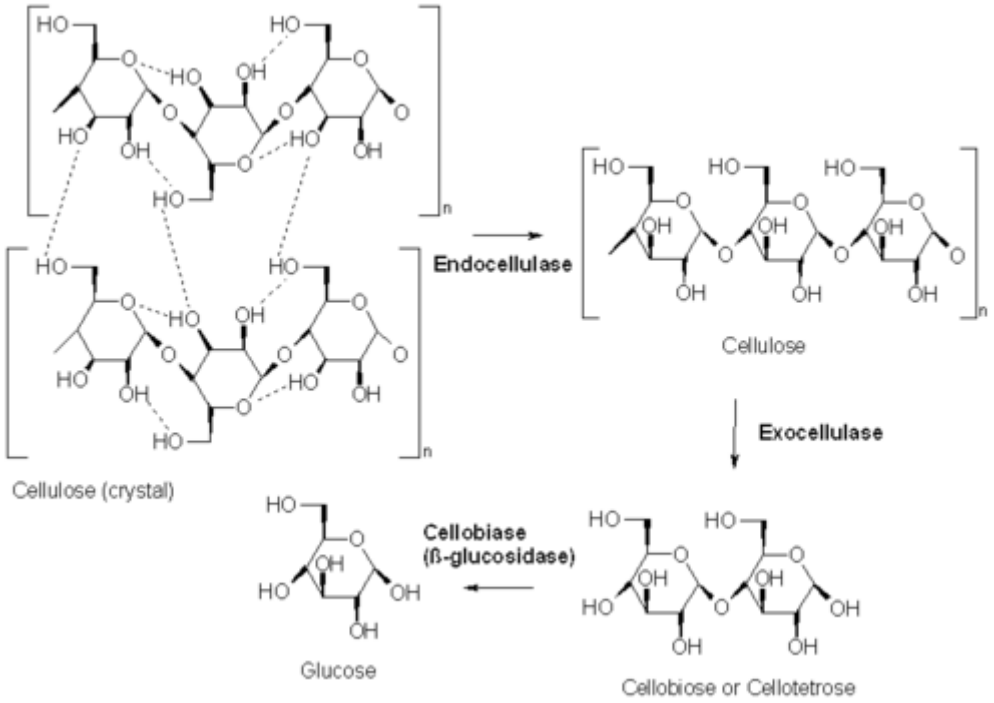

Figure 2.5 The three types of reactions catalyzed by cellulases (Bayer et al., 1998). 1. Breakage of the noncovalent interactions present in the crystalline structure of cellulose by endocellulase 2. Hydrolysis of the individual cellulose fibers to break it into smaller sugars (exocellulase) 3. Hydrolysis of disaccharides and tetrasaccharides into glucose (beta-glucosidase).

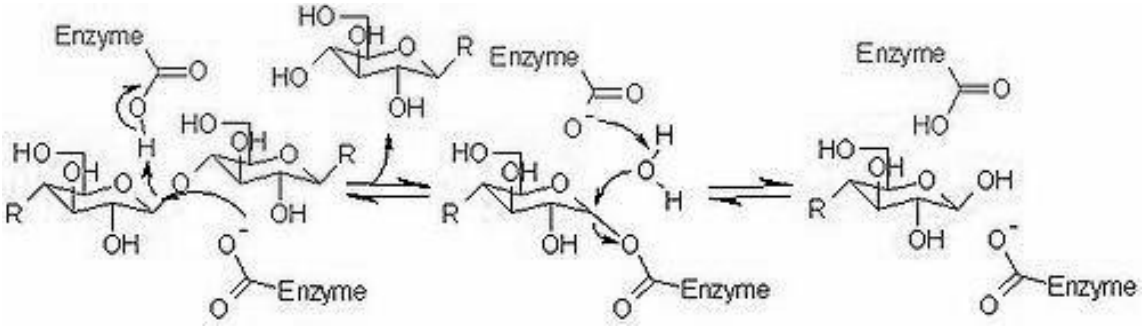

Figure 2.6 Mechanistic details of beta-glucosidase activity of cellulase (Bayer et al., 1998).

Cellulase is one of many different fibrolytic enzymes applied to feed stuffs to break down the cell wall and improve digestibility. Researchers use a mixture of different enzymes in most studies of xylanase additives with the hope one or a combination would have an impact. Elwakeel et al. (2007) concluded cellulases might have the most important activity compared to other enzymes. In an *in vitro* degradation trial using corn silage, the combination of endoglucanase and exoglucanase explained 87% of the variation in the improvement in NDF degradability (Eun and Beauchemin, 2007). Krueger et al. (2006) found cellulase improved fermentation and digestion of bermudagrass hay after a 24 hour (**h**) enzyme substrate interaction period. Application of cellulase to livestock feedstuffs improves nutritive value and ensiling characteristics.

Xylanases

Hemicellulose is composed of many polysaccharides arranged into a xylan core polymer and arabinose branches from this core may be present (Bhat and Hazlewood, 2001). Xylanases are the primary enzymes involved in degrading hemicellulose to soluble sugars (Beauchemin et al., 2003). Xylanses are specific for the internal β-1,4 linkages and are generally, considered endoxylanses (Figure 2.7; Bhat and Hazlewood, 2001). There are two types of endoxylanases, debranching or nondebranching, based on their ability to release arabinose in addition to hydrolyzing the main chain xylan (Beauchemin et al., 2003). The amount and types of xylanases produced by ruminal microbial organisms is dependent on the type of feedstuff being digested. The many different types of microbial organisms in the rumen are affected differently by feed type;

therefore, amounts and types of xylanases being produced are constantly changing (Bhat and Hazlewood, 2001). Factors which affect the activity of xylanases are temperature, pH, ionic strength, substrate concentration, and substrate type, therefore, xylanase activity must be measured in similar conditions for accuracy (Beauchemin et al., 2003).

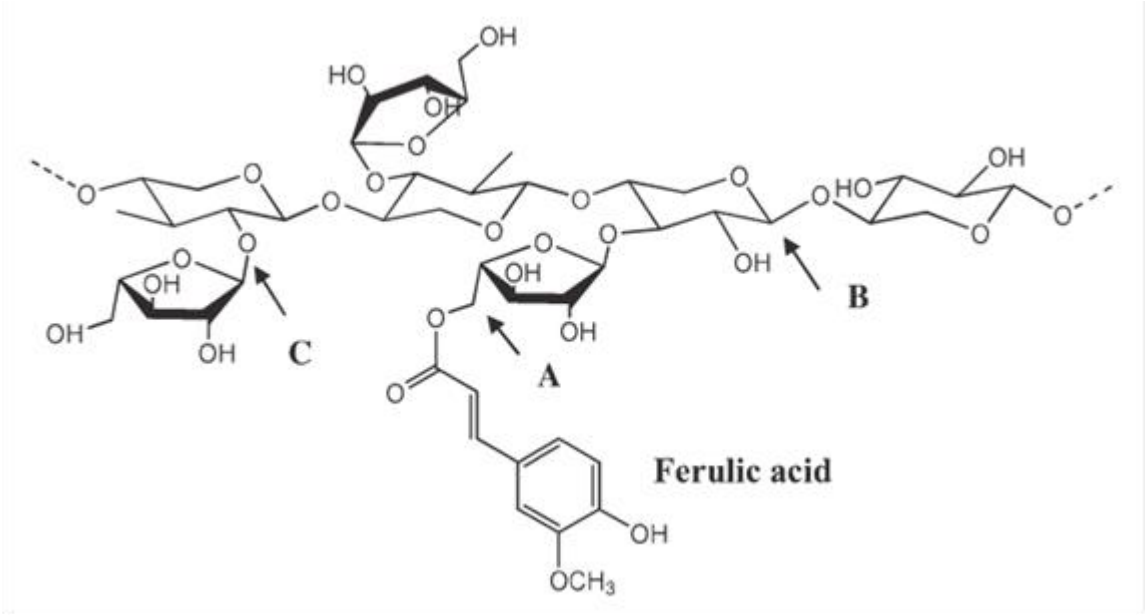

Figure 2.7 Structure of ferulic acid esterified to arabinose units of arabinoxylan (Buanafina, 2009). A. ferulic acid linked to O-5 of arabinose chain of arabinoxylan; B. β-1,4-linked xylan backbone; C: α-1,2- linked L-arabinose.

A fibrolytic enzyme mixture of cellulose and hemicellulase improved the ensiling process and aerobic stability of silage and decreased the ensiling dry matter

losses, structural carbohydrate, and ammonia nitrogen content (Adogla-Bessa et al., 1999).

Several studies by Krueger et al. (2008 a,b) and Krueger and Adesogan (2008) found xylanase in combination with other enzymes did improve fermentation and digestion of bahiagrass (*Paspalum notatum* Flueggé) and bermudagrass. Results of a study which evaluated application of enzymes to a dairy diet with a 50:50 (concentrate:forage) ratio data found IVDMD was increased by the addition of FP800 (β-glucanase, xylanase, and cellulase) even when FP800 was added in smaller amounts than other enzymes (Elwakeel et al., 2007).

Elwakeel et al. (2007) found the IVDMD of four different fibrous dairy feedstuffs was increased by addition of enzyme mixtures (β-glucanase, xylanase, and cellulase). There have also been consistent results of increased digestibility of high grain diets because of the addition of xylanase (Beauchemin et al., 2003). When barley is fed at (95% of the total diet) feed efficiency is improved by 6-12% and the percentage of increase depends on the application timing and level of enzyme addition (Beauchemin et al., 1997; Iwaasa et al., 1997). Addition of a similar enzyme product to a high concentrate diet resulted in a 28% increase in ADF digestibility (Krause et al., 1998). Average daily gain was increased by 10% when enzymes were added to rye-grass silage (30% of the total diet) and barley (70% of the total diet) based diets (McAllister et al., 1999). However, when the same enzyme was added to a barley-based finishing diet containing 17% forage (DM basis) there was no improvement in digestibility due to the

addition of the enzyme (ZoBell et al., 2000). This conflicting data could be due to differences between the nutritive values of diets.

When xylanase is added to high concentrate diets the fiber degradation is usually increased, however, the increased fiber degradation does not always translate into an improvement in animal performance such as increased ADG. Further research should be conducted to determine inoculant rates which consistently provide animal improvements.

Microbial Inoculation with Lactobacillus buchneri

It is common for farmers to apply *Lactobacillus buchneri,* which is prone to forage aerobic spoilage. *Lactobacillus buchneri* is classified as a heterofermentative LAB (McDonald, 1981). Forage conserved thru the ensiling process with greater than 35% DM, including silage, high moisture corn, and cereal grains are prone to aerobic spoilage. Lactic acid bacteria, is applied to stimulate or ensure rapid fermentation and inhibit aerobic spoilage by decreasing the time to reduce pH during fermentation (Kung and Charley, 2010). To promote acetic acid production, *Lactobacillus buchneri* should be applied to silage because it converts LA to acetic acid (Jones, 2012).

Aerobic stability is increased since acetic acid inhibits molds and yeasts, however, the silage must be at the proper pH for acetic acid to be in an active form inhibiting molds and yeasts. At a pH of 4.79 the ratio of active to inactive acetic acid is 50% which is known as the pKa of the acid (Jones, 2012). Applying bacterial inoculants at ensiling will promote fermentation.

Lactobacillus buchneri increased residual WSC concentration, enhanced homofermentation, and reduced pH, DM losses, and proteolysis when applied to bermudagrass silage (Dean et al., 2005). The inoculants used in the study by Dean et al., (2005), were PRO (main ingredient *L. buchneri*), Biocellulase X-20, Biocellulase A-20, and enzyme CT and they were, applied, at varying rates. PRO showed the most significant results in this study.

The effects of *Lactobacillus buchneri* and *L. plantarum*, applied at ensiling, on the fermentation and aerobic stability of wheat and sorghum silage was evaluated by Weinberg et al. (1999). *Lactobacillus buchneri* inoculant was effective at protecting the aerobic stability of wheat and sorghum whole-plant silage exposed to air under laboratory conditions (Weinberg et al., 1999). *Lactobacillus buchneri* inoculant protected the silage in the presence of *L. plantarum* and yeasts which decrease feed quality. *Lactobacillus buchneri* inoculant could also serve as a silage additive along with homofermentative LAB (Weinberg et al., 1999).

Inoculating alfalfa silage with LAB can inhibit the growth of harmful microorganisms such as *Enterobacterium* and *Klebsiella pheumoniae* (Zhang et al., 2009). When a combination of *L. buchneri* and *L. plantarum* were added to alfalfa silage aerobic stability is further improved versus LAB alone (Zhang et al., 2009). However, there was no effect on *in situ* rumen DM and NDF degradability of alfalfa silage when using this inoculant. Nevertheless, application of *L. buchneri* added to alfalfa silage greatly improves the end product, nutritive value, and aerobic stability.

Summary

The overall objective of this dissertation project was to evaluate the effect of enzymatic or microbial inoculation on sorghum silage and small-grain hay. Inoculation may reduce cellulose, hemicellulose, and lignin concentrations of forage, which increases the stored energy available for livestock feed and biofuel. Also, inoculant with *Lactobacillus buchneri* (the main ingredient in PRO) may increase silage aerobic stability which reduced DM loses of stored forage. Our results are relevant to improving the value of feedstuff and feedstock in Texas.

The first experiment determined the effect of inoculation with enzyme or LAB on nutritive value, ensiling characteristics, and *in situ* disappearance kinetics of four forage sorghum cultivars. The hypothesis was addition of cellulase:xylanase or microbial inoculant would increase fiber degradation to WSC resulting in improved ensiling characteristics and aerobic stability. The cultivars were a PPS cultivar with greater yield but lesser digestibility than varieties without the photoperiod trait (PS 747 [**PS**]), a BMR cultivar with lesser yield but greater nutritive value than conventional sorghum (Dairy master BMR [**DBMR**], and two conventional forage cultivars currently grown in Texas (Dairy Silo 700D [**Silo**] and MMR 381/73 [**MMR**].

The second experiment determined the effect of inoculation with enzyme or LAB on nutritive value and *in situ* degradation kinetics of two cultivars of oat and wheat. The hypothesis was addition of cellulase:xylanase or microbial inoculant would enhance the fiber degradation rate and extent, thus indicating potential of additives to enhance C:N ratio of small-grain hay. Wheat cultivars Fannin and TAM 203 and oat cultivars TAMO

606 and Harrison were chosen for the second experiment because they are adapted to Texas' climate and used by local farmers as a dual purpose crop (Texas AgriLife, 2011). Fannin and TAMO 606 had greater DM yields in variety trials compared to TAM 203 and Harrison (Texas AgriLife, 2011).

CHAPTER III

NUTRITIVE VALUE, FERMENTATION CHARACTERISTICS, AND *IN SITU* DISAPPEARANCE KINETICS OF SORGHUM SILAGE TREATED WITH INOCULANTS

Introduction

Sorghum silage can be used as biomass for ethanol production or fed to livestock. In 2010 there were 13,102 ha harvested for silage production in Texas (USDA, 2011). Sorghum is well adapted to the warm climate, produces a high yield, and is drought tolerant making it an excellent crop to meet the grain and forage needs of the livestock industry (Prostko et al., 1998). However, acceptance of sorghum silage for livestock feed has been limited due to its greater ADF and ADL levels than corn silage (Prostko et al., 1998). The greater fiber levels found in sorghum reduce forage digestibility and may compromise milk production (Prostko et al., 1998). Bolsen et al. (1989) reported grain sorghum silage can be substituted for corn silage in mid-lactation dairy cattle diets with no adverse effects on milk production, whereas, others reported silage from BMR sorghum supports milk production similar to corn silages (Lusk et al., 1984; Grant et al., 1995; Oliver, 2004).

During the ensiling process, DM is lost if fermentation does not occur immediately and aerobic stability is not maintained during storage and feedout (Jones, 2012). Therefore, limitations to the use of sorghum silage include storage constraints and fiber degradation, and both may be improved by the application of fibrolytic enzyme or bacterial inoculant, thereby, increasing the value of sorghum silage. Treating silage

with fibrolytic enzymes breaks bonds between hemicelluloses and lignin in order for sugars to be extracted from hemicelluloses (Han et al., 2007). Elwakeel et al. (2007) found *in vitro* DM digestibility of four different fibrous dairy feedstuffs were improved by addition of fibrolytic enzyme mixture containing β-glucanase, xylanase, and cellulase. Bacterial inoculants containing LAB increase the aerobic stability by inhibiting aerobic spoilage because the time to reduce pH is decreased during fermentation (Kung and Charley, 2010).

Sorghum has been bred to favor improved nutritive value and yield. Photoperiod sensitive varieties yield well and use water efficiently, however, they have lesser digestibility and greater fiber than conventional sorghum cultivars which limits their broad application (McCollum et al., 2012). Increased fiber concentrations and reduced digestibility of PPS silage may reduce its feedstock and feedstuff value since fiber degradation is one of the major limitations to value. However, Murray et al. suggests yield traits be selected over composition traits for maximizing energy yield of sorghum biomass (2008). This suggestion from Murray et al. (2008) is similar to the conclusion by McCuistion et al. (2011) in that PPS varieties fed more cattle than varieties with greater nutritive value. Varieties selected for BMR traits have less ADL and may be 10 to 30% more digestible, however, DM yield may be 15 to 20% less and lodge more easily (Ball et al., 2007).

Despite the large acreage of sorghum grown in the United States, there is limited information about the ensiling characteristics, nutritive value, and *in situ* kinetics of sorghum silage pretreated with fibrolytic enzymes or bacterial inoculants. The lack of

information is especially apparent in regards to the genetically improved PPS and BMR types. Thus, the goal of this study was to determine the nutritive value, fermentation characteristics, aerobic stability, and *in situ* ruminal disappearance kinetics of conventional, PPS, and BMR sorghum silage pretreated with fibrolytic enzymes (cellulase:xylanase) or bacterial inoculant.

Materials and Methods

Sorghum cultivars Dairy Master BMR (brown midrib), PS 747 (photoperiod sensitive) , Dairy Silo 700 (conventional forage type), and MMR 381/73 (conventional forage type) were grown at Texas AgriLife Research Station in Beeville (**Bee**) (28°N, 98°) and Texas AgriLife Research Station in College Station (**CS**) (30°N, 96°W), TX. At planting, sorghum was sprayed with a tank mix of 0.575 L/ha^{-1} of atrazine + 0.339 L/ha^{-1} of metolachlor, and no fertilizer was applied. Harvest occurred during mid-dough stage at which time four random height measurements were recorded, 7.3-m of sorghum was cut from the 2 center rows to 10-cm stubble height, and a sub-sample (3.66-m row length) weighed to calculate yield. Material was chopped into at least 13-mm particle size using a chopper shredder (Earthquake, Cumberland, WI). A 1-kg sub-sample of forage was dried at 65°C until weight loss ceased. Samples were ground to 4-mm and subsamples used for nutritive value analysis of pre-ensiled sorghum were ground to pass through a 2-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA).

Chopped sorghum (5 kg) was sprayed with either 200 ml distilled water (**control**), 1.34 ml fibrolytic enzymes (XC), or 16.5 mg bacterial inoculant (PRO; *Lactobacillus buchneri, Pediococcus acidilactici, P. pentosaceous, L. plantarum,*

Enterococcus faecium) mixed with 200 ml distilled water. Silage was hand mixed and packed into mini-silos, which were 17.6 L containers with lids and were lined with 38.1 × 22.9 × 61 cm polyethylene bags sealed for at least 120 d. After 120 days, silos were opened and four separate subsamples were taken. The first subsample was dried at 65°C and ground to pass through a 4-mm screen and then a subsample ground to pass through a 2-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) for nutritive value analysis.

Laboratory Analyses

The second silage subsample was sent to Dairy One (Ithica, New York) for analyses of WSC, ammonia- N (**NH_3-N**), pH, and VFA. Water soluble carbohydrates were determined by incubation in water (40°C) followed by acid hydrolysis with sulfuric acid and colorimetric reaction with potassium ferricyanide (Hall et al., 1999). Ammonia-N was analyzed with a Timberline TL-2800 analyzer (Timberline instruments, Boulder, CO) with the method described by Liu (1998). Silage pH was analyzed by placing wet samples into beaker with deionized water and analyzed using a Thermo Orion Posi-pHlo SympHony Electrode and Thermo Orion 410A meter (Thermo Fisher Scientific, Waltham, MA). Acetate, propionate, butyrate, and iso-butyrate were measured with a Perkin Elmer Autosystem XL Gas Chromatograph (Perkin Elmer, Waltham, MA) containing a Supelco packed column (Sigma Aldrich, St. Louis, MO) with procedures adapted from Supelco (1990). Lactate was determined by analyzing silage extract for L-Lactate using a YSI 2700 SELECT Biochemistry Analyzer (YSI, Inc. Yellow Springs, OH). The third silage subsample was sent to Ag Source

Laboratories in Bonduel, WI, for yeast and mold count analysis (Tournas et al., 1998). The last silage subsample was used to measure aerobic stability by placing type k thermocouple wires (Omega Engineering, Stamford, CT) at the center of approximately 800 g of silage stored in a polyethylene bag, within an open-top polystyrene box covered with 2 layers of cheesecloth to prevent drying. Thermocouple wires were connected to a DT80 series 2 data taker (Scoresby, Victoria, Australia) which recorded temperature readings every 15 min until the silage reached 2°C above ambient temperature (18-25°C).

Fresh and ensiled subsamples previously ground to 2-mm, were dried at 135°C for 8 h for DM determination (AOAC, 1990). Concentrations of NDF and ADF were measured using the Van Soest et al. (1991) method in an ANKOM 200 Fiber Analyzer (ANKOM Technologies, Macedon, NY). The Van Soest et al. (1966) method and an ANKOM Daisy^{II} Incubator (ANKOM Technologies) were used to determine IVTD. Lignin was measured using the ANKOM (2011) procedure based on Van Soest et al. (1967) procedure. Nitrogen was determined by rapid combustion using a Macro elementar N analyzer (Elementar Americas, Mt. Laurel, NJ) and CP was calculated as N × 6.25. The ADIN was determined by rapid combustion of ADF residue obtained through the method previously stated.

In Situ Incubation Procedures

The sorghum cultivars exhibiting the greatest (Silo) and least (MMR) fiber concentrations were milled to pass thru a 4 mm screen were used for *in situ* incubation. Approximately 4.5 g (as fed) of sample was weighed into 10 × 20 cm polyester bags (53

± 10 µm pore size; Bar Diamond, Inc., Parma, ID) in triplicate. Bags were incubated in the ventral rumen of 3 Angus cross steers (453 ± 25 kg BW) and removed after 0, 4, 8, 16, 24, 48, and 72 h. The trial began after a 10-d adaptation to 8 kg hd^{-1} d^{-1} sorgo-sudan (*Sorghum* spp.) hay (6.4% CP, 55.8% NDF, and 32.7% ADF, DM basis) along with 1.7 kg hd^{-1} d^{-1} cotton (*Gossypium hirsutum* L.) seed meal (42.4 % CP, 24.6% NDF, 15.2% ADF, DM basis). Water and a trace mineralized salt block (minimum 1.8% Ca, 90.0% NaCl, 1.0% S, 25 ppm Co, 150 ppm Cu, 90 ppm I, 1,500 ppm Fe, 3,000 ppm Mn, 10 ppm Se, and 2,500 ppm Zn) were provided *ad libitum*. Animal Care and Use regulations set by animal welfare committee at Texas A&M University Kingsville were followed. Immediately after removal, bags were placed in ice water, rinsed with tap water (39°C), placed in plastic bags, and frozen (-20°C) until all bags were incubated. All bags were washed with one cycle in a commercial washing machine and dried at 55°C to a constant weight. Dried residues were analyzed for DM, CP, NDF, and ADF. *In situ* rumen DM, NDF, and ADF degradation data were fitted to the first order exponential model with discrete lag (Mertens, 1977) using the iterative Marqardt method and the NLIN procedure of SAS (SAS Institute, Cary, NC). The model is of the form

$$R_{(t)} = B \times (e^{-k\,d(t-L)}) + C,$$

where $\boldsymbol{R(t)}$ = Total indigested residue at any time t, $\boldsymbol{B}$ = insoluble potentially digestible fraction, $\boldsymbol{k_d}$ = fractional rate of digestion of B, t = time incubated in the rumen in h, $\boldsymbol{L}$ = discrete lag time in h, and $\boldsymbol{C}$ = fraction not digested after 96 h of incubation. The wash fraction $\boldsymbol{A}$ was the percentage of substrate washed out of the bag at 0 h. *In situ* rumen CP degradation data were fit to a similar model which excluded the discrete lag time

(Mertens, 1977). Effective ruminal degradability (extent of digestion, **ERD**) was calculated using the model of Ørskov and McDonald (1979):

$$\text{ERD} = A + \{B \times [k_d / (k_d + k_p)]\},$$

Where, $\boldsymbol{k_p}$ = assumed ruminal passage rate of 0.05.

Statistical Analyses

Statistical analyses were analyzed as a factorial design with GLIMMIX procedure of SAS (SAS, Inst. Inc., Cary, NC) and the model included cultivar, location, treatment, and their interactions. Where significant ($P < 0.05$) effects were observed among the treatments least square means were compared with Fisher's LSD test for multiple comparisons.

Results

Yield and Chemical Composition of Pre-Ensiled Sorghum

The height of DBMR was greater ($P < 0.01$) than MMR at both locations, whereas PS and Silo were intermediate (Figure 3.1). Herbage mass of PS was greater ($P < 0.05$) than MMR, the least yielding cultivar, at both locations and was not different ($P > 0.76$) between locations.

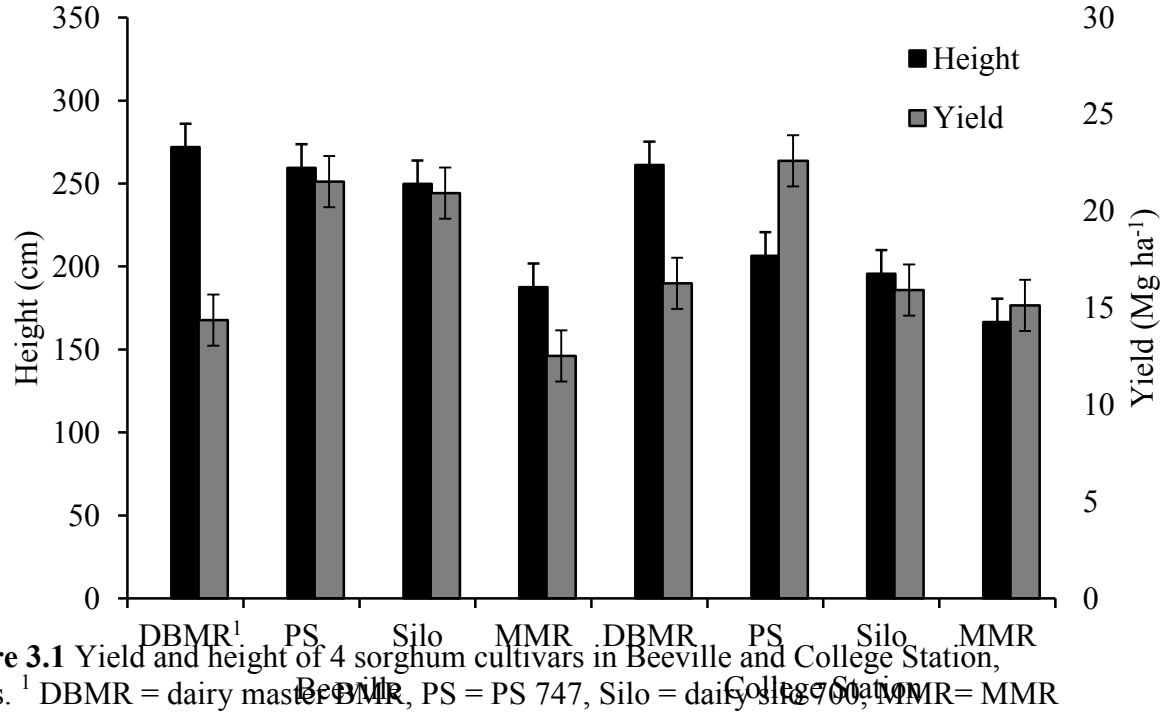

Figure 3.1 Yield and height of 4 sorghum cultivars in Beeville and College Station, Texas. [1] DBMR = dairy master BMR, PS = PS 747, Silo = dairy silo 700, MMR= MMR 381/73

Among pre-ensiled sorghum, DM concentration was not different ($P > 0.17$) among cultivars (Table 3.1). Crude protein concentration was least ($P < 0.03$) in Silo and not different ($P > 0.06$) among the other cultivars. The NDF and ADF concentrations were greatest ($P < 0.03$) in MMR. The PS cultivar had the least ($P < 0.03$) NDF concentration, and the other cultivars were intermediate. The DBMR and PS cultivars had the least ($P < 0.03$) ADF concentrations and Silo was intermediate. Concentration of WSC was greater ($P < 0.02$) in DBMR and PS than MMR. The cultivar DBMR had greater ($P < 0.01$) IVTD than Silo or MMR. The concentration of

ADL was greater ($P < 0.01$) in Silo and MMR than DBMR. The ADIN concentration of Silo was lesser ($P < 0.04$) than other cultivars.

Table 3.1 Chemical composition of pre-ensiled sorghum cultivars

	Cultivars[1]				
Item	DBMR	PS	Silo	MMR	SEM
DM%	35.17[a]	37.33[a]	34.33[a]	31.00[a]	0.02
CP, % of DM	6.36[a]	6.32[a]	5.59[b]	6.94[a]	0.29
NDF, % of DM	53.85[b]	50.55[c]	52.90[bc]	57.66[a]	1.49
ADF, % of DM	30.32[b]	30.57[b]	31.53[ab]	32.99[a]	0.99
WSC,[2] % of DM	21.33[a]	19.92[a]	17.2[ab]	13.32[b]	3.11
IVTD,[3] % of DM	65.45[a]	63.15[ab]	62.05[bc]	59.82[c]	1.18
ADL, % of DM	4.61[b]	5.84[ab]	5.87[a]	6.43[a]	0.58
ADIN, % of DM	2.99[a]	2.52[a]	2.06[b]	3.12[a]	0.42

a-c Within a row, means without a common superscript letter differ ($P < 0.05$)
[1] DBMR = dairy master BMR, PS = PS 747, Silo = dairy silo 700, MMR= MMR 381/73
[2]WSC = water soluble carbohydrates
[3]IVTD = *in vitro* true digestibility

Chemical Composition of Ensiled Sorghum

The DM of PS silage was greater ($P < 0.01$) than DBMR and MMR silages and treatments did not affect ($P > 0.08$) any of the cultivars (Table 3.2). The CP

concentration of MMR silage was greater ($P < 0.01$) than other cultivars, and was not affected ($P > 0.05$) by treatment. Neutral detergent fiber concentration was less ($P < 0.01$) for MMR silage than Silo and DBMR silages, and treatment with XC decreased ($P < 0.04$) the NDF concentration compared to control for all cultivars. Promote decreased ($P < 0.01$) the NDF concentration of DBMR silage. Among cultivars, Silo silage had the greatest ($P < 0.03$) ADF concentration and MMR silage the least ($P < 0.02$). The ADF concentration was lesser ($P < 0.01$) for all cultivars, except MMR silage, treated with XC compared with control. Treatment with PRO decreased ($P < 0.01$) the ADF concentrations of DBMR and PS silages compared to control. The WSC concentration of PS silage was greater ($P < 0.04$) than DBMR or MMR silages, and treatment with XC increased ($P < 0.02$) the WSC of PS and Silo silages versus control. Treatment with PRO increased ($P < 0.02$) the WSC concentration of DBMR, PS, and Silo silages. The IVTD was not different ($P > 0.25$) among cultivars, and treatment with PRO increased ($P < 0.05$) the IVTD of DBMR silage compared to control. The ADL concentrations of PS and Silo silages were greater ($P < 0.03$) than DBMR silage and was not affected ($P > 0.17$) by treatment for any cultivar. Concentration of ADIN in PS silage was greater ($P < 0.04$) than MMR and DBMR silages, and was not different ($P > 0.09$) between treatments within cultivars.

Table 3.2 Chemical composition of ensiled sorghum with and without treatment with inoculant

	Cultivar[1] × inoculant[2]												
	DBMR			PS			Silo			MMR			
Item	C	XC	PRO	C	XC	PRO	C	XC	PRO	C	XC	PRO	SEM
DM %	24.92^{c-e}	23.52^{ef}	25.95^{a-d}	27.30^{a}	26.38^{a-c}	26.78^{ab}	26.00^{a-d}	25.10^{b-e}	26.73^{ab}	23.73^{ef}	22.17^{f}	24.25^{de}	0.87
CP, % of DM	6.03^{de}	6.73^{b-d}	5.70^{e}	6.22^{c-e}	6.53^{b-d}	6.22^{c-e}	6.12^{de}	6.27^{c-e}	5.82^{e}	7.22^{ab}	7.57^{a}	6.88^{a-c}	0.35
NDF, % of DM	61.28^{a}	54.30^{cd}	54.81^{cd}	59.00^{ab}	54.58^{cd}	56.34^{b-d}	61.18^{a}	57.21^{bc}	59.32^{ab}	56.76^{bc}	52.91^{d}	56.05^{b-d}	1.74
ADF, % of DM	36.06^{bc}	32.51^{ef}	32.54^{ef}	37.04^{ab}	34.00^{c-f}	34.06^{c-e}	38.40^{a}	35.29^{b-d}	36.65^{ab}	33.48^{d-f}	31.72^{f}	33.34^{d-f}	1.07
WSC,[3] % of DM	4.80^{de}	7.03^{cd}	8.25^{bc}	7.72^{c}	10.95^{ab}	11.3^{a}	6.22^{c-e}	10.55^{ab}	11.23^{a}	3.53^{e}	3.88^{e}	4.90^{de}	1.38
IVTD,[4] % of DM	57.62^{bc}	59.25^{a-c}	61.24^{a}	58.29^{a-c}	59.29^{a-c}	59.58^{a-c}	56.26^{c}	56.75^{bc}	59.44^{a-c}	58.45^{a-c}	57.85^{a-c}	60.10^{ab}	1.90
ADL, % of DM	5.21^{bc}	5.25^{bc}	4.08^{c}	7.04^{a}	6.98^{a}	5.94^{ab}	6.80^{a}	6.93^{a}	6.55^{ab}	5.73^{ab}	6.66^{ab}	5.67^{ab}	0.71
ADIN, % of DM	2.97^{d}	3.49^{cd}	3.75^{b-d}	4.79^{a}	4.02^{a-c}	4.01^{a-c}	4.64^{ab}	4.77^{a}	4.21^{a-c}	3.84^{b-d}	4.03^{a-c}	3.57^{cd}	0.32

[a-f] Within a row, means without a common superscript letter differ ($P < 0.05$)
[1]DBMR = dairy master BMR, PS = PS 747, Silo = dairy silo 700, MMR= MMR 381/73
[2]Inoculants C = distilled water control, XC= xylanase and cellulase, PRO = promote
[3]WSC = water soluble carbohydrates
[4]IVTD = *in vitro* true digestibility

Fermentation Indices and Aerobic Stability

The pH of MMR silage was greater ($P < 0.01$) than other cultivars, and treatment of MMR silage with either XC or PRO decreased ($P < 0.01$) silage pH compared to control (Table 3.3). Treatment of DBMR silage with PRO also decreased ($P < 0.01$) silage pH versus control. Ammonia-N concentration was lesser ($P < 0.03$) for PS silage than the other cultivars. The MMR control and XC treated silages had the greatest ($P < 0.02$) NH_3-N concentration, and treatment did not affect ($P < 0.09$) NH_3-N concentration of any cultivar. Lactate concentration was least ($P < 0.01$) and acetate concentration greatest ($P < 0.04$) for MMR silage. Treatment of DBMR silage with PRO increased ($P < 0.02$) lactate concentration, whereas, treatment of MMR silage with XC and Silo silage with PRO decreased ($P < 0.01$) acetate concentration versus control. Propionate concentrations were below measurable limits for most samples. Propionate concentration was greater ($P < 0.01$) in MMR silage than the other cultivars, and treatment of MMR silage with both inoculants decreased ($P < 0.01$) propionate concentration compared to control. Iso-butyrate concentration was greater ($P < 0.03$) in Silo silage than the other cultivars, and treatment of Silo silage with PRO decreased ($P < 0.01$) the iso-butyrate concentration compared to control. Similarly treatment of PS silage with PRO decreased ($P < 0.03$) the iso-butyrate concentration compared to control. Butyrate concentrations were not different ($P > 0.34$) among cultivars or affected ($P > 0.46$) by treatment with inoculant. Total VFA concentration was greatest ($P < 0.04$) for Silo, and was decreased ($P < 0.01$) when Silo silage was treated with PRO. When MMR silage was treated with XC the total VFA concentration increased (*P*

< 0.01) compared to control. Yeast counts were not different ($P > 0.55$) among cultivars or affected by inoculant treatment. Mold counts were not different ($P > 1.0$) among cultivars and were increased ($P < 0.02$) in DBMR silage when treated with XC. Aerobic stability was not different ($P > 0.12$) among cultivars and was improved ($P < 0.02$) by over 62 h when MMR silage was treated with XC.

Disappearance Kinetics

The wash DM fraction was greater ($P < 0.01$) for Silo silage than MMR silage and both inoculants decreased ($P < 0.04$) wash DM fraction of Silo versus control (Table 3.4). The potentially degradable DM fraction was greater ($P < 0.01$) for MMR silage than Silo silage and treatment of MMR silage with XC decreased ($P < 0.01$) the potentially degradable DM fraction. The undegradable DM fraction was greater ($P < 0.01$) for Silo silage than MMR silage and reduced ($P < 0.04$) when Silo silage was treated with PRO. The ERD for DM was decreased ($P < 0.01$) when XC was applied to Silo silage. The lag prior to DM degradation was greater ($P < 0.01$) for Silo silage than MMR silage, and was decreased ($P < 0.04$) when Silo silage was treated with XC compared to control. Rate of DM degradation was faster ($P < 0.01$) for Silo control silage than XC treated Silo silage, and not affected by other inoculants.

Table 3.3 Fermentation characteristics of sorghum silage

	Cultivar × inoculant												
	DBMR			PS			Silo			MMR			
Item	C	XC	PRO	C	XC	PRO	C	XC	PRO	C	XC	PRO	SEM
pH	3.83^{cd}	3.77^{de}	3.67^{e}	3.83^{cd}	3.78^{d}	3.78^{d}	3.83^{cd}	3.8^{d}	3.83^{cd}	4.23^{a}	4.00^{b}	3.92^{bc}	0.05
NH_3-N, % of total N	2.67^{a-d}	2.67^{a-d}	2.0^{b-e}	1.0^{e}	1.33^{de}	1.67^{cde}	3.0^{abc}	2.25^{a-e}	1.67^{cde}	3.5^{ab}	3.67^{a}	2.67^{a-d}	0.76
Lactate, % of DM	4.03^{bcd}	5.09^{ab}	5.53^{a}	4.31^{abc}	4.49^{ab}	5.53^{a}	4.75^{ab}	4.97^{ab}	4.92^{ab}	2.06^{e}	2.89^{de}	3.17^{cde}	0.61
Acetate, % of DM	2.42^{de}	2.25^{de}	2.09^{de}	2.635^{cde}	2.76^{cde}	1.64^{e}	4.51^{bc}	3.73^{bcd}	1.26^{e}	5.02^{b}	7.68^{a}	5.01^{b}	0.97
Propionate, % of DM	0.002^{b}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}	0.350^{a}	0.005^{b}	0.000^{b}	0.01
Iso-Butyrate, % of DM	0.16^{ef}	0.19^{def}	0.07^{f}	0.69^{bc}	0.56^{bcd}	0.25^{def}	1.10^{a}	0.96^{ab}	0.36^{cdef}	0.52^{cde}	0.51^{cde}	0.18^{def}	0.20
Butyrate, % of DM	0.01^{a}	0.003^{a}	0.01^{a}	0.01^{a}	0.02^{a}	0.01^{a}	0.02^{a}	0.03^{a}	0.01^{a}	0.02^{a}	0.05^{a}	0.002^{a}	0.02
Total VFA, % of DM	6.63^{d}	7.53^{cd}	7.71^{cd}	7.64^{cd}	7.83^{bcd}	7.44^{cd}	10.38^{ab}	9.69^{abc}	6.54^{d}	7.65^{cd}	11.14^{a}	8.36^{bcd}	1.28
Yeasts, log cfu/g	11.84^{a} ^{b}	6.00^{ab}	5.03^{ab}	10.33^{ab}	5.80^{ab}	14.47^{a}	3.20^{ab}	0.95^{ab}	9.34^{ab}	5.78^{ab}	0.58^{b}	6.17^{ab}	6.85
Molds, log cfu/g	10.0^{b}	175^{a}	10.0^{b}	10.0^{b}	10.0^{b}	10.0^{b}	10.0^{b}	10.0^{b}	10.0^{b}	10.0^{b}	10.0^{b}	10.0^{b}	67.36
Aerobic stability, h	25.37^{b}	22.41^{b}	24.72^{b}	26.47^{b}	32.90^{b}	41.25^{b}	59.56^{ab}	54.81^{ab}	17.97^{b}	34.46^{b}	97.27^{a}	44.97^{ab}	26.25

[a-f] Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 3.4 *In situ* DM disappearance kinetics of two sorghum silage cultivars treated with inoculants prior to ensiling

	Cultivar × inoculant						
	Silo			MMR			
Item	C	XC	PRO	C	XC	PRO	SEM
Wash fraction, %	37.78[a]	28.19[cb]	32.31[b]	29.45[bc]	27.23[c]	29.11b[c]	1.64
Potentially degradable fraction, %	34.99[c]	40.22[bc]	38.29[c]	45.61[ab]	37.77[c]	48.45[a]	1.92
Undegradable fraction, %	38.03[a]	35.97[ab]	35.43[b]	31.75[c]	29.39[c]	31.24[c]	0.8
Extent of digestion, %	57.24[a]	41.17[c]	49.90[ab]	50.51[ab]	44.41[bc]	52.39[a]	2.41
Lag time, h	9.10[a]	0.24[b]	4.50[ab]	0.00[b]	0.00[b]	0.65 [b]	2.08
K_d[1], per h	0.07[a]	0.02[b]	0.05[ab]	0.04[ab]	0.04[ab]	0.05[ab]	0.01

[a-c] Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]Fractional rate of digestion.

The wash NDF fraction was greater ($P < 0.01$) for Silo silage than for MMR silage (Table 3.5). Treatment of Silo silage with XC increased ($P < 0.04$) the wash NDF fraction compared to control. The potentially degradable NDF fraction was greater ($P < 0.03$) for XC treated Silo silage than control. The undegraded NDF fraction was greater ($P < 0.01$) for Silo silage than MMR silage, and treatment of Silo silage with PRO decreased ($P < 0.04$) undegraded NDF fraction compared to control. The ERD for NDF fraction was greater ($P < 0.05$) for Silo silage than MMR silage, and when Silo silage was treated with XC the ERD of NDF was decreased ($P < 0.02$). Lag time of NDF degradation was not different ($P > 0.29$) between cultivars or affected by treatment. Rate of NDF digestion was not different ($P > 0.18$) between cultivars and treatment with XC decreased ($P < 0.04$) the rate of NDF digestion of Silo silage.

The wash ADF fraction was greater ($P < 0.01$) for Silo silage than for MMR silage and not affected ($P > 0.12$) by either inoculant treatment (Table 3.6). The potentially degradable ADF fraction was greater ($P < 0.04$) for MMR than Silo silage, and treatment with XC increased ($P < 0.04$) the potentially degradable ADF fraction of Silo silage compared to control. The undegradable ADF fraction was greater ($P < 0.01$) for Silo silage than MMR silage, and addition of PRO to Silo silage decreased ($P < 0.04$) undegradable ADF fraction. The ERD, lag time, and digestion rate of ADF was not different ($P > 0.05$) between cultivars or affected by treatment with either inoculant.

Table 3.5 *In situ* NDF disappearance kinetics of two sorghum silage cultivars treated with inoculants prior to ensiling

	Cultivar × inoculant						
	Silo			MMR			
Item	C	XC	PRO	C	XC	PRO	SEM
Wash fraction, %	48.78[a]	40.32[bc]	44.53[ab]	37.35[bc]	36.47[c]	34.4[c]	2.61
Potentially degradable fraction, %	48.14[bc]	57.28[a]	46.53[c]	56.08[ab]	52.38[abc]	57.79[a]	2.68
Undegradable fraction, %	38.03[a]	35.97[ab]	35.43[b]	31.75[c]	29.39[c]	31.24[c]	0.8
Extent of digestion, %	74.49[a]	60.00[b]	67.34[ab]	61.96[b]	57.09[b]	59.28[b]	3.96
Lag time, h	8.39[a]	4.40[a]	8.21[a]	5.22[a]	6.35[a]	4.92[a]	2.01
K_d[1], per h	0.06[a]	0.03[b]	0.05[ab]	0.04[ab]	0.03[ab]	0.04[ab]	0.01

[a-c] Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]Fractional rate of digestion.

Table 3.6 *In situ* ADF disappearance kinetics of two sorghum silage cultivars treated with inoculants prior to ensiling

	Cultivar × inoculant						
	Silo			MMR			
Item	C	XC	PRO	C	XC	PRO	SEM
Wash fraction, %	50.48[a]	42.98[ab]	45.21[ab]	36.74[b]	37.80[b]	37.84[b]	3.15
Potentially degradable fraction, %	44.02[b]	55.21[a]	44.11[b]	55.76[a]	49.58[ab]	53.40[ab]	3.51
Undegradable fraction, %	38.03[a]	35.97[ab]	35.43[b]	31.75[c]	29.39[c]	31.24[c]	0.8
Extent of digestion, %	75.67[a]	62.70[a]	66.96[a]	59.66[a]	60.78[a]	63.54[a]	5.14
Lag time, h	9.67[a]	5.61[a]	13.41[a]	6.22[a]	12.16[a]	8.71[a]	3.79
K_d[1], per h	0.07[a]	0.03[a]	0.05[a]	0.04[a]	0.07[a]	0.05[a]	0.02

[a-c] Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]Fractional rate of digestion.

The wash CP fraction was greater ($P < 0.01$) for MMR silage than Silo silage and was increased ($P < 0.01$) when XC was applied (Table 3.7). The potentially degradable CP fraction was not different ($P > 0.37$) between cultivars. Treatment of Silo silage with PRO increased ($P < 0.02$) the potentially degradable CP fraction. The undegradable CP fraction was greater ($P < 0.01$) for Silo silage than MMR silage. Treatment of MMR silage with XC or Silo silage with either inoculant decreased ($P < 0.01$) the undegradable CP fraction. Crude protein ERD was greater ($P < 0.01$) for MMR silage than Silo silage, and MMR silage treated with XC had greater ($P < 0.01$) ERD for CP than control. The rate of CP digestion was not different ($P > 0.81$) between cultivars, and was greater ($P < 0.02$) for MMR silage treated with XC than the control.

Discussion

Yield and Chemical Composition of Pre-Ensiled Sorghum

The PS cultivar is a PPS forage variety and had a greater yield than the MMR forage variety at both locations. This is consistent with previous reports PPS varieties yield greater than other types of sorghums (Bean, 2009). The cultivar with the greatest height was the DBMR forage cultivar which was higher growing at both locations than MMR. These results agree with results from a forage sorghum trial in the Texas Panhandle (Bean, 2009).

Table 3.7 *In situ* crude protein disappearance kinetics of two sorghum silage treated with inoculants prior to ensiling

	Cultivar × inoculant						
	Silo			MMR			
Item	C	XC	PRO	C	XC	PRO	SEM
Wash fraction, %	61.44^{d}	66.69^{b}	61.49^{d}	63.89^{c}	69.10^{a}	56.01^{e}	0.35
Potentially degradable fraction, %	22.21^{bc}	21.90^{bc}	24.63^{a}	23.08^{abc}	21.24^{c}	23.83^{ab}	0.66
Undegradable fraction, %	18.06^{a}	10.22^{c}	14.56^{b}	12.88^{b}	9.87^{c}	19.20^{a}	0.66
Extent of digestion, %	69.18^{c}	73.14^{bc}	72.73^{bc}	75.05^{b}	86.06^{a}	71.78^{bc}	1.33
K_d[1], per h	0.03^{b}	0.02^{b}	0.04^{b}	0.05^{b}	0.29^{a}	0.10^{b}	0.06

[a-c] Within a row, means without a common superscript letter differ ($P < 0.05$).
[1] Fractional rate of digestion.

The pre-ensiled sorghum DM was similar across cultivars and was consistent with the recommended 35% DM pre-ensiling (Kung, 2000). Neutral detergent fiber and ADF concentrations were greatest in the MMR cultivar, however, it is consistent with previously reported values for non-BMR cultivars (Bean and Marsalis, 2012). The structural carbohydrate concentrations of Silo are greater than MMR likely due to the morphology of the taller and greater yielding Silo cultivar (Bean, 2009). The concentration of WSC was greater in DBMR cultivar than MMR, whereas, all cultivars showed greater values than freshly chopped sorghum results from Tabacco et al. (2011). The lesser ADL concentration of DBMR than Silo and MMR and greater WSC concentration of DBMR than MMR contributed to the greater IVTD, which agrees with Bean et al. (2009).

Chemical Composition of Ensiled Sorghum

The DM concentrations among cultivars and between inoculants were not different which agreed with 2004 studies by Colombatto et al. (b, c), which determined fibrolytic enzymes did not affect corn silage DM. Williams and Shinners also found no differences in DM concentrations due to bacterial inoculant or sorghum type (2012). Lack of treatment effect indicates inoculants did not affect potential DM losses due to plant respiration (Muck, 1988). Concentrations of NDF were decreased for all cultivars treated with XC and ADF concentrations decreased in all cultivars, except MMR, which is consistent with previous experiments indicating the effect of XC to reduce the fiberous fraction of corn silage (Colombatto et al., 2004 b,c and Arriola et al., 2011). Inoculant PRO reduced the NDF concentration of DBMR, likely because it is a BMR, which has

lesser NDF concentration and less undegradable components (Bean, 2009). The ADF concentrations of DBMR and PS treated with PRO were reduced, which is consistent with previously published reports indicating ADF concentration of corn silage inoculated with similar bacterial inoculants was reduced (Arriola et al., 2011).

The WSC was greater in DBMR, PS, and Silo inoculated with PRO and cultivars PS and Silo treated with XC, therefore, they were both effective at increasing the availability of fermentation substrates which is consistent with Colombatto et al., 2004b and inconsistent with Colombatto et al., 2004c which found a lesser WSC after treatment with fibrolytic enzymes on corn silage. Kleinschmit and Kung found no difference in WSC after inoculation of corn silage with bacterial inoculants (2006). Pre-ensiled MMR had the least amount of WSC and was the only cultivar unaffected by inoculant treatment, indicating silage bacteria may have had accessibility difficulties due to the reduced polysaccharides inhibiting inoculant effectiveness (William and Shinners, 2012).

The IVTD was greatest for DBMR silage treated with PRO which is reasonable since ADL for DBMR treated with PRO was least among all similarly treated cultivars. Previous studies did not measure ADL or IVTD for comparison with these results. Treating sorghum silage with XC or PRO reduced the NDF and ADF fiber fraction which increased the cell wall degradability. Treatment of DMBR silage with PRO reduced ADL causing an increase of IVTD (McCollum et al., 2012). There were no studies found to compare effects of enzyme or bacterial inoculation of non-BMR to BMR sorghum silage.

Fermentation Indices and Aerobic Stability

Silage pH was only reduced by XC in MMR silage and PRO in DBMR and MMR silages. This is likely due to the increase of lactate concentration of XC treated DBMR silage, treatment of MMR silage with either inoculant only tended to increase lactate concentration of silage. Reduction in pH and improved lactate production has been seen in previous results of sorghum silage treated with bacterial inoculant (Williams and Shinners, 2012; Filya, 2003). Similar to these results, previously reported literature indicates inoculation with fibrolytic enzymes or bacterial inoculants does not affect NH_3-N concentration of corn silage (Shepard and Kung, 1996; Kleinschmit and Kung, 2006).

Acetate concentrations were greatest for XC treated MMR, which explains the 62 h improvement in aerobic stability because acetate is known to inhibit fungi (Moon, 1983). However, the bacterial inoculant PRO did not improve yeast and mold counts or aerobic stability of sorghum silage compared to the control, which may be due to the lesser acetate concentrations, especially of PRO treated Silo silage. Jones (2012) suggested the use of *L. buchneri* on corn silage with a DM content of 32% or less produces inactive acetic acid causing an energy loss without providing significant improvement in aerobic stability. This likely occurred for MMR silage treated with PRO in this experiment. The application of bacterial inoculants to corn and sorghum silage have been successful at improving fermentation products in less than half of the reported studies (Muck and Bolsen, 1991). Williams and Shinners (2012) found that sorghum silage that had a greater DM concentration and greater amount of bacterial

inoculant (38.5-57.9% and 500,000 CFU g^{-1} of silage, respectively) than this experiment (25.5% average of control silage and 100,000 CFU g^{-1} of silage, respectively) exhibited increased production of fermentation products which lowered yeast and mold populations during aerobic exposure and improved aerobic stability so little heating occurred in inoculated sorghum silage.

Disappearance Kinetics

The wash DM, NDF, and ADF fractions were greater and wash CP fraction lesser for Silo silage than MMR silage. This is likely due to the tendency of greater WSC concentration and greater total VFA of Silo silage than for MMR silage. Treating Silo silage with XC decreased the wash DM and NDF fractions. The potentially degradable DM and ADF fractions were greater for MMR silage than Silo silage because of lesser NDF and ADF of MMR silage. Treatment of MMR silage with XC decreased the potentially degradable DM fraction, which is consistent with the decrease of NDF concentration of XC treated MMR silage versus control. The Silo cultivar had greater NDF and ADF concentrations than MMR, and treatment with XC was not successful in increasing potentially degradable DM fraction, but the potentially degradable NDF and ADF fractions were increased. These disappearance kinetics are consistent with *in vitro* results from corn silage showing increased soluble losses compared to controls, indicating fibrolytic enzymes solubilized material that contributed to the increase in the initial organic matter degradation, suggesting enzymes also enhanced accessibility of the insoluble, yet potentially degradable organic matter (Colombatto et al., 2004a,b,c). The extent of DM and NDF digestion for Silo was decreased due to the reduced rate of

digestion of DM and NDF which was caused by decreased structural carbohydrate concentration of XC treated silage. The reduced structural carbohydrate concentration also resulted in a reduced lag time for DM degradation. Since XC reduced the total and less digestible fiber fractions, it could potentially increase intake and digestibility of sorghum silage fed to cattle. Treatment with XC may also reduce structural carbohydrates binding soluble carbohydrates used for ethanol production, thus improving the efficiency of ethanol production from feedstock (Han et al., 2007).

The undegraded DM, NDF, and ADF fractions were greater for Silo than MMR, and greater in untreated sorghum silage than the PRO treated silage. Treatment with PRO also increased the wash DM fraction and potentially degradable ADF fraction of Silo silage. This indicates PRO may be more effective at reducing the undegradable fraction of sorghum silage cultivars having a greater fiber fraction due to greater hemicellulose and lignin bonds (Álvarez et al., 2009; Bean, 2009). Part of the cell wall contents can be loss due to the acidic conditions as well as microbial activity during the ensiling process (Morrision, 1979). An *in vivo* digestibility trial which fed bacterial inoculated corn silage showed similar reductions in NDF and ADF fractions, however, feed efficiency was not affected by treatment (Arriola et al., 2011). Sorghum silage harvested at hard dough stage, which is a later maturity than the sorghum silage in this experiment, did not exhibit decreased cell wall content due to bacterial inoculant (Williams and Shinners, 2012).

The wash CP fraction was greater for MMR silage than Silo silage because the CP concentration of MMR silage was greater. When Silo silage was treated with PRO

the potentially degradable CP fraction was increased, likely due to the reduced undegradable DM and NDF fractions which released bound protein indicated by the ADIN concentrations of silage. Treatment of Silo silage with PRO decreased undegradable CP fraction, whereas, treatment of MMR silage with PRO increased the undegradable CP fraction. Treatment with XC consistently decreased undegradable CP fraction of both cultivars. Generally, inoculants facilitated degradation of cell wall bound proteins which reduced the fiber, releasing available CP (Kohn and Allen, 1992). The extent of CP digestion was increased by XC in MMR silage, likely due to the faster rate of CP digestion. The rate of CP digestion was faster when treated with XC because of the increase of wash CP fraction and decrease of undegradable CP fraction.

Summary

Fibrolytic enzyme XC reduced the NDF concentrations of all cultivars and improved the DM, NDF, ADF, and CP disappearance kinetics of both MMR and Silo, but especially of Silo, the cultivar with the greatest structural carbohydrate concentration. The reduction in potentially degradable NDF fraction should improve silage intake which should associate with increased milk production in dairy cattle (Chen et al., 1994). More research is recommended to determine whether the improved nutritive value of sorghum silage treated with XC inoculation translated into enhanced forage quality and ethanol extraction or not. In addition, XC increased the aerobic stability in MMR silage due to the greater amount of acetate which promotes aerobic stability (Jones, 2012). Bacterial inoculant PRO only reduced the NDF and ADF concentrations of DBMR and PS silages, the cultivars with moderate ADF

concentrations. The undegradable DM, NDF, ADF, and CP fractions of Silo silage were decreased when treated with PRO. Treatment with PRO did not improve ensiling characteristics enough to increase aerobic stability, and only improved lactate in one cultivar, therefore, it is not suggested for improving aerobic stability of sorghum silage. It is most efficient to apply inoculants to sorghum silage with greater structural carbohydrate concentrations such as those that are harvested as a more advanced stage of maturity. Treatment with fibrolytic enzymes XC has more benefit on fermentation characteristics and *in situ* disappearance kinetics than treatment with the bacterial inoculant used in this experiment.

CHAPTER IV

INOCULANTS TO ENHANCE THE ENERGY AND PROTEIN BALANCE OF SMALL- GRAIN HAY

Introduction

Oat and wheat are commonly grown in the southern United States because there are sufficient growing degree days to produce 120 to 150 d of forage that can be grazed, harvested for conserved forage, or harvested for grain making it a dual-purpose crop (Holman et al., 2010). When cool-season forages are fed to ruminant livestock there is typically an asynchrony of C:N, which is due to the greater N concentration than that of energy (Poppi and McLennan, 1995). Typically, the TDN:CP is 3.75 in small-grain forages grazed and cut for hay (Holman, 2010). An asynchronous diet limits the growth of rumen microbes because of an inadequate amount of either energy or N substrates. The reduction in ruminal microbial activity results in decreased degradation of digesta and microbial efficiency (Hersom, 2008). To address this issue, ruminants are usually fed a grain-based supplement. Grain supplementation may shift the rumen microorganism population from cellulolytic to amylolytic microbes, thus reducing forage digestibility (El-Shazly et al., 1961). This reduction in forage digestibility is the cause of the substitution effect, whereby the energy source substitutes for forage by reducing forage intake (Mathis, 2003). The starch content of the energy supplement suppresses intake and digestion, which reduces the energy derived from the basal forage diet. Therefore, the energy intake of the animal may not increase to the desired level because of a reduction in forage intake.

An alternative to grain supplementation is increasing the digestibility of the cell wall portion of forage. The application of fibrolytic enzymes or bacterial inoculants to small-grain forage may increase energy availability by increasing the degradation of hemicellulose and lignin (Beauchemin et al., 2003). This reduction in hemicellulose and lignin binding will make the soluble carbohydrates more available to rumen microbes increasing the energy availability (Elwakeel et al., 2007) and enhancing the synchrony of C:N. Past studies have shown ADG was increased by 10% when enzymes were added to diet containing 30% rye-grass silage (McAllister et al., 1999). Feng et al. found the addition of fibrolytic enzymes to cool-season grass hay before feeding has the potential to enhance intake and digestion (1996). These improvements may be due to fibrolytic enzymes increasing fiber degradation thus enhancing C:N synchrony.

The objective of this study was to determine if fibrolytic enzyme XC or bacterial inoculant PRO would reduce the fiber fraction of wheat and oat hay. Improving fiber degradation *in situ* should improve the availability of energy to the rumen microbes.

Materials and Methods

Wheat (cv. Fannin and TAM 203) and oat (cv. TAMO 606 and Harrison) were planted at College Station, TX, (30°N, 96°W) on September 22, 2011. Four replicate plots were planted as a completely randomized design. Per soil analysis recommendations 22 kg ha^{-1} of N was applied as urea (46-0-0). Clethodim was applied to voluntary corn plants on October 3 with a rope wick at 0.88 L ha^{-1}. On October 11, Chlorsulfuron + Flucarbazone-Sodium was applied at 0.07 L ha^{-1}. On October 25,

Octanoic acid ester of bromoxynil + Heptanoic acid ester of bromoxynil was applied at 1.17 L ha^{-1}. Plots were irrigated (3.8 cm) pre-planting on September 16, and on October 6 (3.8 cm), October 26 (3.8 cm), and November 2 (1.9 cm). Forage was harvested at tillering stage 83 days after planting (**H1**), after 49 days of regrowth at tillering stage (**H2**), and 104 days after H2 the stover remaining after grain harvest (**H3**). Plant material from H1, H2, and H3 was harvested from each plot to a stubble height of 5 cm. Three random samples of 1 kg of material from each plot was sprayed with either 40 ml distilled water (control), 0.268 ml XC kg^{-1} in 40 ml distilled water, or 3.3 mg PRO kg^{-1} in 40 ml distilled water. When the harvested material was less than 1 kg, only 0.5 kg was collected and treated with half of the spray treatment. After treatment, samples were dried at 65°C until weight loss ceased, weighed, and milled to 4-mm in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) for *in situ* incubation and a subsample of approximately 200 g was then ground to 1-mm for nutritive value analyses. Growing season and 30-year average temperature was obtained from NOAA (2012).

Laboratory Analyses

Hay samples were analyzed for DM, CP, NDF, ADF, ADL, and IVTD, and post-*in situ* samples were analyzed for DM, NDF, and ADF. Forage samples were dried at 135°C for 8 h for DM determination (AOAC, 1990). Nitrogen was determined by rapid combustion using a Macro elementar N analyzer (Elementar Americas, Mt. Laurel, NJ), and CP was calculated as N × 6.25.

Concentrations of NDF and ADF were measured using the Van Soest et al. (1991) method in an ANKOM 200 Fiber Analyzer (ANKOM Technologies, Macedon, NY). Lignin was measured using the ANKOM (2011) procedure for determining ADL in beakers based on Van Soest et al. (1967) procedure. The Van Soest et al. (1966) method and an ANKOM Daisy^{II} Incubator (ANKOM Technology) were used to determine IVTD. Total digestible nutrients was estimated based on ADF (Bull, 1981).

In Situ Incubation Procedures

An *in situ* study was conducted to determine the ruminal degradability and degradation rates of Harrison oat and Fannin wheat hay pretreated with fibrolytic enzymes or microbial inoculant. Harrison and Fannin cultivars were selected because these cultivars contained the greatest NDF and ADF concentrations within each forage species. Because of limited forage harvested at H1 and H2, samples were composited so tillering and stover maturity stages were represented. Six ruminally fistulated and cannulated Angus crossbred steers weighing approximately 907.18 ± 45 kg were used for the study. Animal care and use protocols are on file at Texas A&M University-Kingsville and all regulations for the proper care of livestock were followed. The trial began after a 10-day adaptation to small-grain hay (8.69% CP, 65.34% NDF, and 40.75% ADF [DM basis]), water, and trace mineral salt block (Ca, NaCl, S, Co, Cu, I, Fe, Mn, Se, and Zn) provided *ad libitum*. During the 6-day trial steers consumed 92.5 kg hd^{-1} d^{-1}. During *in situ* incubation, daily samples of hay and supplement were collected, dried to constant weight at 65°C, ground, composited, and subsampled (400 g) for laboratory analysis (DM, CP, NDF, and ADF).

Approximately 3.5 g (as fed) of material was weighed in sextuplet polyester bags (10 × 20 cm; 53 ± 10 μm pore size; Bar Diamond, Inc., Parma, ID). All samples (except 0 h) were incubated during 2 periods with 42 samples period^{-1} steer^{-1} in the rumen and removed at 3, 6, 12, 24, 36, and 60 h. Immediately after removal bags were placed in ice water, rinsed with approximately (39°C) tap water, placed in plastic bags, and frozen (-20°C) until all bags were incubated. Incubated and 0 h bags were washed with one cycle in a front load commercial washing machine (Speed Queen, Ripon, WI). Following *in situ* incubation and washing, bags were dried at 60°C to constant weight. Remaining sample material for each forage, treatment, and incubation time was composited among animal (n=6).

In situ rumen DM, NDF, and ADF degradation data were fitted to the first order exponential model with discrete lag (Mertens, 1977) using the iterative Marqardt method and the NLIN procedure of SAS (SAS Institute, Cary, NC). The model is of the form:

$$R_{(t)} = B \times (e^{-kd(t-L)}) + C,$$

where $\boldsymbol{R(t)}$ = total indigested residue at any time t, $\boldsymbol{B}$ = insoluble potentially digestible fraction, $\boldsymbol{k_d}$ = fractional rate of digestion of B, $\boldsymbol{t}$ = time incubated in the rumen in h, $\boldsymbol{L}$ = discrete lag time in h, and $\boldsymbol{C}$ = fraction not digested after 96 h of incubation. The wash fraction $\boldsymbol{A}$ was the percentage of substrate washed out of the bag at 0 h. The ERD was, calculated using the model of Ørskov and McDonald (1979):

$$\text{ERD} = A + (B \times [k_d / (k_d + k_p)])$$

where $\boldsymbol{k_p}$ = assumed ruminal passage rate of 0.05.

Statistical Analyses

Statistical analyses were conducted utilizing SAS (SAS, Inst. Inc., Cary, NC). Nutritive value (DM, CP, NDF, ADF, ADL, IVTD, TDN, and TDN:CP) and *in situ* degradation parameters (DM, NDF, and ADF) were analyzed with PROC GLIMMIX. The model included cultivar × harvest × inoculant. Where significant ($P < 0.05$) effects were observed least square means were compared with LSMEANS (Fisher's LSD test for multiple comparisons).

Results

Chemical Composition of Small-Grain Hay

The average monthly temperature from December to the final harvest was greater than the 30-yr average during this experiment (Figure 4.1). The temperature was warmer during growth of H1 forage than during regrowth of H2 forage. The chemical composition of hays was not affected ($P > 0.28$) by treatment, so data shown is the average of each cultivar and maturity across treatment (Table 4.1). Crude protein concentration was greater ($P < 0.01$) at H1 and H2 than H3 and not different ($P > 0.05$) between cultivars at H1or H3. Crude protein concentration at H2 was greater ($P < 0.01$) for Harrison oat than Fannin wheat, and intermediate for TAMO 606 oat and TAM 203 wheat. Generally, NDF and ADF concentrations were greater ($P < 0.01$) and IVTD and ADL concentration lesser ($P < 0.01$) at H3 than at either H1 or H2.

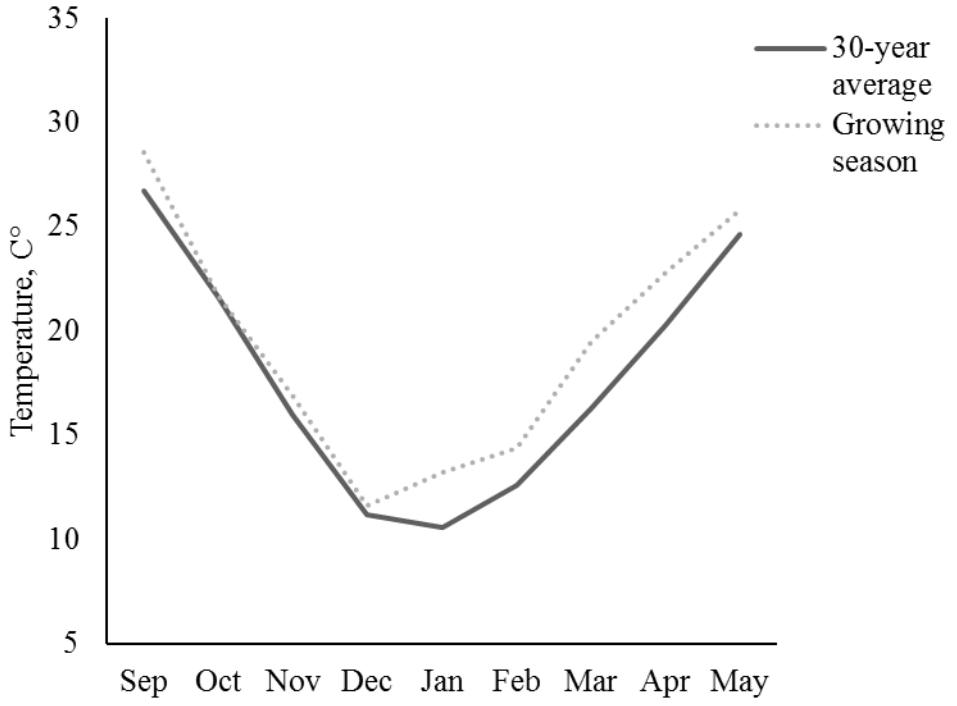

Figure 4.1 Growing season and 30-year average temperature at College Station, TX.

The NDF concentration was greater ($P < 0.01$) in Fannin wheat than either oat cultivar at H1. The wheat cultivars had greater ($P < 0.01$) NDF concentrations than the oat cultivars at H2 and H3. Acid detergent fiber concentrations were greater ($P < 0.04$) for both wheat cultivars than TAMO 606 oat at H1, and either oat cultivar at H2 and H3. Oat cultivars had greater ($P < 0.04$) IVTD during H1 and H3 than the wheat cultivars. Harrison oat had greater ($P < 0.02$) IVTD than Fannin wheat, and the other cultivars were intermediate at H2. At H1, ADL concentration was greater ($P < 0.02$) in TAM 203 wheat than either oat cultivar, and not different ($P > 0.08$) among cultivars at H2 or H3. The ADL concentration among cultivars was greatest ($P < 0.02$) at H1 and least at H3,

whereas, H2 was intermediate. The TDN concentration was not different (P > 0.05) among cultivars or harvest maturity, and was greater (P < 0.05) for oat at H2 maturity than wheat at H3 maturity. The TDN:CP was not different (P > 0.05) between cultivars, whereas, wheat from H3 had greater (P < 0.05) TDN:CP than wheat and oat from H1 and H2.

Disappearance Kinetics

At each harvest, the wash DM fraction was not different ($P > 0.08$) for any of the cultivars, but was greater ($P < 0.01$) at tillering than stover maturity stage (Table 4.2). Treatment of Fannin stover with XC resulted in a greater ($P < 0.01$) wash DM fraction than control or PRO, whereas, Harrison stover was not affected ($P > 0.53$) by treatment. The potentially degradable DM fraction was greater ($P < 0.01$) at stover than at tillering maturity stage. Potentially degradable DM fraction of untreated Fannin at tillering was greater ($P < 0.02$) than untreated Harrison. The potentially degradable DM fraction of Harrison at tillering was greater ($P < 0.01$) for XC treated hay than untreated hay, whereas, PRO treated hay was not different ($P < 0.14$). There was no treatment effect ($P > 0.43$) for Fannin at tillering, however, stover treated with PRO had greater ($P < 0.01$) potentially degradable DM fraction than untreated hay. The undegradable DM fraction was not different ($P > 0.09$) between hays or treatments at tillering, and was greater ($P < 0.01$) at stover than at tillering maturity stage. Stover hay undegradable DM fraction of Harrison was not effected by treatment ($P > 0.27$), however, PRO treated Fannin hay had lesser ($P < 0.01$) undegradable DM fraction than untreated hay. The extent of DM digestion was greater ($P < 0.01$) at tillering than at stover maturity stage, and was not

different ($P > 0.33$) between either cultivar at tillering. Although there was no effect ($P > 0.70$) of treatment on extent of DM digestion for stover hay made from Harrison, treatment with XC increased ($P < 0.04$) extent of DM digestion versus untreated Fannin stover. Lag prior to DM degradation of Fannin was greater ($P < 0.02$) than Harrison hay at both maturity stages. Inoculant treatment did not improve ($P > 0.31$) DM lag time for either hay type or maturity. Lag time prior to DM degradation for Harrison hay was not effected ($P > 0.07$) by inoculant treatment, nor were there differences ($P > 0.13$) across Fannin treatments. Rate of DM degradation was faster ($P < 0.01$) at tillering maturity stage than stover maturity stage, and fastest for Harrison. Treatment of Harrison with either inoculant at tillering increased ($P < 0.01$) rate of DM degradation.

The wash NDF fraction was greater ($P < 0.01$) at tillering than at stover maturity stages, and was greater ($P < 0.01$) for Harrison than Fannin at tillering, but not different ($P > 0.25$) between cultivars at stover maturity (Table 4.3). Both treatments increased ($P < 0.01$) wash NDF fraction of both cultivars at tillering, whereas, only PRO treated Harrison increased ($P < 0.03$) wash NDF fraction at stover maturity stage. Potentially degradable NDF fraction was greatest ($P < 0.01$) for Fannin at tillering than at stover or either maturity of Harrison hays. The potentially degradable NDF fraction of Harrison at tillering was not effected ($P > 0.47$) by treatment, however, XC treated stover had greater ($P < 0.01$) potentially degradable NDF fraction than untreated hay. Both treatments decreased ($P < 0.02$) potentially degradable NDF fraction of Fannin at tillering, whereas, stover treated with PRO was greater ($P < 0.01$) than untreated hays.

Table 4.1 Chemical composition of hays of two oat cultivars and two wheat cultivars at three harvests

	Cultivar × harvest												
	Oat						Wheat						
	Harrison			TAMO 606			Fannin			TAM 203			
Item	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3	SEM
CP, % of DM	16.71^{cde}	19.79^{a}	6.85^{f}	15.96^{de}	19.2^{ab}	7.45^{f}	15.75^{de}	17.4^{bcd}	5.67^{f}	14.96^{e}	18.36^{abc}	5.63^{f}	0.94
NDF, % of DM	43.05^{d}	37.06^{f}	65.30^{b}	40.28^{e}	37.54^{f}	65.05^{b}	45.99^{c}	43.17^{d}	72.63^{a}	45.61^{cd}	43.12^{d}	73.12^{a}	1.37
ADF, % of DM	30.42^{de}	26.04^{g}	43.00^{b}	28.61^{ef}	26.59^{fg}	42.07^{b}	32.45^{cd}	29.37^{e}	47.75^{a}	33.06^{c}	29.09^{e}	48.05^{a}	1.19
IVTD[1], %	80.11^{b}	84.81^{a}	57.07^{d}	79.49^{b}	82.42^{ab}	55.70^{d}	74.99^{c}	79.91^{b}	50.05^{e}	74.58^{c}	81.29^{ab}	44.49^{e}	2.11
ADL, % of DM	17.94^{bc}	14.49^{d}	8.50^{e}	17.73^{bc}	14.46^{d}	7.49^{e}	19.44^{ab}	16.57^{cd}	9.08^{e}	20.79^{a}	16.64^{cd}	9.17^{e}	0.86
TDN[2]	66.76^{ab}	68.12^{a}	62.88^{ab}	67.32^{ab}	67.95^{a}	63.16^{ab}	66.14^{ab}	67.09^{ab}	61.41^{b}	65.95^{ab}	67.17^{ab}	61.32^{b}	2.53
TDN: CP	4.00^{b}	3.44^{b}	9.18^{ab}	4.22^{b}	3.54^{b}	8.48^{ab}	4.20^{b}	3.86^{b}	10.83^{a}	4.41^{b}	3.66^{b}	10.89^{a}	3.00

[a-g] Within row, means without a common superscript letter differ ($P < 0.05$).

[1] In vitro true digestibility.

[2] Total digestible nutrients.

Undegradable NDF fraction was greater ($P < 0.01$) at stover than tillering maturity stages. Treatment with XC decreased ($P < 0.05$) undegradable NDF fraction of Harrison at both maturities. At tillering undegradable NDF fraction of Fannin was not affected ($P > 0.22$) by treatment, but treatment with PRO at stover maturity decreased ($P < 0.01$) the undegradable NDF fraction. Extent of NDF digestion was greater ($P < 0.01$) at tillering, and greater ($P < 0.01$) for Harrison than Fannin. Extent of NDF digestion for Harrison at tillering was not different ($P > 0.27$) among treatments, whereas, both treatments increased ($P < 0.01$) extent of NDF digestion at stover maturity. Extent of NDF digestion for Fannin at tillering was greater ($P < 0.01$) when treated with PRO, whereas, stover was not different ($P > 0.13$) among treatments. Lag time of NDF degradation was generally greater at stover than tillering maturity stage, and not different ($P > 0.50$) between cultivars at each maturity. Lag of NDF degradation was decreased ($P < 0.01$) when Harrison stover was treated with XC and Fannin stover treated with PRO. Rate of NDF digestion among cultivars was faster ($P < 0.01$) at tillering than at stover maturity, and fastest for Harrison at tillering maturity stage and slowest for Fannin at stover maturity stage. Rate of NDF digestion for XC treated Harrison at tillering was slower ($P < 0.01$) than untreated hay, whereas, rate of NDF digestion among Fannin harvests and treatments were not affected by treatment ($P > 0.12$).

Table 4.2 *In situ* DM disappearance kinetics of hays of oat and wheat cultivars at tillering and stover

	Cultivar × harvest												
	Harrison oat						Fannin wheat						
	Tillering			Stover			Tillering			Stover			
	C	XC	PRO	C	XC	PRO	C	XC	PRO	C	XC	PRO	SEM
A[1], %	58.61[a]	56.71[a]	58.74[a]	9.87[bcd]	11.92[bc]	9.0[bcd]	53.84[a]	55.45[a]	53.55[a]	3.86[cd]	15.81[b]	2.45[d]	3.25
B[2], %	33.47[d]	38.91[c]	35.79[cd]	44.77[b]	45.07[b]	45.46[b]	38.46[c]	36.63[cd]	38.30[c]	47.81[b]	46.93[b]	54.4[a]	1.47
C[3], %	6.43[d]	4.38[d]	5.0[d]	45.36[abc]	43.09[bc]	45.55[abc]	7.7[d]	7.92[d]	8.16[d]	46.92[ab]	47.76[a]	41.68[c]	1.55
ERD[4], %	82.83[a]	79.81[a]	80.81[a]	30.73[bc]	34.31[b]	29.17[bc]	76.77[a]	77.01[a]	77.42[a]	20.72[c]	34.00[b]	29.17[bc]	4.37
L[5], h	0.95[e]	1.64[cde]	0.91[e]	1.59[cde]	2.91[a-d]	2.13[b-e]	2.72[a-d]	1.65[cde]	1.55[de]	2.99[abc]	4.07[a]	3.50[ab]	0.50
K_d[6], per h	0.13[a]	0.09[b]	0.08[b]	0.04[cd]	0.05[c]	0.05[c]	0.08[b]	0.08[b]	0.09[b]	0.03[cd]	0.03[cd]	0.04[d]	0.01

[a-e] Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]Wash fraction.
[2]Potentially degradable fraction.
[3]Undegradable fraction.
[4]Extent of digestion.
[5]Lag time.
[6]Fractional rate of digestion.

The wash ADF fraction was greater ($P < 0.01$) at tillering than at stover maturity, but not different ($P > 0.75$) between cultivars at each maturity (Table 4.4). Treatment did not affect ($P > 0.31$) wash ADF fraction of Harrison, whereas, Fannin treated with PRO at tillering and XC at stover maturity had greater ($P < 0.04$) wash ADF fraction than untreated hays. The potentially degradable ADF fraction of Harrison at either maturity and Fannin at tillering were not ($P > 0.13$) affected by treatment, whereas, Fannin stover treated with PRO had greater ($P < 0.04$) potentially degradable ADF fraction than untreated hay. The undegradable ADF fraction was greater ($P < 0.01$) at stover than at tillering maturity stage, and not different ($P > 0.33$) for either cultivar at tillering stage. Harrison stover treated with XC and Fannin stover treated with PRO had lesser ($P < 0.02$) undegradable ADF fraction than untreated hay. Extent of ADF digestion was greatest ($P < 0.01$) at tillering and not different ($P > 0.7$) between cultivars at each harvest. Extent of ADF digestion not affected ($P > 0.07$) by treatment. Lag prior to ADF degradation was greater ($P < 0.02$) for Fannin than Harrison at both maturities. Lag of ADF degradation was only different ($P < 0.02$) for Harrison stover treated with PRO, which had greater ($P < 0.02$) lag of ADF degradation than untreated hay. Rate of ADF digestion was faster ($P < 0.05$) at tillering than stover maturity for Harrison. Rate of ADF digestion was not affected ($P > 0.06$) by treatment.

Table 4.3 *In situ* NDF disappearance kinetics of hays of oat and wheat cultivars at tillering and stover

	Cultivar × harvest												
	Harrison oat						Fannin wheat						
	Tillering			Stover			Tillering			Stover			
	C	XC	PRO	C	XC	PRO	C	XC	PRO	C	XC	PRO	SEM
A[1], %	37.78^{cd}	43.45^{a}	41.65^{ab}	1.22^{g}	3.89^{fg}	4.69^{f}	25.26^{e}	34.79^{d}	39.21^{bc}	2.99^{fg}	3.34^{fg}	2.41^{fg}	1.08
B[2], %	47.49^{def}	49.49^{cd}	48.28^{de}	43.49^{ef}	51.15^{bc}	42.36^{f}	61.56^{a}	54.73^{bc}	49.90^{cd}	47.25^{def}	49.87^{cd}	58.33^{ab}	1.94
C[3], %	11.90^{e}	7.22^{f}	9.25^{ef}	50.55^{ab}	45.58^{c}	53.90^{a}	13.18^{e}	10.33^{ef}	10.80^{ef}	48.26^{bc}	46.90^{bc}	39.72^{d}	1.63
ERD[4], %	69.67^{a}	70.98^{a}	72.05^{a}	22.38^{d}	27.96^{c}	29.49^{c}	59.92^{b}	61.58^{b}	67.95^{a}	23.21^{d}	23.27^{d}	20.05^{d}	1.50
L[5], h	1.09^{c}	1.98^{c}	1.70^{c}	7.51^{a}	2.18^{c}	7.70^{a}	1.78^{c}	1.54^{c}	2.84^{c}	5.44^{b}	5.27^{b}	1.88^{c}	0.73
K_d[6], per h	0.10^{a}	0.07^{bc}	0.09^{ab}	0.05^{cde}	0.05^{def}	0.07^{bc}	0.07^{bcd}	0.05^{cde}	0.07^{bc}	0.04^{ef}	0.04^{ef}	0.02^{f}	0.01

[a-g] Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]Wash fraction.
[2]Potentially degradable fraction.
[3]Undegradable fraction.
[4]Extent of digestion.
[5]Lag time.
[6]Fractional rate of digestion.

Discussion

Chemical Composition of Small-Grain Hay

Chemical composition of small-grain forages was not affected by inoculant treatment, and the effects of maturity on nutritive value were apparent. Improvements seen at H2 were due to the lower temperatures during regrowth. The reduction in ADL at H3 may have positive implications to using small-grain stover in lignocellulosic feedstock production. Oat showed greater nutritive value than wheat, thus it is recommended for livestock with greater nutrient demands.

Generally, the NDF, ADF, and lignin concentrations decreased from H1 to H2, and then increased at H3. These structural carbohydrates were greater in wheat than oat, whereas, similar species harvested in Minnesota did not show differences between species in ADF and ADL concentrations, or IVDMD (Cherney and Marten, 1982a). Another trial comparing these species showed greater NDF in oat than wheat, however, this may have been due to a greater number of grain type cultivars than forage types (Coblentz and Walgenbach, 2010a). Previous studies measuring nutritive value in vegetative winter wheat cultivars reported static or reduced concentrations of NDF from the first to the second harvest, whereas, herbage yield continued to increase (Coblentz and Walgenbach, 2010a). For fall grown wheat and oat forage, the ADF concentration increased to the first harvest interval and declined thereafter (Coblentz and Walgenbach, 2010a). This phenomena was reported previously (Dennis, 1984) and may be explained by the suppression of physiological development due to colder temperatures following

Table 4.4 *In situ* ADF disappearance kinetics of hays of oat and wheat cultivars at tillering and stover

	Cultivar × harvest												
	Harrison oat						Fannin wheat						
	Tillering			Stover			Tillering			Stover			
	C	XC	PRO	C	XC	PRO	C	XC	PRO	C	XC	PRO	SEM
A[1], %	50.47^{ab}	57.49^{a}	58.26^{a}	1.23^{d}	8.28^{cd}	4.37^{d}	44.40^{b}	52.11^{ab}	54.66^{a}	6.77^{d}	16.27^{c}	2.33^{d}	3.26
B[2], %	38.15^{c-f}	35.39^{d-f}	33.05^{f}	43.78^{bc}	45.78^{b}	40.31^{b-e}	41.44^{bcd}	37.06^{c-f}	33.90^{ef}	40.83^{b-e}	43.96^{bc}	56.21^{a}	2.51
C[3], %	11.38^{ef}	7.32^{f}	8.30^{ef}	54.31^{a}	46.40^{c}	55.77^{a}	14.03^{e}	10.46^{ef}	12.38^{ef}	52.50^{ab}	47.02^{bc}	39.87^{d}	2.11
ERD[4], %	75.59^{ab}	77.83^{a}	78.23^{a}	22.91^{c}	31.32^{c}	28.90^{c}	66.37^{b}	71.37^{ab}	76.76^{ab}	26.27^{c}	33.71^{c}	27.36^{c}	3.93
L[5], h	1.05^{d}	3.06^{cd}	1.52^{d}	2.91^{cd}	3.80^{bcd}	7.93^{ab}	6.05^{abc}	3.73^{bcd}	4.41^{bcd}	6.72^{abc}	8.77^{a}	5.25^{a-d}	1.50
K_d[6], per h	0.10^{a}	0.08^{a-d}	0.09^{abc}	0.05^{c-f}	0.05^{c-f}	0.08^{a-d}	0.07^{b-e}	0.07^{b-e}	0.10^{ab}	0.05^{def}	0.04^{ef}	0.02^{f}	0.01

[a-g] Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]Wash fraction.
[2]Potentially degradable fraction.
[3]Undegradable fraction.
[4]Extent of digestion.
[5]Lag time.
[6]Fractional rate of digestion.

the first harvest which occurred on December 1, 2011 (Figure 4.1). Lignification of the plant cell wall is a function of both maturity and increasing ambient temperatures (Van Soest, 1982), therefore, the relatively short duration of regrowth and the reduction in temperature between H1(22.37°C) and H2 (12.41°C) was likely integral in limiting the concentrations of NDF, ADF, ADL and increasing the IVTD (Figure 4.1).

The reduction of lignin concentration at H3 may be explained by the dilution of forage fiber as plants partitioned nonfibrous DM, primarily carbohydrate, into the filling grain head (Cherney and Marten, 1982b; Coblentz et al., 2000; Coblentz and Walgenbach, 2010a). However, the increase in NDF and ADF concentrations and decrease in IVTD at H3 was due to an increase in stage of maturity at harvest. From H2 to H3, all forages underwent stem elongation and heading which leads to rapidly increased fiber concentrations as reported in the literature (Cherney and Marten, 1982 a,b; Coblentz et al., 2000, 2002). The primary reduction in nutritive value of H3 was due to increased maturity, which results in a decrease in the leaf:stem ratio (Ugherughe, 1986) where the stem has a greatly reduced nutritive value. The decline in CP from H1 and H2 to H3 is also directly related to an increase in forage maturity (Cherney and Marten, 1982a). Concentration of CP was greater in Harrison oat than the Fannin wheat at H2, which agrees with reports by Coblentz and Walgenbach whom also reported greater CP in oat than wheat (2010a). Analyzed TDN is an estimate of the amount of energy found in feed (Holman et al., 2010). The TDN:CP for small-grain hay from H1 and H2 maturity was similar with previous reported values (Holman et al., 2010),

however, it was greater in stover from H3 maturity due to a greater amount of structural carbohydrates which increased energy and a simultaneous decrease in CP.

Disappearance Kinetics

Oat had greater i*n situ* degradation than wheat, and Small-grain forage at tillering maturity was more degradable than stover which agrees with the results from a previous study by Coblentz and Walgenbach (2010b). *In situ* results indicate improvements in several degradation parameters due to the application of fibrolytic enzyme XC and bacterial inoculant PRO to small-grain forage at tillering and stover maturity. The effect on NDF and ADF parameters were more pronounced indicating change in the fiber degradation may not be reflected in the overall DM degradation, and this effect has been seen in similar cool-season *in situ* trials (Feng et al., 1996; Álvarez et al., 2009). Treatment with XC and PRO increased the wash NDF fraction of Harrison oat harvested at tillering, and the XC treatment reduced undegradable NDF fraction but slowed the rate of NDF digestion. Harrison stover was most positively affected by XC treatment as indicated by the increased potentially degradable NDF fraction, reduced undegradable NDF and ADF fractions, increased extent of NDF digestion, and reduced lag time for NDF degradation. Treatment of Harrison stover with PRO increased extent of NDF digestion and lag time for ADF degradation. Treatment of Fannin wheat at tillering with either XC or PRO increased wash NDF fraction, but only PRO treatment increased wash ADF fraction and extent of NDF degradation, due to the decreased potentially degradable NDF fraction. Reduced degradable fraction often prevents the improvement of other degradation parameters such as wash, extent, and rate of degradation (Feng et

al., 1996). Fannin wheat stover had increased wash ADF fraction when treated with XC and treatment with PRO resulted in an increase of potentially degradable NDF and ADF fractions and NDF lag time resulting in a decrease of undegradable ADF and NDF fractions. Microbial inoculants decreasing the undegradable fraction can increase the potentially degradable fraction (Mandebvu et al., 1999).

Results from this study agree with results from a similar cool-season forage study reporting improved rates of *in situ* NDF disappearance and total tract DM and NDF digestibility (Feng et al., 1996). Álvarez also reported improved DM disappearance, NDF and ADF disappearance rate, and ADF potential disappearance of oat straw due to the application of fibrolytic and bacterial inoculant (2009). Treatment with XC improved the DM degradation of Harrison at tillering due to the improvement of NDF degradation and of Fannin at stover maturity stage due to the improvement of ADF degradation. This improved DM degradation due to improvement in the soluble cell wall and undigestible components has been reported in oat straw (Álvarez et al., 2009). Treatment of Harrison at tillering with XC and PRO had enhanced DM and NDF rate of degradation due to the improvement in NDF wash fraction degradation.

Summary

Inoculant treatments did not have pre-*in situ* differences, but the enhanced *in situ* disappearance kinetics indicate inoculants may have increased available energy to rumen microbes. Increasing the available energy to rumen microbes would improve the C:N synchrony resulting in improved DM, NDF, and ADF disappearance (Chumpawadee et al., 2006).

Since improvements were seen in *in situ* fiber degradation, a feeding trial to evaluate the effects of inoculant application to oat and wheat hay on milk and meat production. Future studies should also measure rumen function and metabolism to determine if the inoculants effected C:N synchrony. Small-grain stover pretreated with inoculants should also be evaluated for use as lignocellulosic feedstock since improvements were seen in fiber degradation.

CHAPTER V

CONCLUSIONS & RECOMMENDATIONS

Sorghum Silage

Fibrolytic enzyme XC had the greatest reduction in structural carbohydrates in the sorghum silage experiment. Bacterial inoculant PRO reduced the structural carbohydrate in cultivars with the least undigestible fraction. Inoculant XC increased the aerobic stability in the MMR cultivar. Sorghum silage treated with XC had the greatest reduction in the degradable fraction, which decreases the digestion time. This reduction in digestion rate may increase feed intake of ruminant livestock. Inoculant PRO improved ensiling characteristics, however, it was ineffective at increasing the aerobic stability. The results from this experiment indicated XC and PRO may be used on sorghum silage as a pretreatment to reduce the fiber fraction. A feeding trial to measure feed intake, milk production, or ADG would determine whether XC or PRO would improve the quality of sorghum silage.

Due to the reduction of fiber fractions, XC and PRO may also be a viable option for improving the value of sorghum as lignocellulosic feedstock. Therefore, it is recommended sorghum silage treated with XC or PRO be compared with untreated sorghum silage in the production of ethanol from lignocellulosic feedstock.

Small-Grain Hay

Since nutritive value was greater in oat than wheat, managers should consider planting oat when planting small-grain forage to meet the nutritional demands of

livestock with greater nutrient requirements. In central Texas, small-grain forage grown during lower temperatures of December and January may have improved nutritive value compared with the same forage grown during warmer temperatures of October and November (Van Soest, 1982; Dennis 1984). The reduction in ADL at H3 and improved degradation of stover due to XC and PRO treatment may improve the feedstock value of small-grain stover. Therefore, it is recommended an experiment be conducted to compare the differences XC and PRO would make in treating small-grain stover for use as lignocellulosic feedstock for ethanol production.

Although the application of fibrolytic enzyme XC and bacterial inoculant PRO was successful in reducing the structural carbohydrate and improving the degradation of sorghum silage and small-grain hay, the economics of such a practice are important to managers (Table 5.1). The cost of the product is low, and the added value of greater nutritive value forage may make the return on investment worth purchase and application of the product. Promote is a product that has been on the market for several years, and can be easily purchased. Xylanase PLUS and cellulase PLUS are new products without an extensive distribution program, therefore, it is sold in large quantities. This may give farming co-ops the opportunity to sell smaller quantities to customers interested in the application of fibrolytic enzymes to forages.

Table 5.1 Cost of XC and PRO forage inoculant

Item	$ per treated Mg of forage
XC	2.41
PRO	0.42 – 1.60

(Cargill Promote prices; Dyadic International September 28, 2012)

REFERENCES

Aden, A., M. Ruth, K. Ibsen, J. Jechura, K. Neeves, J. Sheehan, and B. Wallace. 2002. Lignicellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. National Renewable Energy Laboratory. Accessed May 1, 2012. http://www1.eere.energy.gov/biomass/pdfs/32438.pdf.

Adler P.R., S.J. Del Grosso, and W.J. Parton. 2007. Life cycle assessment of net greenhouse gas flux for bioenergy cropping systems. Ecol. Appl. 17:675-691.

Adogla-Bessa, T., E. Owen, and A.T. Adesogan. 1999. Ensiling of whole crop wheat with cellulose-hemicellulase based enzymes: 3. Comparing effects of urea or enzyme treatment on forage composition and stability. Anim. Feed Sci. Technol. 82:51-56.

Álvarez, G., J.M. Pinos-Rodríguez, J.G. Herrera, J.C. García, S.S. Gonzalez, and R. Bárcena. 2009. Effects of exogenous fibrolytic enzymes on ruminal digestibility in steers fed high fiber rations. Livest. Sci. 121:150-154.

ANKOM. 2011. Method for determining acid detergent lignin in beakers. Accessed Oct. 15, 2012. http://www.ankom.com/media/documents/Method_8_Lignin_in_beakers_4-13-11.pdf.

AOAC. 1990. Official Methods of Analysis. Vol. I. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Arriola, K.G., S.C. Kim, C.R. Staples, and A.T. Adesogan. 2011. Effect of applying bacterial inoculants containing different types of bacteria to corn silage on the performance of dairy cattle. J. Dairy Sci. 94:3973-3979.

Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2007. Southern Forages. 4th ed. International Plant Nutrition Institute, Norcross, GA.

Bayer, E.A., H. Chanzy, R. Lamed, and Y. Shoham. 1998. Cellulose, cellulases, and cellulosomes. Curr. Opin. Struct. Biol. 8:548-557.

Beauchemin, K.A., S.D.M. Jones, L.M. Rode, and V.J.H. Sewalt. 1997. Effects of fibrolytic enzyme in corn or barley on diets on performance and carcass characteristics of feedlot cattle. Can. J. Anim. Sci. 77:645-653.

Beauchemin, K.A., D. Colombatto, D.P. Morgavi, and W.Z. Yang. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81(Suppl. 2):E37-E47.

Bean, B., T. McCollum, B. Villarreal, J. Blumenthal, J. Robinson, R. Brandon, E. Buttrey, R. Van Meter, and D. Pietsch. 2009. Texas panhandle forage sorghum silage trial. AgriLife Research and Extension Center at Amarillo. Accessed May 1, 2012. http://amarillo-tamu-edu.wpengine.netdna-cdn.com/files/2010/11/Forage-Sorghum-2009-Variety-Report-Final.pdf.

Bean, B., and M. Marsalis. 2012. Corn and sorghum silage production considerations. The High Plains Dairy Conference, Texas Animal Nutrition Council, Amarillo. Accessed October 1, 2012. http://www.highplainsdairy.org/2012/18_Bean_Corn%20and%20Sorghum%20Silage%20Production%20Considerations_2012%20HPDC_Final.pdf.

Bhat, M.K., and G.P. Hazlewood. 2001. Enzymology and other characteristics of cellulases and xylanases. Pages 11-60 in Enzymes in Farm Animal Nutrition. M. Bedford and G. Partridge, ed. CAB International, Wallingford, UK.

Bodine, T.N., H.T. Purvis II, and D.L. Lalman. 2001. Effects of supplement type on animal performance, forage intake, digestion, and ruminal measurements of growing beef cattle. J. Anim. Sci. 79:1041-1051.

Bodine, T.N., and H.T. Purvis II. 2003. Effects of supplemental energy and (or) degradable intake protein on performance grazing behavior, intake, digestibility, and fecal and blood indices by beef steers grazed on dormant native tallgrass prairie. J. Anim. Sci. 81:304-317.

Bolensen, K.K., K.J. Moore, W.K. Coblentz, M.K. Siefers, and J.S. White. 2003. Sorghum silage. Pages 609-632 in Silage Science and Technology. L. Al-Amoodi, ed. ASA-CSSA-SSSA, Madison, WI.

Bolsen, K.K., J.E. Sherley, A. Laytimi, and J. Dickerson. 1989. Whole-plant grain sorghum and inoculated corn silages in mid-lactation dairy cows diets. Pages 32-37 in Annual Kansas State Dairy Day Proc. Agriculture Experiment Station, Kansas State University, Manhattan. Accessed May 1, 2012. http://krex.k-state.edu/dspace/bitstream/handle/2097/14666/Dairy89pg32-37.pdf?sequence=1.

Buanafina, M.M.O. 2009. Feruloylation in grasses: Current and future perspectives. Mol. Plant. 2: 861–872.

Bull, H. S. 1981. Estimating the nutrient value of corn silage. Pages 15-19 in Proc. of 41st Semi Annual Meeting of American Feed Manufacturers Association, Lexington, KY.

Chen, J., M.R. Stokes, and C.R. Wallace. 1994. Effects of enzyme inoculant systems on preservation and nutritive value of hay crop and corn silage. J. Dairy Sci. 77:501-512.

Cherney, J.H., and G.C. Marten. 1982a. Small grain crop forage potential: I. Biological and chemical determinants of quality and yield. Crop Sci. 22:227-231.

Cherney, J.H., and G.C. Marten. 1982b. Small grain crop forage potential: II. Interrelationships among biologica, chemical, morphological, and anatomical determinants of quality. Crop Sci. 22:240-245.

Chesson, A., and C.W. Forsberg. 1988. Polysaccharide degradation by rumen microorganisms. Pages 251-284 in The rumen microbial ecosystem. P.N. Hebson, ed. Elsevier Applied Science, London, UK.

Chumpawadee, S., K. Sommart, T. Vongpralub, and V. Pattarajinda. 2006. Effects of synchronizing the rate of dietary energy and nitrogen release on ruminal

fermentation, microbial protein synthesis, blood urea nitrogen and nutrient digestibility in beef cattle. Asian-Aust. J. Anim. Sci.19:181-188.

Coblentz, W.K., K.P. Coffrey, J.E. Turner, D.A. Scarbrough, J.S. Weyers, K.F. Harrison, Z.B. Johnson, J.B. Daniels, C.F. Rosenkrans Jr., D.W. Kellogg, and D.S. Hubbell III. 2000. Effect of maturity on degradation kinetics of sod-seeded cereal grains in northern Arkansas. J. Dairy Sci. 83:2499-2511.

Coblentz, W.K., K.P. Coffrey, J.E. Turner, D.A. Scarbrough, J.V. Skinner, D.W. Kellogg, and J.B. Humphry. 2002. Comparisons of in situ DM disappearance of wheat forages harvested by various techniques and evaluated in confined and grazing steers. J. Dairy Sci. 85:854-865.

Coblentz, W.K., and R.P. Walgenbach. 2010a. Fall growth, nutritive value, and estimation of total digestible nutrients for cereal-grain forages in the north-central United States. J. Dairy Sci. 88:383-399.

Coblentz, W.K. and R.P. Walgenbach. 2010b. In situ disappearance of dry matter and fiber from fall-grown cereal-grain forages in the north-central United States. J. Dairy Sci. 88:3992-4055.

Coleman, S.W., and K.M. Barth. 1973. Quality of diets selected by grazing animals and its relation to quality of available forage and species composition of pastures. J. Anim. Sci. 36:754-761.

Collar, C., S. Orloff, M. Mathews, S. Wright, and L. Jackson. 2006. Small grain forages. Small Grain Production Manual, University of California, Division of Agriculture and Natural Resources, Davis. Accessed May 1, 2012. http://anrcatalog.ucdavis.edu//pdf/8208.pdf.

Colombatto, D., F.L. Mould, M.K. Bhat, R.H. Phipps, and E. Owen. 2004a. *In vitro* evaluation of fibrolytic enzymes as additives for maize (*Zea mays* L.) silage: I. Effects of ensiling temperature, enzyme source and addition level. Anim. Feed. Sci. Technol. 111:111-128.

Colombatto, D., F.L. Mould, M.K. Bhat, R.H. Phipps, and E. Owen. 2004b. *In vitro* evaluation of fibrolytic enzymes as additives for maize (*Zea mays* L.) silage: II. Effects on rate of acidification, fibre degradation during ensiling and rumen fermentation. Anim. Feed Sci. Technol. 111:129-143.

Colombatto, D., F.L. Mould, M.K. Bhat, R.H. Phipps, and E. Owen. 2004c. *In vitro* evaluation of fibrolytic enzymes as additives for maize (*Zea mays* L.) silage: III.

Comparison of enzymes as derived from psychrophilic, mesophilic or thermophilic sources. Anim. Feed Sci. Technol. 111:145-159.

Courtin, M.G., and S.F. Spoelstra. 1990. A simulation model of the microbiological and chemical changes accompanying the initial stage of aerobic deterioration of silage. Grass Forage Sci. 45:153-165.

Cowan, W.D. 1994. Factors affecting the manufacture, distribution, application and overall quality of enzymes in poultry feeds. Pages 175-184 in Joint Proc. 2nd Int. Roundtable on Animal Feed Biotechnology. Probiotics and Workshop on Animal Feed Enzymes, S.K. Ho, D.A. Leger, and E.E. Lester, ed. Ottawa, Canada.

Cusi, D.S. 1979. Nonwood plant fiber pulping progress report No. 10. TAPPI Press, Atlanta, GA.

Dahlberg, J., E. Wolfrum, B. Bean, and R.L. Rooney. 2011. Composition and agronomic evaluation of sorghum biomass as a potential feedstock for renewable fuels. J. Biobased Mater. Bio. 5:507-513.

Danner, H., M. Holzer, E. Mayrhuber, and R. Braun. 2003. Acetic acid increases stability of silage under aerobic conditions. Appl. and Environ. Microb. 69:562-567.

Dean, D. B., A. T. Adesogan, N. Krueger, and R. C. Littell. 2005. Effect of fibrolytic enzymes on the fermentation characteristics, aerobic stability, and digestibility of bermudagrass silage. J. Dairy Sci. 88:994-1003.

Dennis, F.G., Jr. 1984. Flowering. Pages 237-264 in Physiological Basis of Crop Growth and Development. M.B. Tesar, ed. ASA-CSSA-SSSA, Madison, WI.

Desai, JD, A.J. Desai, and N.P. Patel. 1982. Production of cellulases and glucosidase by shake culture of *Scytalidium lignicola*. J. Ferment. Technol. 60:117-124.

DOE (US Department of Energy). 2007. Breaking the biological barriers for cellulosic ethanol; A joint research agenda. US Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy. Accessed March 12, 2009. www.doegenomestolife.org/biofuels.

Donohue, S.J., R.L. Harrison, and H.E. White (Ed.). 2000. Table 20.1 Annual yield and composition of silage crops. Page 164 in Southern Forages. 4th ed. Ball, D.M., C.S. Hoveland, and G.D. Lacefield. International Plant Nutrition Institute, Norcross, GA.

Dubbs, T.M., E.S. Vanzant, S. E., Kitts, R.F. Bapst, B.G. Fieser, and C.M. Howlett. 2003. Characterization of season and sampling method effects on measurement of forage quality in fescue-based pastures. J. Anim. Sci. 81:1308-1315.

Eggeman T., and T.R. Elander. 2005. Process and economic analysis of pretreatment technologies. Bioresource Technol. 8:2019-2025.

El-Shazly, K., B.A. Dehority, and R.R. Johnson. 1961. Effect of starch on the digestion of cellulose *in vitro* and *in vivo* by rumen micro-organisms. J. Anim. Sci. 20:268-273.

Elwakeel, E.A., E.C. Titgemeyer, B.J. Johnson, C.K. Armendariz, and J.E. Shirley. 2007. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. J. Dairy Sci. 90:5226-5236.

Environmental Protection Agency. 2012. Fuels and fuel additives renewable fuel standard Accessed October 17, 2012. http://www.epa.gov/otaq/fuels/renewablefuels/index.htm.

Esteghlalian, A.R., M. Bilogesu, S.D. Mansfield, and J.N. Saddler. 2001. Do enzymatic hydrolyzability and simons' strain reflect the changes in the accessibility of

lignocellulosic substrates to cellulase enzymes? Biotechnol. Progr. 17:1049-1054.

Eun, L.S., and K.A. Beauchemin. 2007. Enhancing in vitro degradation of alfalfa hay and corn silage using feed enzymes. J. Dairy. Sci. 90:2839-2851.

Farmer, C.G., R.C. Cochran, D.D. Simms, E.A. Klevesahl, T.A. Wickersham, and D.E. Johnson. 2001. The effects of several supplementation frequencies on forage use and the performance of beef cattle consuming dormant tallgrass prairie forage. J. Anim. Sci. 79:2276-2285.

Farrell, A.E., R.J. Plevin, B.T. Turner, A.D. Jones, M. O'Hare, and D.M. Kammen. 2006. Ethanol can contribute to energy and environmental goals. Science. 27:506-508.

Feng, P., C.W. Hunt, G.T. Pritchard, and W.E. Julien. 1996. Effect of enzyme preparations on in situ and in vitro degradation and in vivo digestive characteristics of mature cool-season grass forage in beef steers. J. Anim. Sci. 74:1349-1357.

Filya, I. 2003. The effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal

degradability of wheat, sorghum, and maize silage. J. Appl. Microbiol. 95:1080-1086.

Fisher, D.S., J.C. Burns, K.R. Pond, R.D. Mochrie, and D.H. Timothy. 1991. Effects of grass species on grazing steers: I. Diet composition and ingestive mastication. J. Anim. Sci. 69:1188-1198.

Gallagher, P., M. Dikeman, J. Fritz, E. Wailes, W, Gauther, and H. Shapouri. 2003. Biomass from crop residues: Cost and supply estimates. U.S. Department of Agriculture, Office of the Chief Economist, Office of Energy Policy and New Uses. Agricultural Economic Report. Accessed May 1, 2012 http://www.usda.gov/oce/reports/energy/AER819.pdf.

Garcés-Yépez P., W.E. Kunkle, D.B. Bates, J.E. Moore, W.W. Thatcher, and L.E. Sollenberger. 1997. Effects of supplemental energy source and amount on forage intake and performance by steers and intake and diet digestibility by sheep. J. Anim. Sci. 75:1918-1925.

Grant, R.J., S.G. Haddad, K.J. Moore, and J.F. Pedersen. 1995. Brown midrib sorghum silage for mid lactation dairy cows. J. Dairy Sci. 78:1970-1980.

Greenhill, W.L. 1959. The respiration drift of harvested pasture plants during drying. J. Sci. Food Agr. 10:495-501.

Hall, M.B., W.H. Hoover, J.P. Jennings, and T.K. Miller Webster. 1999. A method for partitioning neutral detergent soluble carbohydrates. J. Sci. Food Agric. 79:2081.

Han, K.-H. J.-H. Ko, and S.H. Yang. 2007. Optimizing lignocellulosic feedstock for improved biofuel productivity and processing. Biofuels, Bioprod. Biorefin. 1:135-146.

Hart, R.H., and G.W. Burton. 1967. Curing coastal bermudagrass hay: effect of weather, yield, and quality of fresh herbage on drying rate, yield, and quality of cured hay. Agron. J. 59:367-371.

Herrera-Saldana, R., R. Gomez-Alarcon, M. Torabi, and J.T. Huber. 1990. Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. J. Dairy Sci. 79:142-148.

Hersom, M.J. 2008. Opportunities to enhance performance and efficiency through nutrient synchrony in forage-fed ruminants. J. Anim. Sci. 86(E.Suppl.):E306-E317.

Holman, J.D., C.R. Thompson, R.L. Hale, and A.L. Schlegel. 2010. Forage yield and nutritive value of hard red and hard white winter wheat. Agron. J. 120:759-773.

Hoover, W.H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755-2766.

Huston, J.E., H. Lippke, T.D.A. Forbes, J.W. Holloway, and R.V. Machen. 1999. Effects of supplemental feeding interval on adult cows in western Texas. J. Animal. Sci. 77:3057-3067.

Iwaasa, A.D., L.M. Rode, K.A. Beauchemin, and S. Eivemark. 1997. Effect of fibrolytic enzymes in barley-based diets on performance of feedlot cattle and *in vitro* gas production. Joint Rowett Res. Inst. Natl. de Recherche Agronomique Rumen Microbial. Symp., Aberdeen, Scotland.

Jessup, R. 2009. Development and status of dedicated energy crops in the United States. *In Vitro* Cell. Deve.-Pl. 45:282-290.

Jirjis, R., and O. Theander. 1990. The effect of seasonal storage on chemical composition of forest residue chips. Scan. J. For. Res. 5:345-448.

Jones, L. 2012. Inoculants: Insurance or strategy? Accessed August 1, 2012. http://www.progressiveforage.com/index.php?option=com_content&view=article&id=4572:inoculants-insurance-or-strategy&catid=81:silage&Itemid=135.

Kim, K.H., J. Choung, and D.G. Chamberlain. 1999a. Effects of varying the degree of synchrony of energy and nitrogen release in the rumen of the synthesis of microbial protein in lactating dairy cows consuming a diet of grass silage and a cereal-based concentrate. J. Sci. Food Agric. 79:1441-1447.

Kim, K.H., Y. Oh, J. Chourng, and D.G. Chamberlin. 1999b. Effects of varying degree of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in cattle consuming grass silage. J. Sci. Food Agric. 79:833-838.

Kleinschmit, D.H., and L. Kung, Jr. 2006. A meta-analysis of the effects of lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89:4005-4013.

Kohn, R. A., and M.S. Allen.1992. Storage of fresh and ensiled forages by freezing affects fibre and crude protein fractions. J. Sci. Food Agric. 58:215-220.

Krause, M., K.A. Beauchemin, L.M. Rode, B.I. Farr, and P. Norgaard. 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. J. Anim. Sci. 76:2912-2920.

Krueger, N.A., A.T. Adesogan, and R.C. Littell. 2006. Effect of multienzyme cocktails on the digestion and fermentation of bermudagrass hay. J. Dairy Sci. 89(Suppl.1):254. (Abstr.)

Krueger, N. A., and A.T. Adesogan. 2008. Effects of different mixtures of fibrolytic enzymes on digestion and fermentation of bahiagrass hay. Anim. Feed Sci.Tech. 145:84-94.

Krueger, N.A., A.T. Adesogan, C.R. Staples, W.K. Krueger, D.B. Dean, and R.C. Littell. 2008a. The potential to increase the digestibility of tropical grasses with a fungal ferulic acid esterase enzyme preparation. Anim. Feed Sci. Tech. 145:95-108.

Krueger, N.A., A.T. Adesogan, C.R. Stapes, W.K. Krueger, S.C. Kim, R.C. Littell, and L.E. Sollenberger. 2008b. Effect of method of applying fibrolytic enzymes or ammonia to Bermudagrass hay on feed intake, digestion, and growth of beef steers. J. Anim. Sci. 86:882–889.

Kumar, R., S. Sing, and O.V. Singh. 2008. Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. J. Ind. Microbiol. Biotechnol. 35:377-391.

Kung, L., Jr. 2000. Silage fermentation & additives. Direct-fed Microbial, Enzyme & Forage Additive Compendium, Miller Publishing Co., Minnetonka, MN. Accessed May 1, 2012. http://www1.foragebeef.ca/$Foragebeef/frgebeef.nsf/all/frg69/$FILE/silagefermentationadditives.pdf.

Kung, L. Jr., M.R. Stokes, and C.J. Lin. 2003. Silage additives. Accessed May 1, 2012. http://ag.udel.edu/anfs/faculty/kung/articles/analyzing_silages_for_fermentati.htm.

Kung, L., and R. Charley. Forage inoculants, key silage management topics. Accessed June 18, 2010. http://www.qualitysilage.com/inoculants/PDF/Forage_Inoculants.pdf

Lardy, G.P., D.N. Ulmer, V.L. Anderson, and J.S. Caton. 2004. Effect of increasing level of supplemental barley on forage intake, digestibility, and ruminal fermentation in steers fed medium quality grass hay. J. Anim. Sci. 82:3662-3668.

Liu. L. 1998. Determination of ammonium-nitrogen in plant tissue. Pages 89-92 in Handbook of Reference Methods for Plant Analysis. Y.P. Kalra, ed. Soil and Plant Analysis Council, Inc., CRC Press, Boca Raton, FL.

Lusk, J.W., P.K. Karau, D.O. Balogu, and L.M. Gourley. 1984. Brown midrib sorghum or silage for milk production. J. Dairy Sci. 67:1739-1744.

Mackowiak, C.L., R.O. Myer, A.R. Blount, J.L. Foster, and R.D. Barnett. 2011. Yield and mineral concentrations of southeastern United States oat cultivars used for forage. J. of Plant Nutrition. 34:1828-1842.

Mandebvu, P., J.W. West, M.A. Froetschel, R.D. Hatfield, R.N. Gates, and G.M. Hill. 1999. Effect of enzyme or microbial treatment of bermudagrass forages before ensiling on cell wall composition, end products of silage fermentation and *in situ* digestion kinetics. Anim. Feed Sci. Tech. 77:317-329.

Mathis, C. 2003. Protein and energy supplementation to beef cows grazing New Mexico rangelands. Accessed April 10, 2012. http://aces.nmsu.edu/pubs/_circulars/Circ564.pdf.

McAllister, T.A., S.J. Oosting, J.D. Popp, Z. Mir, L.J. Yanke, A.N. Hristov, R.J. Treacher, and K.J. Cheng. 1999. Effect of exogenous enzymes on digestibility of

barley silage and growth performance of feedlot cattle. Can. J. Anim. Sci. 79:353-360.

McCollum, T. III., K. McCuistion, and B. Bean. 2012. Brown Mid-rib and photoperiod-sensitive forage sorghums. Texas Agricultural Research and Extension Center Amarillo, Texas A&M University System. Accessed April 5, 2012. http://amarillo.tamu.edu/files/2010/11/brownmidrib.pdf.

McCuistion, K.C., F.T. McCollum, L.W. Greene, J. MacDonald, B. Bean. 2011. Performance of stocker cattle grazing 2 sorghum-sudangrass hybrids under various stocking rates. Prof. Anim. Sci. 27(Suppl. 2):92-100.

McDonald, P. 1981. The Biochemistry of Silage. Wiley Interscience, New York.

Mertens, D.R. 1977. Dietary fiber components: relationship to the rate and extent of ruminal digestion. Fed. Proc. 36:187-192.

Messman, M.A., W.P. Weiss, and M.E. Koch. 1994. Changes in total and individual proteins during drying, ensiling, and ruminal fermentation of forages. J. Dairy Sci. 77:492-500.

Moon, N.J. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate, and their synergistic mixture. J. Appl. Bacteriol. 55:453-460.

Moore, J.A., M.H. Brant, W. E. Kunkle, and D.I. Hopkins. 1999. Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance. J. Anim. Sci. 77(Suppl. 2):122-135.

Morgan, P.W., S.A. Finlayson, K.L. Childs, J.E. Mullet, and W.L. Rooney. 2002. Opportunities to improve adaptability and yield in grasses: Lessons from sorghum. Crop Sci. 42:1791-1799.

Morrison, I.M.1979. Change in the cell wall components of laboratory silages and the effect of various additives on these changes. J. Agr Sci. 93:581-586.

Moser, L.E. 1980. Quality of forage as affected by post-harvest storage and processing. Page 227 in Crop Quality, Storage, and Utilization. C.S. Hoveland, ed. ASA-CSSA-SSSA, Madison, WI.

Muck, R.E. 1988. Factors influencing silage quality and their implications for management. J. Dairy Sci. 71:2992-3002.

Muck R.E., and K.K. Bolsen. 1991. Silage preservation and silage additive products. Pages 105-126 in Field Guide for Hay and Silage Management in North America. K.K. Bolsen, ed. NFIA, Des Moines, IA.

Muck, R.E., and R.E. Pitt. 1992. Aerobic losses at the silo face. Am. Soc. Agric. Eng., St. Joseph, MI.

Murray, S., W.L. Rooney, S. E. Mitchell, A. Sharma, P. E. Klein, J.E. Mullet, and S. Kresovich. 2008.Genetic improvement of sorghum as a biofuel feedstock: II. QTL. For stem and leaf structural carbohydrates. Crop Sci. 48:2180-2193.

NOAA. 2012. National weather service weather forecast. Accessed September 15, 2012. http://www.srh.noaa.gov/hgx/?n=climate_cll.

National Research Council. 2000. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

NRCS (National Resource Conservation Service). 2006. Crop residue removal for biomass energy production; effects on soils and recommendations. Soil Quality Technical Note. Accessed March 12, 2009. http://www.soils. Usda.gov/sqi.

Oliver, A.L., R.J. Grant, J.F. Pedersen, and J.O. Rear. 2004. Comparison of brown midrib-6 and -18 forage sorghum with conventional sorghum and corn silage in diets of lactating dairy cows. J. Dairy Sci. 87:637-644.

Ørskov, E. R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499-503.

Parkes, M.E., and D.J. Greig. 1974. The rate of respiration of wilted ryegrass. J. Agric. Eng. Res. 19:259-263.

Pereira, A. 2009. Performance of forage-finished beef cattle grazing ryegrass, rye or oat, and forage quality measured by a high-throughput procedure. Dissertation. Auburn University, AL. Accessed October, 2012. http://etd.auburn.edu/etd/handle/10415/1865.

Pinchak, W.E., W.D. Worrall, S.P. Caldwell, L.J. Hunt, N.J. Worrall, and M. Conoly. 1996. Interrelationships of forage and steer growth dynamics on wheat pasture. J. Range Mang. 49:126-130.

Pitt, R.E., R.E. Muck, and N.B. Pickering. 1991. A model of aerobic fungal growth in silage: 2. Aerobic stability. Grass Forage Sci. 46:301-312.

Poppi, D.P., and S.R. McLennan. 1995. Protein and energy utilization by ruminants at pasture. J. Anim. Sci. 73:278–290.

Powell, J.M., M.A. Wattiaux, G.A. Broderick, V.R. Moreira, and M.D. Casler. 2006. Dairy diet impacts on fecal chemical properties and nitrogen cycling in soils. Soil Sci. Soc. Am. J. 70:786-794.

Prostko, E.P., Muir, J.P., and Stokes, S.R. 1998. The influence of harvest timing on forage sorghum silage yield and quality. Texas Agric. Exp. Stn., Stephensville, TX. Accessed June 15, 2012. http://articlesearchdatabase.tamu.edu/article.cfm?WhichArticle=1394.

Ren, H., T.L. Richard, Z. Chen, M. Kuo, Y. Bian, and K.J. Moore. 2006. Ensiling corn stover: effect of feedstock preservation on particle board performance. Biotechnol. Prog. 22:78-85.

Rentizelas, A.A., A.J. Tolis, and I.P. Tatsiopoulous. 2009. Logistics issues of biomass: the storage problem and the multi-biomass supply chain. Renew. Sust. Energy Rev. 13:887-894.

Richardson, J.M., R.G. Wilkinson, and L.A. Sinclair. 2003. Synchrony of nutrient supply to the rumen and dietary energy source and their effects on the growth and metabolism of lambs. J. Animal. Sci. 81:1332-1347.

Rooney, W.L., J. Blumenthal, B.Bean, and J.E. Mullet. 207. Designing sorghum as a dedicated bioenergy feedstock. Biofuel. Bioprod. Bior. 1:147-157.

Rotz , C.A., and S.M. Abrams. 1988. Losses and quality changes during alfalfa hay harvest and storage. Trans. ASAE. 31:350-355.

Rotz, C.A., and R.E. Muck. 1994. Chapter 20 Changes in forage quality during harvest and storage. Pages 828-868 in Forage Quality, Evaluation, and Utilization. G.C. Fahey, Jr., ed. ASA-CSSA-SSSA, Madison, WI.

Sanderson, M.A., R.M. Jones, J. Ward, and R. Wolfe. 1992. Silage sorghum performance trial at Stevensville: Forage research in Texas. Texas Agric. Exp. Stn., Stephenville.

Sartori F., R. Lal, M.H. Ebinger, and D.J. Parrish. 2006. Potential soil carbon sequestration and CO_2 offset by dedicated energy crops in the USA. Crit. Rev. Plant Sci. 25:441-472.

Shepard, A.C., and L. Kung, Jr. 1996. Effects of an enzyme additive on composition of corn silage ensiled at various stages of maturity. J. Dairy Sci. 79:1767-1773.

Sipos, B., J. Réczey, Z. Somorai, Z. Kádár, D. Dienes, and K. Réczey. 2009. Sweet sorghum as feedstock for ethanol production: Enzymatic hydrolysis of steam-pretreated bagasse. Appl. Biochem. Biotech. 153:151-162.

Smith, S.R., B. Benson, and W. Thomason. 2009. Growing small grains for forage in Virginia. College of Agriculture and Life Sciences. Accessed Jan. 20, 2012. http://pubs.ext.vt.edu/424/424-006/424-006.html.

Somerville, C. 2007. Biofuels. Curr. Biol. 17:R115-R119.

Sree, N. K., M. Sridhar, L.V. Rao, and A. Pandey. 1999. Ethanol production in solid fermentation using thermotolerant yeast. Process Biochem. 34:115-119.

Supelco, 1990. Analyzing fatty acids by packed column gas chromatography. Supelco GC Bulletin 856A.

Tabacco, E., F. Righi, A. Quarantelli, and G. Borreani. 2011. Dry matter nutritional losses during aerobic deterioration of corn and sorghum silages influenced by different lactic acid bacteria inocula. J. Dairy Sci. 94:1409-1419.

Texas AgriLife Research and Extension Small Grain Variety Trial Information. Accessed Nov. 4, 2011. http://varietytesting.tamu.edu/wheat/docs/2011/2011%20Wheat.pdf.

Tournas, V., M. E. Stack, P. B. Mislivec, H. A. Koch, and R. Bandler. 1998. Yeasts, molds and mycotoxins. Pages 18.01-18.11 in Food and Drug Administration Bacteriological Analytical Manual. AOAC Int., Gaithersburg, MD.

Trostle, Calvin. 2012. BioEnergy and the Texas South Plains. AgriLife Extension Service, Texas A&M System, Lubbock. Accessed October 17, 2012. http://lubbock.tamu.edu/programs/crops/bioenergy/.

Ugherughe, P.O. 1986. Relationship between digestibility of *Bromus inermis* plant parts. Quantifying forage protein quality. J. Agron. Crop. Sci. 157:136-143.

Undersander, D.J., L.H. Smith, A.R. Kaminski, K.A. Kelling, and J.D. Doll. 1991. Alternative field crops manual. Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN. Accessed Sep. 25, 2012. http://www.hort.purdue.edu/newcrop/afcm/guar.html.

Undersander, D.J. 2003. The new relative forage quality index-concept and use. World's forage superbowl contest, UWEX. Accessed Sep. 25, 2012. http://www.dfrc.ars.usda.gov/WDExpoPdfs/newRelative FQindex.pdf.

Undersander, D.J. 2012. Sorghums, sudangrasses, and sorghum sudangrass hybrids. University of Wisconsin. Accessed Aug. 1, 2012. http://www.uwex.edu/ces/crops/uwforage/SorghumsFOF.htm.

USDA (United States Department of Agriculture). 2011. National agricultural statistics service. Accessed June 24, 2011. http://www.nass.usda.gov/Statistics_by_State/Ag_Overview/AgOverview_TX.pdf.

Van Soest, P.J., R.H. Wine, and L.A. Moore. 1966. Estimation of the free digestibility of forages by the in vitro digestion of cell walls. Pages 438-411 in Proc. 10th Intl. Grassl. Congr., Helsinki, Finland.

Van Soest, P.J. 1967. Development of a comprehensive system of feed analyses and its application to forages. J. Anim. Sci. 26:119-128.

Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. Cornell Univ. Press, Ithaca, NY.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell Univ. Press, Ithaca, NY.

Vendramini, J.M.B., L.E. Sollenberger, J.C.B. Dubeux, Jr., S.M. Interrante, R.L. Stewart, Jr., and J.D. Arthington. 2006. Concentration supplementation effects on forage characteristics and performance of early weaned calves grazing rye-ryegrass pastures. Crop Sci. 46:1595-1600.

Virginia Tech. 2009. Controlled grazing of Virginia's pastures. Forages, Crop and Soil Environmental Sciences Department, Virginia Tech. Accessed Sep. 19, 2012. http://pubs.ext.vt.edu/418/418-012/418-012.html.

Volden, H., L.T. Mydland, and V. Olaisen. 2002. Apparent ruminal degradation and rumen escape of soluble nitrogen fractions in grass and grass silage administered intraruminally to lactating dairy cows. J. Dairy Sci. 80:2704-2716.

Weinberg, Z.G., G. Szakacs, G. Ashbell, and Y. Hen. 1999. The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* applied at ensiling, on the ensiling fermentation and aerobic stability of wheat and sorghum silages. J. Ind. Microbiol. Biotechnol. 23:218-222.

Williams, S.D. and K.J. Shinners. 2012. Farm-scale anaerobic storage and aerobic stability of high dry matter sorghum as a biomass feedstock. Biomass and Bioenerg. In Press. Accessed Oct. 1, 2012. http://dx.doi.org/10.1016/j.biombioe.2012.08.010.

Wiselogel A.E., F.A. Agblevor, D.K. Johnson, S. Deutch, J.A. Fennell, and M.A. Sanderson. 1996. Compositional change during storage of large round switchgrass bales. Bioresource Technol. 56:103-109.

Woolford, M.K. 1990. The detrimental effects of air on ensilage. J. Appl. Bacteriol. 68:101-116.

Zhang, T., L. Li, X. Wang, Z. Zeng, Y. Hu, and Z. Cui. 2009. Effects of *Lactobacillus buchneri* and *Lactobacillus plantarum* on fermentation, aerobic stability, bacteria diversity and ruminal degradability of alfalfa silage. World J. Microbiol. Biotechnol. 25:965-971.

ZoBell, D.R., R.D. Weidneier, K.C. Olson, and R.J. Treacher. 2000. The effect of an exogenous enzyme treatment on production and carcass characteristics of growing and finishing steers. Anim. Feed Sci. Tech. 87:279-285.

APPENDIX A

SAS CODES USED FOR CHAPTER III

The SAS codes used to analyze sorghum height and yield are presented here.

```
Data 2010 sorghum height and yield;
Input location$ cultivar$ rep height yield;
Cards;

Proc print;
Proc glimmix;
Class location cultivar;
Model height yield = location| cultivar;
Random rep;
Lsmeans location cultivar location*cultivar/diff lines;
Run;
```

The SAS codes used to analyze pre-ensiled sorghum DM, CP, NDF, ADF, WSC, in vitro true digestibility (IVTD), ADL, ADIN are presented here.

```
Data 2010 fresh sorghum chemical composition;
Input sample ID location$ cultivar$ rep DM CP NDF ADF WSC IVTD ADL ADIN;
Cards;

Proc print;
Proc sort;
By location;
Proc means;
Var DM CP NDF ADF WSC IVTD ADL ADIN;
Proc glimmix;
Class location cultivar rep;
Model DM CP NDF ADF WSC IVTD ADL ADIN=location cultivar;
Random rep;
Lsmeans location cultivar/diff lines;
Run;
```

The SAS codes used to analyze ensiled sorghum after pre-treatment with inoculant DM, CP, NDF, ADF, WSC, IVTD, ADL, ADIN are presented here.

```
Data 2010 sorghum silage chemical composition;
Input ID location$ cultivar$ inoculant$ rep DM CP NDF ADF WSC IVTD ADL ADIN;
Cards;

Proc print;
Run;
Proc sort;
By location;
Run;
Proc means;
Var DM CP NDF ADF WSC IVTD ADL ADIN;
Run;
Proc glimmix;
Class location cultivar inoculant rep;
Model DM CP NDF ADF WSC IVTD ADL ADIN =location| cultivar | inoculant;
Random rep;
Lsmeans location inoculant cultivar location*cultivar*inoculant/diff lines inoculant;
Run;
```

The SAS codes used to analyze silage fermentation characteristics, yeast/mold counts, and elapsed time of sorghum silage

```
Data 2010 sorghum silage VFA;
Input ID location$ cultivar$ inoculant$ rep pH lactate butyrate LA ratio acetate
propionate iso-butyrate Total VFA yeast mold elapsed time;
Cards;

Proc print;
Run;
Proc sort;
By location;
Run;
Proc means;
Var pH lactate butyrate LA ratio acetate propionate iso-butyrate Total VFA yeast mold
elapsed time;
Run;
Proc glimmix;
Class location cultivar inoculant rep;
Model pH lactate butyrate LA ratio acetate propionate iso-butyrate Total VFA yeast
mold elapsed time =location| cultivar| inoculant;
Random rep;
Lsmeans location cultivar inoculant cultivar* inoculant/diff lines;
run;
```

The SAS Codes used to analyze sorghum silage in situ DM, NDF, and ADF remaining parameters a, L, b, and k are presented here.

```
Data 2011 sorghum silage in situ DM NDF ADF remaining parameters;
Input cultivar$ inoculant$ animal time DM remaining, NDF remaining, ADF remaining;
Cards;

Proc sort;
By inoculant cultivar animal;
Proc nlin data=allphin best = 20 method = marquardt;
Parms a=45 L=5 b=20 k=0.01;
By inoculant cultivar animal;
If t<L then do;
Model DM remaining NDF remaining ADF remaining= a + b;
Bounds a <= 100;
Bounds L >= 0;
Bounds b >= 0;
Bounds k >= 0;
End;
Else do;
Model DM remaining NDF remaining ADF remaining = b*(exp(-k*(t-L)))+a;
End;
```

The SAS codes used to analyze sorghum silage in situ DM, NDF, and ADF degradation parameters are presented here.

```
Data 2011 sorghum silage in situ DM NDF ADF degradation parameters;
Input inoculant$ cultivar$ animal a b L k c;
Cards;

Proc print;
Proc glimmix;
Class inoculant cultivar;
Model a= inoculant| cultivar;
Lsmeans cultivar inoculant cultivar* inoculant/diff lines;
Run;
Proc glimmix;
Class inoculant cultivar;
Model b= inoculant| cultivar;
Lsmeans cultivar cultivar* inoculant/diff lines;
Run;
Proc glimmix;
Class inoculant cultivar;
```

```
Model L= inoculant| cultivar;
Lsmeans cultivar inoculant cultivar*inoculant/diff lines;
Run;
Proc glimmix;
Class inoculant cultivar;
Model k= inoculant| cultivar;
Lsmeans cultivar inoculant cultivar* inoculant/diff lines;
Run;
Proc glimmix;
Class inoculant cultivar;
Model c= inoculant| cultivar;
Lsmeans cultivar inoculant cultivar* inoculant/diff lines;
Run;
```

The SAS codes used to analyze sorghum silage in situ DM, NDF, and ADF ERD

```
Data 2011 sorghum silage degradation parameters ERD;
Input treatment$ cultivar$ animal$ Extent;
Cards;

Proc glimmix;
Class inoculant cultivar;
Model extent= inoculant| cultivar;
Lsmeans cultivar inoculant cultivar* inoculant/diff lines;
Run;
```

The SAS codes used to analyze post in situ crude protein are presented here.

```
Data 2011 sorghum silage post in situ CP no lag;
Input cultivar$ inoculant$ animal time CP;
Cards;

Proc sort;
By cultivar inoculant animal time;
Proc nlin data=silage post in situ CP best = 20 method = marquardt;
Parms a=45 b=20 k=0.01;
By cultivar inoculant animal;
Model CP = b*(exp(-k*t))+a;
Bounds a <= 100;
Bounds a >0;
Bounds b >= 0;
Bounds k >= 0;
Run;
```

APPENDIX B

SAS CODES FOR CHAPTER IV

The SAS codes used to analyze oat and wheat yield are presented here.

```
Data 2011 Cool season forage yield;
Input cultivar$ rep harvest yield;
Cards;

Proc print;
Proc glimmix;
Class cultivar rep harvest;
Model yield =cultivar| harvest;
Lsmeans cultivar harvest cultivar*harvest/diff lines;
Run;
```

The SAS codes used to analyze oat and wheat DM, CP, NDF, ADF, in vitro true digestibility (IVTD) and ADL are presented here.

```
Data 2011 Cool season forage chemical composition;
Input cultivar$ harvest$ rep inoculant$ DM CP NDF ADF DM IVTD ADL;
Cards;

Proc print;
Proc sort;
By variety;
Proc means;
Var DM CP NDF ADF DM IVTD ADL;
Run;
Proc glimmix;
Class cultivar harvest inoculant rep;
Model DM CP NDF ADF DM IVTD ADL = cultivar| harvest| inoculant;
Random rep;
Run;
```

The SAS codes used to analyze oat and wheat in situ DM, NDF, ADF remaining parameters are presented here.

```
Data 2011 cool season forage in situ disappearance;
```

```
Input cultivar$ inoculant$ animal time DM remaining NDF remaining ADF remaining;
Cards;

Proc sort;
By inoculant cultivar animal;
Proc nlin data= in situ disappearance best = 20 method = marquardt;
Parms a=45 L=5 b=20 k=0.01;
By inoculant cultivar animal;
If t<L then do;
Model DM remaining NDF remaining ADF remaining = a + b;
Bounds a <= 100;
Bounds L >= 0;
Bounds b >= 0;
Bounds k >= 0;
End;
Else do;
Model DM remaining NDF remaining ADF remaining = b*(exp(-k*(t-L)))+a;
End;
```

The SAS codes used to analyze oat and wheat in situ DM, NDF, ADF degradation parameters are presented here.

```
Data 2011 Cool season forage in situ DM NDF ADF degradation parameters;
Input cultivar$ inoculant$ harvest animal b k L r a;
Cards;

Proc print;
Proc glimmix;
Class cultivar inoculant harvest;
Model a=inoculant| cultivar| harvest;
Lsmeans cultivar inoculant harvest cultivar*inoculant*harvest/diff lines;
Run;
Proc glimmix;
Class inoculant cultivar harvest;
Model b=inoculant| cultivar| harvest;
Lsmeans cultivar inoculant harvest cultivar*inoculant*harvest/diff lines;
Run;
Proc glimmix;
Class inoculant cultivar harvest;
Model L=inoculant| cultivar| harvest;
Lsmeans cultivar inoculant cultivar*inoculant*harvest/diff lines;
Run;
Proc glimmix;
Class inoculant cultivar harvest;
```

```
Model k=inoculant| cultivar | harvest;
Lsmeans cultivar inoculant cultivar*inoculant*harvest/diff lines;
Run;
Proc glimmix;
Class inoculant cultivar harvest;
Model r=inoculant| cultivar | harvest;
Lsmeans cultivar inoculant cultivar*inoculant*harvest/diff lines;
Run;
```

The SAS codes used to analyze oat and wheat in situ DM, NDF, ADF, extent of degradation are presented here.

```
Data 2011 Cool season forage in situ Extent of digestion parameters;
Input cultivar$ inoculant$ harvest$ animal$ Extent;
Cards;

Proc glimmix;
Class inoculant cultivar harvest;
Model extent=inoculant| cultivar| harvest;
Lsmeans cultivar inoculant harvest cultivar*inoculant*harvest/diff lines;
Run;
```