MANAGEMENT IMPACTS ON SORGHUM SILAGE RUMINAL DIGESTIBILITY

by

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ABSTRACT

The impetus for sorghum (*Sorghum bicolor L.*) silage to replace corn silage in beef cattle and dairy rations has increased due to sorghum's ability to grow well under water-limited conditions. Two experiments were conducted to evaluate the effects of maturity, mechanical processing, ensiling duration, and hybrid on *in situ* ruminal digestibility. In Exp. 1, three ruminally cannulated beef steers (BW = 463 ± 15 kg) were used to evaluate the effect of maturity stage, mechanical processing, and ensiling duration on *in situ* digestibility of forage sorghum silage. A brown midrib (**BMR**) forage sorghum (Alta Seed AF7401) was harvested at soft dough (**SD**) and hard dough (**HD**). Within each maturity stage, sorghum kernels were processed (**C**) using a chipper shredder or left unprocessed (**W**), and were subjected to 0, 30, 60, or 120 d of ensiling. In Exp. 2, three ruminally cannulated steers (BW = 570 ± 17.9 kg) were used to evaluate sorghum hybrids and ensiling duration on *in situ* silage digestibility. A BMR forage sorghum (Alta Seed AF7401), conventional forage sorghum (Sorghum Partners NK300), and a grain sorghum (Sorghum Partners SP33S40) were ensiled for 0 or 60 d, which was the optimal ensiling duration for Exp. 1. In Exp. 1, kernel processing increased ($P < 0.01$) the effective ruminal degradability (**ERD**) of DM, OM, CP, and starch in SD sorghum silage and increased the ERD of starch in HD silage. Ensiling duration affected ($P < 0.01$) the ERD of all *in situ* disappearance variables for SD silage, except ($P = 0.40$) the ERD of

starch. The ERD of *in situ* variables in HD silage was affected ($P < 0.01$) by ensiling duration. *In situ* ERD of DM, OM, CP, and starch was different ($P < 0.01$) between hybrid type. Ensiling affected ($P < 0.01$) the ERD of CP and starch, with no differences observed ($P \geq 0.32$) in the ERD of DM and OM. Kernel processing and increased ensiling duration improved *in situ* digestibility of both early and late matured sorghum silage. Hybrid type may influence silage digestibility, thus livestock producers should consider hybrid selection to maximize ration digestibility.

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LIST OF ABBREVIATIONS

BMR = brown midrib
C = kernel processed sorghum silage
ERD = effective rumen degradability
HD = hard dough
HMC = high moisture corn
IVTD = in vitro true digestibility
NDFD = neutral detergent fiber disappearance
PS = photoperiod sensitive
SD = soft dough
VFA = volatile fatty acid
W = unprocessed kernel sorghum silage

CHAPER I

REVIEW OF LITERATURE

Introduction

Many dairies and concentrated beef cattle operations commonly utilize silage-fermented feedstuffs as a roughage source for cattle rations. Ensiling forages is an approach that uses high-moisture forage crops to attain preservation of nutrients and forage value for long-term storage. Consequently, ensiling forages provides a means to feed livestock during periods of inadequate pasture growth and insufficient pasture quality (Romero et al., 2015). Corn (*Zea mays L*) silage is generally the most predominant crop grown for silage production due to the consistency it provides from a nutritive value standpoint (Bean and Mersalis, 2012). Conversely, sorghum's (*Sorghum bicolor* (L) Moench) highly variable composition and feeding value has deemed it to be of lesser value than corn (Hibberd et al., 1982). However, sorghum silage has received renewed interest due to its drought tolerance and reduced water requirements.

Optimization of sorghum's feeding value depends on management decisions including hybrid selection, maturity at harvest, method of processing, and the manner of which the silage is preserved. Multiple studies in corn have demonstrated that digestibility is altered by ensiling, mechanical processing, and maturity stage (Der Bedrosian et al., 2012; Ebling and Kung, 2004; Johnson et al., 2002; Philippeau and

Michalet-Doreau, 1998). However, few studies have assessed the combined effects of these variables on sorghum digestibility. As consideration towards feeding sorghum silage increases, there is a need to better understand methods that could potentially increase its feeding value.

Forage Sorghum

Sorghum Characteristics

Many forage sorghums contain less grain as compared to traditional grain sorghums; however, forage sorghums typically produce more leaves and are taller. Forage sorghums are classified by the genotypic traits they carry or lack thereof. Traits, which genetically differentiate forage sorghum hybrids, are BMR, non-BMR, photoperiod sensitive (**PS**), and non-PS (Bean et al., 2013).

Improved sorghum hybrids appear to have the greatest impact on enhancing digestibility and nutrient availability (Oliver et al., 2005). Cell wall digestibility is primarily dependent on the lignin, or the extent of cross-linking between lignin with cell wall carbohydrates (Oliver et al., 2005). Having lower lignin content, forage sorghum BMR hybrids are utilized when increased fiber digestibility is desired; such is the case when feeding silage to ruminants. Because BMR forage sorghums generally have lower lignin contents, the stalks of the sorghum plant are prone to bending, breaking, or lodging (Bean and Marsalis, 2012). BMR hybrids were developed by Porter et al. (1978), who developed 19 sorghum BMR mutants by treating two grain sorghum varieties with diethyl sulfate. Seeds from the two sorghum varieties were soaked in diethyl sulfate for 3 h in flasks, which were agitated every 30 min. Seeds were then washed in distilled water for 5 min, air-dried, and immediately planted. Brown midrib-6, BMR-12, and BMR-18

were then selected for further evaluation due to the decreased lignin content and increased digestibility that each mutant displayed (McCollum et al., 2012).

Many forage producers select forage sorghums for their ability to yield well and withstand lodging; whereas, digestibility and nutrient composition are the primary concern to livestock feeding operations (Bean et al., 2009). Distinct differences in yield and digestibility between forage sorghum hybrids were demonstrated by McCollum et al. (2012). McCollum et al. (2012) collected and summarized from sorghum variety trial test plots over five production years. Overall, PS varieties tended to produce higher yields than BMR and non-BMR varieties. Broad averages of the IVTD using a 48-h ruminal fluid incubation, indicated that the BMR group was 5.3% more digestible than the non-BMR varieties; however, the IVTD for the non-BMR varieties to be 10.1% greater than the PS varieties (McCollum et al., 2012). Nonetheless, McCollum et al. (2012) concluded that there were instances when similar yield and IVTD between BMR, non-BMR, and PS were observed. The authors recommended that decisions should not be based on genotype designation, but rather on the specific hybrid.

Comparison of Sorghum and Corn

Corn silage is a dominant forage crop commonly utilized in dairy and feedlot operations across the High Plains region of the US. There were 2,603,747 ha of corn silage harvested in the US in 2017, totaling 128,356,000 tons of silage (NASS, 2017). Corn silage typically contains 50% corn grain, generating the greatest amount of digestible energy per acre of any other forage annually (Martin et al., 2008). One disadvantage in producing corn silage adequate in nutritional value is the substantial amount of water required to fulfill its genetic potential. Across the Southern High Plains

(SHP), declining irrigation capacities and intermittent droughts limit corn silage production. Additionally, underwater limited water, poor grain development limits corn silage quality. Consequently, forage sorghums are a viable alternative to corn silage because of its' lower water requirement. In 2017, sorghum silage production totaled 3,772,000 tons of silage, encompassing 114,931 ha of land (NASS, 2017). Bean and Marsalis (2012) estimated that 20 to 25 inches of irrigation water is needed for full production corn silage, while only 14 to 18 inches is needed for sorghum silage. This suggests that there is potential for an increase in sorghum silage production to meet the forage demands of the High Plains dairy industry.

Corn silage is generally considered a higher quality feed compared to sorghum silage. The kernel structure and gross composition of corn and sorghum are similar; however, differences in the type and distribution of proteins surrounding the starch in the endosperm are significant (Rooney and Plugfelder, 1986). Although it has been well-documented that achieving optimum digestibility of the sorghum grain requires vigorous processing because the grain endosperm and protein-starch matrix of whole grain inhibit digestion, previous research has reported some varieties of sorghum produce silage that meets or exceeds the nutritional values of corn (Bernard, 2015; Rooney and Pflugfelder, 1986). Weight gain in feeder cattle and milk production in dairy cattle have also shown to be similar when fed either sorghum silage or corn silage of comparable quality (Bean et al., 2013).

In review of digestibility values between corn silage and sorghum silage conducted by McCollum and Bean (2003), the IVTD of corn silage averaged 81.3%, while IVTDs of BMR forage sorghum silage and non-BMR forage sorghum silage

averaged 80.2 and 77.6%, respectively. Ranges for the sorghum silages surpassed the IVTD for corn silage, validating that sorghum silage digestibility can meet or exceed the digestibility of corn (McCollum and Bean, 2003). Corn must produce large amounts of grain to yield silage high in nutritional value. Contrary to corn, BMR hybrids have attained IVTD of 80.8% when as little as 2.0% of the total weight was grain content (Bean and Marsalis, 2012).

Oliver et al. (2004) reported the effects of conventional forage sorghum, BMR-6 forage sorghum, BMR-18 forage sorghum, and a dual-purpose corn hybrid on milk yield, ruminal fermentation and total tract digestibility in 16 multiparous Holstein dairy cows. Differences within the lignin biosynthesis pathway differentiate the BMR-6 and BMR-18 sorghum mutants. Treatment diets were fed in a replicated Latin square design with 4-wk periods, and consisted of 40% test silage, 10% alfalfa silage, and 50% concentrate mix. The grain mixture of the concentrate mix consisted of 52.1% ground dry corn. No differences were observed in DMI between the treatment diets. Cows consuming the BMR-6 or corn silage had the greatest levels of milk production, with cows consuming the conventional sorghum produced the least amount of milk (Oliver et al., 2004). Both BMR-6 and corn silage diets resulted in a greater total tract neutral detergent fiber (NDF) digestibility compared to conventional sorghum. In contrast, Lusk et al. (1984) reported an increase in BMR-12 sorghum silage consumption over corn silage consumption in dairy cows. However, Lusk et al. (1984) observed no differences in milk production between the BMR sorghum silage and the corn silage, similar to the results of Oliver et al. (2004). The authors of this study speculated the difference in intake between the BMR

sorghum and the corn silage were due to possibly an off odor and heat damage to the corn silage attributed to its high dry matter at ensiling.

Grant et al. (1995) utilized 12 dairy cows during midlactation to measure milk production, chewing activity, ruminal pH, rumen ammonia and VFA concentrations, and fiber digestibility of non-BMR, BMR, corn and alfalfa silage treatment diets. The specific BMR mutant type utilized in this experiment was not specified. The cows were fed diets consisting of 65% (DM Basis) silage. Dry matter intake for BMR sorghum and corn silage treatments were greater than alfalfa and conventional sorghum silage treatments (Grant et al., 1995). Fat corrected milk production for cows fed sorghum silage was comparable to cows fed alfalfa and corn silage treatment diets. Three ruminally fistulated cows were assigned to the same treatment diets. Results from the *in situ* portion of the trial concluded that the BMR sorghum silage exhibited greater ruminal fiber digestion than the non-BMR sorghum and alfalfa.

Ensiling Process

Phases of Silage Fermentation

The ensiling process is initiated as a result of epiphytic microflora, primarily lactic acid bacteria, converting water-soluble carbohydrates into organic acids (Filya, 2003). Successful silage preservation is reliant on anaerobic conditions that stimulate lactic acid production and reduce forage pH, which then inhibits aerobic microorganisms and naturally occurring plant proteolytic enzymes (Muck and Pitt, 1994). Four primary phases involved in the ensiling process are the pre-seal, active fermentation, stable phase, and feed out phase (Barnhart, 2008). The pre-seal phase is primarily characterized by plant reparation prior to sealing the silo, silage pit, or silage bag. Plants and microbes

continue to respire during the chopping, filling, and packing stages. Carbon dioxide, water, and heat are continuously produced as a product of respiration, which continues to utilize oxygen and sugars (Barnhart, 2008; Muck, 1988). During the active fermentation phase, an anaerobic environment suitable for silage preservation is formed when: 1) oxygen is eliminated by this respiration process, and 2) anaerobic bacteria ferment sugars producing lactic acid and lowering the silage pH. The active fermentation phase is characterized by a decline in pH to as low as 4.0, which typically occurs within one to four wks of ensiling (Barnhart, 2008). When forage is harvested too early, the forage DM is low. Subsequently, the active fermentation phase can become a wasteful process because there are highly digestible sugars being degraded by clostridial bacteria and forming butyric acid, which results in reduced palatability.

There are also DM and energy losses due to proteolysis; the breakdown of the proteins. Minimizing DM and energy losses, along with maintaining feeding characteristics, should be of primary concern when utilizing silage as a method to preserve forages (Muck, 1988). Extensive proteolysis typically occurs at a pH of 5.5 to 6.0 and is greatest during the first day after sealing. Proteolysis is inversely related with oxygen consumption; therefore, oxygen rapidly decreases as a more defined anaerobic environment is created. Furthermore, the feeding value of an ensiled crop may be lowered considering proteolytic enzymes possess the ability to convert protein nitrogen to non-protein nitrogen. Non-protein nitrogen can range from 20 to 85% of total nitrogen after ensiling (Muck and Dickerson, 1988; Barnhart, 2008). The stable phase of the ensiling process begins as water soluble carbohydrates in the crop are completely used, or when the pH is reduced enough to hinder the growth of lactic acid bacteria (Barnhart,

2008). Biological activity of microorganisms is minimal provided that the seal holds its integrity.

If silage is not properly sealed, air seepage through seals or silo openings is detrimental to silage quality because aerobic microorganisms such as yeast and molds are allowed an opportunity to become active (Filya, 2003). The internal temperature of the silage rises as a result of these processes, potentially allowing other detrimental microbial organisms to accumulate (Johnson et al., 2002). If silage temperatures rise to 60°C, the formation of Maillard products could be accelerated, thus causing further damage to the silage by heat damage, foul odor, and loss in palatability (Muck, 1988). Research indicates that opening any silage containment should only occur once the fermentation phase has been completed (Romero et al., 2015). Silage spoilage by aerobic microorganisms is also seen during the feedout phase. Muck and Pitt (1994) suggested that losses in aerobic feedout are greater than plant respiration, fermentation, and infiltration losses prior to feedlot. Aerobic deterioration of silage during the feedout can result in the loss of up to 20% of stored sorghum DM (Tabacco et al., 2011). Due to DM losses attributable to spoilage by yeast and aerobic bacteria, Barnhart (2008) suggested removing a minimum of 2 inches of silage per day in the winter and 4 inches of silage per day in the summer while in the feedout phase.

Factors That Effect Silage Quality

Silage quality can be influenced by moisture, composition of the microbial population, harvest maturity, particle size, packing density, seal integrity, and temperature (Johnson et al., 2002; Romero et al., 2015). Ensiling forages with greater than 70% moisture risks potential seepage losses and undesirable bacteria growth.

Achieving optimal DM concentrations for ensiling is generally affected by the maturity stage of the crop (Romero et al., 2015). The maturity stage of a crop is also a primary factor in determining silage digestibility. Silage digestibility can also be associated with cell wall digestibility, which decreases as the plant matures (Di Marco et al., 2002). For corn silage, maturity stage for harvest is defined by evaluation of kernel-milk line. Kernels advancing in maturity display lower milk lines and eventually reach full maturity with no milk line present. For sorghum silages, the milk stage is achieved when a milk-like liquid is obtained from squeezing the kernel between the fingers. The SD and HD stages are generally reached when the kernel can be squeezed with little or no liquid, and when the kernel cannot be compressed by the fingers, respectively (Gerik et al., 2015).

Di Marco et al. (2002) evaluated the effect of corn maturity on silage chemical composition and digestibility using *in vivo* and *in vitro* digestibility methods. The crops were harvested at silking, milk, and half milk line with DM contents of 20, 26, and 32%, respectively. Plants were chopped to a length of 5 to 7 mm and approximately 560 kg of fresh sample were placed and ensiled in containers for 4 months. Dry matter yield increased from 125 to 236 kg as crop maturity progressed from silking to half milk line. As crop maturity increased silage starch content increased and NDF content decreased, having no effects on lignin (Di Marco et al., 2002). Soluble carbohydrates were least in the silking stage with increased levels in the milk and half milk line stages. Soluble carbohydrate levels are crucial in maintaining well-preserved silage, as silage spoilage is heavily dependent on pH, which is lowered by lactic acid bacteria that convert water-soluble carbohydrates into lactic acid (Filya, 2003). Nine lambs fed at maintenance were used to conduct the *in vivo* portion of this trial (three replicated 3 × 3 Latin squares).

Silage treatments were supplemented with sunflower meal and urea to achieve a crude protein content of 12%. Lambs were adapted to treatments for 10 d with an additional 7 d for data collection. Diets were offered twice a day, and total feces were collected daily. Fecal samples were collected by animal and period for DM, starch, and NDF analysis (Di Marco et al., 2002). Results from this experiment concluded that *in vivo* DM digestibility was not affected by the stage of maturity; however, NDF digestibility decreased 52% in the silking stage, 46% in the milk stage, and 29% in the half milk line stage (Di Marco et al., 2002). An increase in the starch content of silage was observed with increasing maturity; therefore, NDF digestibility did not affect DM digestibility. No differences in DM digestibility were detected for the *in vitro* portion of the trial. Maturity stage also had no effect on digestibility values for the *in vitro* experiment. Neutral detergent fiber digestibility decreased with maturity for both the *in vivo* and *in vitro* experiments. In conclusion, DM yield, DM content, and starch accumulation decreased and NDF digestibility increased with younger stages of maturity. The authors concluded that the lack of differences in DM digestibility were due to the counteraction between the increased NDF digestibility and lower starch contents (Di Marco et al., 2002).

Particle size of chopped forage plays an essential role for both silage packing density and effective fiber supplied for feeding (Romero et al., 2015). An increased packing density allows for a better anaerobic environment due to the increased exclusion of air (Johnson et al., 2002). Furthermore, air allows for the oxidation of fermentation acids and other substrates by aerobic bacteria, yeast, and molds (Wilkinson and Davie, 2012).

Starch Digestion

Starch digestibility is directly influenced by the physical form and composition of the starch, cellular integrity of the starch-containing units, protein-starch interactions, and the antinutritional factors and physical form of the feed (Rooney and Pflugfelder, 1986). Bacterial attachment to feed particles is an initial, critical step in ruminal starch digestion. Bacterial attachment and microbial fermentation results in the production of cells and volatile fatty acids, further influencing enzymatic digestion in the small intestine to produce glucose (Waldo, 1973). Ruminal protozoa and fungi are also equipped with aiding in starch digestion. Protozoa are capable of ingesting and digesting starch granules with or without bacteria attached. Fungi assist digestion by creating lesions, allowing a greater degree of bacterial attachment. The more highly processed a starch molecule is the better chance bacteria will attach and utilize starch. This is rather important as starch is a primary source of energy in diets that include grain.

Optimizing production in both feedlot and dairy industries is heavily reliant on the ability for the animals to efficiently utilize cereal grains as a source of energy and protein. Finishing diets typically contain 70 to 90% grain inclusion, with starch roughly 60 to 80% of the composition of the grain (Philippeau et al., 1999; Kotarski et al., 1992). The composition and structure of cereal starches, along with numerous metabolic interactions that occur during digestion, play a significant role in the digestibility and feeding value of grain for livestock (Rooney and Pflugfelder, 1986).

Chemical Characteristics of Starch

Starch is comprised of hydrogen-bonded amylopectin and amylose molecules within highly organized pseudo-crystal granules (Rooney and Pflugfelder, 1986). Starch

mass represents approximately 70 to 80% of most cereal grains. Amylopectin and amylose are both polysaccharides that compose starch; however, amylopectin is the more digestible fraction due to its branching characteristics (Sang et al., 2008). Grains and varieties are categorized by descriptors such as vitreous, flinty, waxy, non-waxy, and opaque by the relative abundance of endosperm layers that are present (Huntington, 1997). The differences in grain source and species are the basis for reported differences in digestion and performance within in vitro and in vivo experiments (Huntington, 1997). Waxy starches predominantly contain amylopectin and have minimal amounts of amylose. Cereal starches containing floury endosperms typically contain 20 to 30% amylose and are generally characteristic of less digestible sources of starch (Van Soest, 1982). In general, cereal grains containing a waxy endosperm are typically more digestible than non-waxy grains, which is a result from the increased amounts of amylopectin compared to amylose in non-waxy grain (Rooney et al., 2005; Huntington, 1997). Containing a greater degree of polymerization (1000 to 2000 units), hydrogen bonding becomes stronger with amylose compared to amylopectin (Van Soest, 1982). Amylopectin, which accounts for 70 to 80% of most cereal starches, can be distinguished from amylose by its α-1,6 branch points within the α-1,4 linked D-glucose units. Amylose is a linear polymer consisting of α-1,4-linked D-glucose units (Rooney and Pflugfelder, 1986). Starch granules encompass both crystalline and amorphous areas. The crystalline area is characterized by being resistant to both water and enzymatic attack, along with the refraction of the granule. The amorphous area is an amylose rich, less dense region where water moves freely and the amylase digestion of the granule begins.

Starch Hydrolysis

Depolymerization of starch molecules by starch hydrolases is vital in starch digestion, which results in the release of sugars used in various metabolic processes (Muralikrishna and Nirmala, 2005). Enzyme molecules are capable of affecting starch granules by either degrading the outer surface of the starch granule or causing disruption at the center of the granule (Lin et al., 2016). Amylases, which are widely distributed in microbes, plants and animals, are a class of hydrolases capable of cleaving the O-glycosidic bonds in starch (Muralikrishna and Nirmala, 2005). Starch degrading enzymes can be classified as endoamylases, exoamylases, or debranching amylases (Muralikrishna and Nirmala, 2005). Endoamylases such as α-amylase are liquefying enzymes whose responsibility is to randomly cleave α-1,4 glycosidic bonds in amylose, amylopectin and other related polysaccharides (Muralikrishna and Nirmala, 2005). Oligosaccharides such as maltose and linear dextrins are products produced as a result of starch hydrolysis (Muralikrishna and Nirmala, 2005; Rooney and Pflugfelder, 1986). Unlike endoamylases that target glycosidic bonds, exoamylases cleave α-1,4 glucosidic bonds in amylose, amylopectin, and glycogen. Exoamylases such as β-amylases cleave units from the ends of chains, and they are capable of degrading amylose, but are limited to parts of amylopectin (Van Soest, 1982). Alpha and β-amylases are incapable of attacking α-1,6 glycosidic linkages; therefore, debranching enzymes such as pullulanases are produced which possess the specific capability to attack α-1,6 linkages (Muralikrishna and Nirmala, 2005; Rooney and Pflugfelder, 1986).

Starch-Protein Matrix

Kernel structure and gross composition for corn and sorghum are similar. Additionally, starch granules of corn and sorghum are similar in size and shape, yet ruminal starch digestibility is lower in sorghum. This reduction in starch digestibility is partially attributed to the lower digestibility of sorghum protein that surrounds the starch granules (Rooney and Pflugfelder, 1986). Zein and kafirin proteins are prolamin storage proteins that function as a primary nitrogen repository for corn and sorghum, respectively. Innately, zein and kafirin proteins are largely resistant to digestion; however, the reduced digestibility is more pronounced in sorghum (Holding, 2014). Differences in protein digestibility between corn and sorghum can be explained by intermolecular crosslinks, called cross-liked kafirins, which adhere more tightly in sorghum to endosperm starch and protein (Rooney and Pflugfelder, 1986).

The starch-protein matrix hinders microbial attachment; subsequently altering rumen fermentation and hydrolytic and enzymatic digestion in the abomasum and small intestine (Ferraretto et al., 2015). The peripheral and corneous endosperms are the endosperm layers that contain the majority of the starch granules embedded within the protein-rich matrix (Kotarski et al., 1992; Huntington, 1997). These proteins are not intrinsic within the starch granule but are localized on the exterior of the starch granules (Hoffman et al., 2011). This interaction between starch and protein significantly reduces the susceptibility of both unprocessed and processed starches to enzymatic hydrolysis (Rooney and Pflugfelder, 1986).

One promising method to attaining an increased rate of starch hydrolysis is to increase proteolysis through the addition of protease. Kung et al. (2014) conducted an

experiment to evaluate the effects of high-moisture corn (HMC) treated with exogenous protease on in vitro ruminal starch degradation. In this experiment, HMC (73% DM) was left untreated or treated with exogenous protease to attain a final concentration of 2,000 mg of protease/kg of fresh corn. Corn was ensiled for 70 and 140 d in laboratory-scale bags. After 70 and 140 d of ensiling, protease-treated HMC attained increased concentrations of soluble protein and ammonia-nitrogen (NH_3N) and decreased protein concentration. In all protease-treated samples, the in vitro rumen degradability of starch was greatest compared to the untreated HMC samples. Soluble protein and NH_3N concentrations were positively correlated with in vitro starch degradation. Furthermore, prolamin protein concentrations were inversely related to in vitro starch degradation. Results from this experiment indicated that treating HMC with exogenous protease could increase ruminal starch fermentation (Kung et al., 2014). Feraretto et al. (2015) evaluated the effects of inoculation with one of three microbial inoculants and exogenous protease on in vitro starch digestion was evaluated. Results from this experiment concluded that protease, but not inoculation, increased in vitro starch digestion by 7.5 percentage units, which aligns with results found by Kung et al. (2014).

Increasing proteolytic mechanisms to increase starch digestibility may be achieved through ensiling. Hoffman et al. (2011) evaluated the effects of ensiling time and inoculation on zein proteins in HMC. Two random corn hybrids (A and B) containing 25.7 and 29.3% moisture, respectively, were ground and inoculated with or without 600,000 cfu/g of *Lactobacillus buchneri* 40788. Quadruplicate samples of inoculated and non-inoculated treatments were vacuum-sealed in plastic bags for 0, 15, 30, 60, 120, or 240 d at 22°C (Hoffman et al., 2011). Borate-phosphate buffer-soluble CP

concentrations increased from 2% of DM at d 0 to greater than 4% at d 240 in response to ensiling time. Buffer-soluble CP was not influenced by inoculation. Inoculation showed no effects on decreasing hydrophobic zein proteins in the endosperm of HMC (Hoffman et al., 2011). Ensiling from d 0 to 240 decreased all zein subunits with the exception of 2 α and 1 δ subunits. Alpha and Gamma zein subunits are primarily responsible for cross-linking in the starch-protein matrix. Gamma zein subunits decreased (42.2 to73.2%) in response to ensiling during this trial. The authors attributed the observed degradation of hydrophobic zein proteins to chronic proteolytic activity during the ensiling process (Hoffman et al., 2011). Additionally, it was hypothesized that degradation of starch-matrix proteins in the ensiling process may allow increased access to starch granules in HMC (Hoffman et al., 2011). Further research is needed to asses ensiling time on starch-protein degradation across multiple plant species.

Extended ensiling periods have shown to increase ruminal in vitro starch digestibility (Ferraretto et al., 2015). Der Bedrosian et al. (2012) evaluated the effects of hybridization, maturity at harvest, and length of storage on the composition and nutritive value of corn silage. Conventional and BMR hybrids, harvested at 32 or 41% DM were ensiled for 0, 45, 90, 180, 270, or 360 d. In vitro starch digestion (7 h) and NDFD (30 h) were used to measure differences in digestion between treatments. Neutral detergent fiber digestion was not affected for either the normal or BMR hybrids between 45 and 270 d of ensiling (Der Bedrosian et al., 2012). The NDFD of BMR hybrids was consistently greater at all times of ensiling. Neutral detergent fiber digestion for the conventional hybrid was not affected by maturity; however, NDFD of the BMR hybrid was lower in more mature silage. As ensiling duration increased, *in vitro* starch digestion increased,

which was attributed to potential proteolytic mechanisms. Results from this experiment indicate that starch digestion can improve by extending periods of ensiling (Der Bedrosian et al., 2012).

Intestinal Starch Digestion

Pre-digestion of starch in the rumen influences both the quantity and composition of starch reaching the small intestine; therefore, small intestinal digestion of starch should not be overlooked (Owens et al., 1986). A primary factor affecting the efficiency of digested energy to meet the ruminant's metabolic needs is the site at which starch is digested (Church, 1988). The introduction of starch into the small intestine from concentrate-containing diets should be more efficient than those extensively fermented in the rumen (Church, 1988; Owens et al., 1986). Losses in fermentation heat and methane account for 12 to 20% of the ingested energy, so it is desirable for starch to escape ruminal fermentation if starch digestion in the small intestine is maximized (Ørskov, 1986). Generally, starch digested in the small intestine accounts for 5 to 20% of the starch consumed (Owens, 1986). Continuous flow of digesta to the duodenum preludes the concept that there is neuro-endocrine control of pancreatic secretion; therefore, secretion and production of enzymes active in starch digestion respond more to the amount of energy consumed than by the amount of dietary starch (Huntington, 1997).

Kernel Processing

The primary purpose of processing cereal grains is to increase starch availability in diets fed to livestock by decreasing particle size (Pritchard and Stateler, 1997). Mechanically isolated starch from plants cannot be degraded by amylase until the starch grains have ruptured (Van Soest, 1982). Because whole grain with an intact pericarp is

largely resistant to bacterial attachment, processing grain increases digestibility by breaking the seed coat and allowing an opportunity for a greater extent of bacterial attachment to starch granules (Firkins et al., 2001; Huntington, 1997). Subsequently, as particle size of grain decreases, the rate of digestion of starch from the grain in the rumen increases (Owens et al., 1986). Starch utilization is also improved by processing through the disruption of the protein matrix surrounding the starch granules (Theurer, 1986). The magnitude of the enhancement in starch utilization is dependent on the grain processing method, ruminant species, and grain source (Theurer, 1986). It has been reported that processing sorghum grain can increase digestibility to levels comparable to corn (Rooney and Plugfelder, 1986). Most processing techniques will increase access to starch granules by reducing particle size or altering the endosperm protein to solubilize or denature the gelatinous proteins (Owens et al., 1986). Many improvements in increasing feed values through grain processing are present in sorghum grain (Rooney and Pflugfelder, 1986).

Standard processing methods to increase the digestibility of grains are steam flaking, micronizing, popping, reconstitution, rolling, and grinding (Rooney and Pflugfelder, 1986). Oliveira et al. (1995) evaluated the effects of sorghum grain processing on the site and extent of digestion of starch in lactating dairy cows. Four lactating Holstein cows fitted with duodenal cannulas were used in a 4 × 4 Latin square design. Treatment diets were steam-rolled corn, dry-rolled sorghum, steam-flaked sorghum, and an equal mixture of steam-flaked and dry-rolled sorghum as 43% of DM in a TMR. Nutrient flow to the small intestine and total tract digestibility were estimated by using chromium oxide as a marker. Results from this study indicated that apparent total tract digestibility of starch for the diets previously mentioned were 81, 60, and 70, and

60%, respectively. Ruminal digestibility of starch for the treatment diets averaged 97, 85, 91, and 89%, respectively. Intestinal starch digestibility for steam-flaked corn was greater than both dry-rolled and mixed sorghum (83 vs. 63 and 61%). Steam flaking resulted in less postruminal passage as compared to the alternate treatments. Steam flaking increased starch digestibility in the rumen from 60 to 81%, and starch digestibility in the small intestine from 63 to 83% compared to dry rolling of sorghum. Digestible starch intake increased 9% (6.4 vs. 5.9 kg/d) with steam flaking compared to dry rolling sorghum. Steam-rolled values were intermediate between the steam-flaked and dry-rolled sorghum treatments (Oliveira et al., 1995).

A study conducted by Hinman and Johnson (1974) evaluated the influence of processing methods on digestion of sorghum starch in high concentrate beef cattle rations. Sorghum grain was dry-rolled, micronized, steam-flaked, or ground and fed in a 4 × 4 Latin square design. Four steers having both rumen and abomasal cannulas were utilized to determine the digestion of starch in the rumen and lower digestive tract (Hinman and Johnson, 1974). Sorghum was included in the ration at 84% DM. A 14 d adjustment period was used for each of the four ration changes. Automatic feeders designed to deliver feed at hourly intervals were used to maintain a constant flow of digesta though the digestive tract and minimize sampling variation of abomasal contents. No differences in the amount of starch digested in the rumen were found between ration; however, reduced intestinal digestion of starch was observed with dry rolled sorghum. Starch digestion from ground sorghum grain was greater than that of dry rolled sorghum grain. *In vitro* dry matter disappearance (IVDMD) at 12 h indicated that micronized and steam-flaked sorghum were digested at a faster rate than non-heat treated sorghum grain

(Hinman and Johnson, 1974). Results from this study indicate that there was reduced accessibility for enzymatic attack on raw starch from dry rolled sorghum in the small intestine. Increased starch digestion for ground sorghum indicates that particle size is an important factor in the rate and extent of starch digestion (Hinman and Johnson, 1974).

Increasing silage digestibility through management practices such as hybrid selection, kernel processing, and ensiling durations have been extensively studied in corn. Minimal data regarding whether these practices apply to sorghum silage have been published.

Therefore, the objectives of the current research are to:

1) Determine optimal sorghum harvest stage, kernel processing method, and ensiling duration to enhance in situ degradability of nutrients for one BMR forage sorghum.
2) Evaluate the optimal plant maturity, processing, and ensiling duration for three different sorghum types: a grain sorghum, BMR forage sorghum, and a non-BMR forage sorghum.

Literature Cited

Barnhart, S. K. 2008. The ensiling process and additives. Iowa State Univ. Ext.

Bean, B.W, R. L. Baumhardt, F. T. Mccollum, and K. C. Mccuistion. 2013. Comparison of sorghum classes for grain and forage yield and forage nutritive value. Field Crops Res. 142:20–26.

Bean, B., and M. Marsalis. 2012. Corn and sorghum silage production considerations. 2012 High Plains Dairy Conference., Amarillo, Texas.

Bean, B., F. McCollum, B. Villarreal, J. Blumenthal, J. Robinson, R. Brandon, E. Buttrey, R. VanMeter, and D. Pietsch. 2009. 2009 Texas panhandle forage sorghum silage trial. Texas Agric. Ext. Serv., Texas A&M Univ., College Station.

Bernard, J. K. 2015. Comparison of sorghum silage vs corn silage. Univ. of Georgia. https://www.txanc.org/docs/Bernard-Comparison-of-Sorghum-silage-vs-Corn-silage_MSRNC2015-Author-Approved.pdf. (Accessed 3 February 2018).

Church, D. C. 1998. The Ruminant Animal: Digestive Physiology and Nutrition. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Der Bedrosian, M. C., K. E. Nestor, and L. Kung. 2012. The Effects of Hybrid, Maturity, and Length of Storage on the Composition and Nutritive Value of Corn Silage. J. Dairy Sci. 95:5115-5126.

Di Marco, O. N., M. S. Aello, M. Nomedeu, S. Van Houtte. 2002. Effect of maize crop maturity on silage chemical composition and digestibility (in vivo, in situ, and in vitro). Anim. Feed Sci. Technol. 99:37-43.

Ebling, T. L., and L. Kung. 2004. A comparison of processed conventional corn silage to unprocessed and processed brown midrib corn silage on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 87: 2519-2527.

Ferraretto, L. F., S. M. Fredin, and R. D. Shaver. 2015. Influence of ensiling, exogenous protease addition, and bacterial inoculation on fermentation profile, nitrogen factors, and ruminal in vitro starch digestibility in rehydrated and high-moisture corn. J. Dairy Sci. 98:7318-7327.

Filya, I. 2003. The effect of lactobacillus buchneri and lactobacillus plantarum on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. J. Dairy Sci. 86:3575–3581.

Firkins, J. L., M. L. Eastridge, N. R. St-Pierre, and S. M. Noftsger. 2001. Effects of grain variability and processing on starch utilization by lactating dairy cattle. J. Anim. Sci. 79: E218-E238.

Gerik, T., B. Bean, and R. Vanderlip. Sorghum growth and development. Texas Cooperative Extension. Accessed June 10, 2018. http://glasscock.agrilife.org/files/2015/05/Sorghum-Growth-and-Development.pdf.

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

Hale, W. H. 1973. Influence of processing on the utilization of grains (starch) by ruminants. J. Anim. Sci. 37: 1075-1080.

Hibberd, C. A., D. G. Wagner, R. L. Schemm, E. D. Mitchell, R. L. Hintz, and D. E. Weibel. 1982. Nutritive characteristics of different varieties of sorghum and corn grains. J. Anim. Sci. 55: 665-672

Hinman, D. D., and R. R. Johnson. 1974. Influence of processing methods on digestion of sorghum starch in high concentrate beef cattle rations. J. Anim. Sci. 39:417-422.

Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblentz, M. P. Scott, A. L. Bodnar, R. J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465-2474.

Holding, D. 2014. Recent advances in the study of prolamin storage protein organization and function. J. Front Plant Sci. 5:276.

Huntington, G. B. 1997. Starch utilization by ruminants: from basics to the bunk. J. Anim. Sci. 75:852-867.

Johnson, L. M., J. H. Harrison, D. Davidson, W. C. Mahanna, K. Shinners, and D. Linder. 2002. Corn silage management: effects of maturity, inoculation, and mechanical processing on pack density and aerobic stability. J. Dairy Sci. 85:434-444.

Kotarski, S. F., R. D. Waniska, and K. K. Thurn. 1992. Starch hydrolysis by the ruminal microflora. J. Nutr. 122:178–190.

Kung Jr, L., M. C. Windle, and N. Walker. 2014. The effect of an exogenous protease on the fermentation and nutritive value of high-moisture corn. J. Dairy Sci. 97:1707-1712.

Lin, L., D. Guo, J. Huang, X. Zhang, L. Zhang, and C. Wei. 2016. Molecular structure and enzymatic hydrolysis properties of starches from high-amylose maize inbred lines and their hybrids. Food Hydocoll. 58:246–254.

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

Martin, N. P., D. R. Mertens, M. B. Hall, and J. G. Lauer. 2008. Fiber digestibility and starch content of corn silage. Idaho Alfalfa and Forage Conference.

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 Jan. 13, 2018. http://amarillo.tamu.edu/ files/2010/11/brownmidrib.pdf.

McCollum, T., III, and B. Bean. 2003. Comparative Value of Silages Based on Digestibility. Texas Cooperative Extension. Accessed April 22, 2018.

Muck, R. E., and R. E. Pitt. 1994. Aerobic deterioration in corn silage relative to the silo face. ASAE. 37(3):735-743.

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

Muck, R. E., and J. T. Dickerson. 1988. Storage temperature effects on proteolysis in alfalfa silage. ASAE 31:1005–1009.

Muralikrishna, G., and M. Nirmala. 2005. Cereal α-amylases—an overview. Carbohydr Polym. 60:163–173.

NASS. 2017 Corn silage production. January 2017. USDA, National Agricultural Statistics Service. Accessed Feb. 7, 2018. http://www.nass.usda.gov.

NASS. 2017 Sorghum silage production. January 2017. USDA, National Agricultural Statistics Service. Accessed Feb. 7, 2018. http://www.nass.usda.gov.

Oliver, A. L., J. F. Pedersen, R. J. Grant, T. J. Klopfenstein, and H. D. Jose. 2005. Comparative effects of the sorghum bmr-6 and bmr-12 genes. Crop Sci. 45:2234–2239.

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.

Oliveira, J. S., J. T. Huber, J. M. Simas, C. B. Theurer, and R. S. Swingle. 1995. Nutrition, feeding, and calves. J. Dairy Sci. 78: 1318-1327.

Ørskov, E. R. 1986. Starch digestion and utilization in ruminants. J. Anim. Sci. 63:1624-1633.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1997. The effect of grain source and grain processing on performance of feedlot cattle: a review. J. Anim. Sci. 75:868-879.

Owens, F. N., R. A. Zinn, and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. J. Anim. Sci. 63:1634-1648.

Philippeau, C., C. Martin, and B. Michalet-Doreau. 1999. Influence of grain source on ruminal characteristics and rate, site, and extent of digestion in beef steers. J. Anim. Sci. 77:1587-1596.

Porter, K., J. Axtell, V. Lechtenberg, and V. Colenbrander. 1978. Phenotype, fiber composition, and in vitro dry matter disappearance of chemically induced brown midrib (bmr) mutants of sorghum. Crop Sci.18:205-208.

Pritchard, R. H., and D. A. Stateler. 1997. Grain processing: effects on mixing, prehension, and other characteristics of feeds. J. Anim. Sci. 1997. 75:880-884.

Romero, J. J., M. Castillo, and J. C. Burns. 2015. Forage conservation techniques: silage and haylage production. NC State Extension. Accessed June. 23, 2017. https://content.ces.ncsu.edu/forage-conservation-techniques-silage-and-haylage-production.

Rooney, W. L., S. Aydin, and L. C. Kuhlman. 2005. Assessing the relationship between endosperm type and grain yield potential in sorghum (*Sorghum bicolor* L. Moench). Field Crops Res. 91:199-205.

Rooney, W. L., and R. L. Pflugfelder. 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. J. Anim. Sci. 63:1607-1623.

Sang, Y., S. Bean, P. A. Seib, J. Pedersen, and Y. Shi. 2008.Structure and functional properties of sorghum starches differing in amylose content. J. Agri. Food Chem. 56: 6680-6685.

Tabacco, E., F. Righi, A. Quarantelli, and G. Borreani. 2011. Dry Matter and Nutritional Losses During Aerobic Deterioration of Corn and Sorghum Silages as Influenced by Different Lactic Acid Bacteria Inocula. J. Dairy Sci. 94:1409-1419.

Theurer, C. B. Grain processing effects on starch utilization by ruminants. 1986. J. Anim. Sci. 63:1649-1662.

Thomas, M. E., J. L. Foster, K. C. McCuistion, L. A. Redmon, and R. W. Jessup. 2013. Nutritive value, fermentation characteristics, and in situ disappearance kinetics of sorghum silage treated with inoculants. J. Dairy Sci. 96:7120-7131.

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

Waldo, D. R. 1973. Extent and partition of cereal grain starch digestion in ruminants. J. Anim. Sci. 37:1062.

Wilkinson, J. M., and D. R. Davies. 2012. The aerobic stability of silage: key finding and recent developments. J. Grass Forage Sci. 68: 1-19.

CHAPTER II

EFFECT OF HARVEST STAGE, KERNEL PROCESSING, AND ENSILING TIME ON DIGESTIBILITY CHARACTERISTICS OF FORAGE SORGHUM SILAGE

Introduction

The ability for forage sorghum (*Sorghum bicolor* (L.) Moench) to efficiently use water, produce high silage yields, and be planted later than corn (*Zea mays L.*) makes it an important forage crop for many concentrated animal feeding operations located within semi-arid environments (Oliver et al., 2004). Previous adoption of sorghum has been marginal compared to corn due to sorghum's generally lower feeding value attributed to its increased fiber levels and kernel maturation as it approaches physiological maturity (Hibberd et al., 1982; Thomas et al., 2013). Increased kernel maturation results in greater starch, but the lower feeding value for sorghum is likely due to endosperm characteristics and processing. In a review of the effect of harvest stage and mechanical processing on corn silage quality, Johnson et al. (1999) stated that mechanically processing corn silage enhances ensiling characteristics and starch and fiber digestion, while also reducing DM losses during ensiling. Johnson et al., (1999) summarized multiple digestion studies that observed decreases in digestibility of corn silage as maturity progressed, indicating a negative relationship between grain development and stover quality as corn matures

Ebling and Kung (2004) observed an increase in DM and starch digestion for processed corn silage compared to unprocessed corn silage. Other studies have also shown effects of corn maturity and ensiling time on corn silage digestibility (Der Bedrosian et al., 2012; Johnson et al., 2002). However, minimal research has been conducted to quantify kernel processing and ensiling time impacts on sorghum silage digestibility. Thus, the objective of this experiment was to investigate the effects of plant maturity, kernel processing, and ensiling time on the *in situ* ruminal degradability of sorghum silage to optimize silage quality.

Materials and Methods

All procedures involving live animals were approved by the West Texas A&M University/Cooperative Research, and Education, and Extension Team (CREET) Institutional Animal Care and Use Committee (approval # 02-06-17).

Animals

This experiment was conducted at the Texas A&M AgriLife Research/USDA-ARS Feedlot in Bushland, TX. Three ruminally cannulated beef steers (BW = 463 ± 15 kg) were used to calculate *in situ* ruminal digestibility of 16 sorghum silage treatments in a 2 × 2 × 4 factorial arrangement. Throughout the experiment, steers consumed a 54% forage based diet consisting of sorghum-sudan grass and corn stalks at 44 and 31.65% (DM), respectively. Due to the limited ruminal capacity of the cannulated beef steers, two consecutive sampling periods were used with each period having a 14 d adaptation period. Steers were individually housed in 2 × 18 m partially covered pens throughout each sampling period. Steers were fed once (0700 h) at maintenance level intake

(BCNRM, 2016) with ad libitum access to water. Individual BW was collected before each adaptation period.

Silage Treatments

A brachytic dwarf, BMR forage sorghum (FS; Alta Seed AF7401), was planted on June 8, 2016 in replicated plots located under center pivot irrigation (Michael Menke Farm, Bushland, Texas). Treatments were arranged in a 2 × 2 × 4 factorial arrangement with harvest maturity, grain processing method, and ensiling duration as factors. Forage biomass was sampled from a 2.3 m^2 area (0.76-m row × 3.0 m) in each plot at two different growth stages. The forage sorghum was harvested at soft dough (**SD**) and late matured forage sorghum at hard dough (**HD**) stages of maturity. Subsamples from the harvested sample were separately chopped using a chipper shredder (model CS3310, Cub Cadet, Cleveland, OH) to simulate mechanical kernel processing. Using the chipper shredder, a forage chop length of 2.0 ± 0.5-cm was achieved, and the sorghum grain was cracked (**C**), which simulates kernel-processed grain. To simulate unprocessed, whole grain (**W**), heads from the second subsample were removed. The separated forage sorghum biomass was chopped using the chipper shredder, and the heads were separately threshed so that the grain was not damaged or processed. The **W** grain was added back to the chopped forage before ensiling. Immediately after chopping and processing, dry matter was obtained by drying a sample at 105°C for 24 h in a forced air oven. The remaining silage samples were vacuum-sealed in polyethylene bags (FoodSaver, series v4840, Sunbeam Products, Inc., Boca Raton, FL). Silage samples were ensiled for 0, 30, 60, or 120 d post-harvest. Silage pH was evaluated post-ensiling as a control measure of silage preservation and quality. The *in situ* procedures in this experiment were adapted

from Vanzant et al. (1998). Samples were dried for 48 h in a forced-air oven at 55 °C (Despatch model LBB2-18-1, Minneapolis, MN) and ground (Wiley Mill, model 4, Thomas Scientific, Swedesboro, NJ) to pass a 2-mm screen.

The *in situ* bags (Ankom Technology, Macedon Ny; 10 x 20 cm, 53 ± 15 μm pore size) were individually labeled with a solvent resistant marker and weights of the bags were recorded (Model MS603S, Mettler Toledo, Greifensee, Switzerland). Five grams of ground sample were weighed in duplicate into *in situ* bags and then sealed using an impulse sealer (American International Electric, model AIE-200, City of Industry, CA). Duplicate *in situ* bags containing each treatment were then placed into mesh laundry bags; therefore, there were two forage bags per treatment, per designated *in situ* incubation time-point, per steer. Prior to ruminal incubation for each sampling period, forage bags were soaked in 39 °C water for 20 min to decrease lag time of microbial attachment. Laundry bags for each *in situ* sample time point were placed in the ventral rumen space (excluding 0 h) of three ruminally cannulated steers. For the first sampling period, bags were removed at 6 and 24 h of incubation and at 12 and 48 h for the second sampling period. Following the removal of the rumen, *in situ* bags were thoroughly rinsed to remove the soluble fraction, which was achieved when the rinsing water remained clear. All bags were dried for 72 h in a forced air oven at 55 °C before weighing. Duplicate bags were composited within animal for subsequent analysis of DM, OM, NDF, ADF, N, and Starch. Dry matter analysis was conducted by drying the sample in an oven (Precision Thelco, model 17, Precision Scientific, Chennai, India) at 105°C for 12 h. Organic matter was determined by ashing samples in a muffle furnace (Thermolyne, model F-A730, Dubuque, IA) at 500 °C for 6 h. Neutral detergent fiber

content was analyzed using an Ankom fiber analyzer with sodium sulfite and amylase (model 200, Ankom Technologies, Fairport, NY). Total nitrogen and starch of samples was analyzed at a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI).

Statistical Analysis

Compositing the *in situ* bags within animal resulted in two observations per treatment per incubation time-point. *In situ* rumen CP and starch degradation data were fitted to the first-order exponential model without discrete lag (Mertens, 1997) using the iterative Marquardt method and the NLIN procedure of SAS (SAS Institute, Cary, NC). The model is as follows:

$$R_{(t)} = b \times \left(e^{-k_d\,(t-L)} \right) + c$$

Variables included: $R_{(t)}$ = total undigested residue at any time, t = time incubated in the rumen in h, b = potentially degradable fraction, c = fraction not digested after 48 h ruminal incubation, L = lag time, and k_d = fractional rate of digestion. In situ rumen DM, OM, NDF, and ADF degradation data were not able to meet the convergence criterion; therefore, did not fit the first-order exponential model, thus discrete lag time (*L*) and the fractional rate of digestion (k_d) were calculated using a logarithmic regression generated by the REG procedure of SAS. The potentially degradable fraction (*b*) was calculated by the difference in residue at 0 h of incubation and 48 h of incubation. Effective ruminal degradability (extent of digestion, **ERD**) was calculated using the model of Ørskov and McDonald (1979):

$$\mathrm{ERD} = a + \{b \times \left[\frac{k_d}{k_d + k_p} \right] \}$$

Where k_p = assumed ruminal passage rate of 0.05, and a = immediately soluble fraction (percentage of substrate washed out of the bag at 0 h; b and k_d described above). The GLIMMIX procedure of SAS was utilized to compare a, b, c, L, k, and ERD between treatments. Treatment means of each explanatory variable was separated by the LSMEANS statement with PDIFF option with a protected F-test ($P \leq 0.05$), $P \leq 0.05$ was considered significant. Main effects of maturity, kernel processing method, ensiling time, and interactions between variables were included in the statistical model.

Results and Discussion

Dry Matter Degradability. Dry matter degradation data is presented in Table 2.1a and Table 2.1b. A maturity × processing effect was observed ($P < 0.01$) for the effective ruminal degradability (ERD) of DM. Processed SD silage had the greatest ERD values, while W treatments tended to have the least. An interaction ($P < 0.01$) between maturity, processing, and ensiling was found for the immediately soluble DM fraction a. The immediately soluble DM fraction a was greatest for SD-C silage that ensiled for 30, 60, and 120 days and was lowest for W silage that ensiled for 0 d. Ensiling increased ($P < 0.01$) the immediately soluble DM fraction and was most pronounced for silage treatments in the HD stage. This illustrates the capability for ensiling to enhance the immediately soluble DM fraction, subsequently, enhancing digestibility. A maturity × ensiling interaction ($P = 0.05$) was observed for the potentially degradable DM fraction b. The potentially degradable DM fraction was greatest for HD silage at all ensiling durations and increased in response to processing. The decrease observed in the potentially degradable DM fraction from C to W silage is possibly related to the decrease in particle size during ensiling as a result of processing. No effects ($P > 0.11$) of maturity

or processing were observed on the undegradable DM fraction *c*; however, ensiling did decrease ($P < 0.03$) the undegradable DM fraction. A maturity × processing interaction ($P = 0.03$) was seen, in which SD-C silage had the lowest amount of undegradable DM fraction compared to SD-W silage and other HD silage treatments. These results suggest that there is a greater chance to manipulate and enhance the feed value of early matured silage through processing and prolonging the ensiling duration as compared to HD silage. Maturity stage, ensiling duration, and processing all affected ($P < 0.01$) the lag before DM degradation *L*. The lag before DM degradation was less for SD silage compared to HD silage and decreased with processing as ensiling duration increased. An effect of maturity was observed ($P = 0.01$) on *in situ* rate of DM degradation k_d; however, no other effects seemed to significantly influence ($P > 0.09$) the rate of DM degradation. Ebling and Kung (2004) reported an increase in DM digestibility for processed BMR corn silage compared to unprocessed BMR corn silage for the *in vivo* portion of their experiment, and the authors found that *in situ* DM digestibility was greater for processed BMR corn silage than for unprocessed BMR corn silage after 3 and 12 h of ruminal incubation, similar to the results of the current experiment. This increase in DM digestibility may be explained by the increase in grain digestibility from the processing machine. Smith et al. (1985) conducted an experiment to determine the apparent digestibility of grain sorghum silage in response to mechanical processing and observed no difference in DM digestibility between processed and unprocessed sorghum silage, which does not concur with the results of this current trial. Differences in results between the current experiment and Smith et al. (1985) may be attributed to the different sorghum varieties used and their potentially unequal nutritive characteristics.

Organic Matter Degradability. Organic matter degradation data is presented in Table 2.2a and Table 2.2b. Similar to the SD and HD DM degradation data, the estimated ruminal degradability (ERD) of OM was affected by a maturity × processing interaction ($P < 0.01$), in which the ERD of SD-C and HD-C silage was greater than the ERD of SD-W and HD-W, respectively. An interaction ($P < 0.01$) for the immediately soluble OM fraction *a* was found between maturity stage, processing method, and ensiling duration. The immediately soluble OM fraction was greatest for SD-C silage ensiled for at least 30 d. The immediately soluble OM fraction was less for the W treatments compared to the C treatments for both maturity stages. An interaction ($P = 0.05$) between maturity stage and ensiling duration was observed for the potentially degradable OM fraction *b*. Processing and prolonging the ensiling duration decreased the potentially degradable OM fraction, which increased from SD to HD silage. No three-way interaction was observed ($P = 0.29$) between maturity stage, processing method, and ensiling duration; however, all three factors independently affected ($P < 0.01$) the lag time before OM degradation *L*. The lag time before OM degradation was less for HD silage than for SD silage, and it was decreased by grain processing and an increased ensiling duration. *In situ* rate of OM degradation k_d was unaffected ($P > 0.12$) by all treatments except maturity ($P = 0.01$). Rate of OM degradation was greater for SD silage treatments than for HD silage treatments. Organic matter digestibility values align similarly with DM digestibility values. There were minimal differences in OM degradation between HD-C and HD-W silage due to the effect of processing, which is in agreement with results reported by Johnson et al. (2002) who found no differences between processed and unprocessed corn silage on OM truly digested in the rumen. Although Johnson et al. (2002) found no

statistical differences between processed and unprocessed corn silage, the authors observed a trend that ruminal OM digestion decreased with processing. The authors speculated that this was because of an increased particle size and slower rate of passage for the unprocessed silage.

Crude Protein Degradability. Crude protein degradation data is presented in Table 2.3a and Table 2.3b. A maturity × ensiling × processing interaction was found to affect ($P < 0.01$) the estimated ruminal degradability (ERD) of CP. The ERD of CP was greatest for processed SD silage that was ensiled for greater than 0 d and was least for HD silage that was ensiled for 0 d. The immediately soluble CP fraction *a* was affected ($P = 0.01$) by a maturity × processing interaction. A maturity × ensiling interaction ($P = 0.01$) was observed for the potentially degradable CP fraction *b*. The potentially degradable CP fraction was greater ($P < 0.01$) for HD silage and for SD silage. Although the undegradable CP fraction *c* was minimal for both SD and HD silage, there was an effect ($P = 0.02$) of maturity observed. A maturity × ensiling ($P = 0.04$) and maturity × processing ($P = 0.01$) interaction was observed for the *in situ* rate of CP digestion k_d. In corn, it has been well documented that hydrophobic zein proteins are degraded during the ensiling process (Hoffman et al., 2011; Ferraretto et al., 2015), which may potentially improve CP degradation in the rumen. In sorghum, kafirin is the main protein influencing both CP and starch digestion. There is a greater proportion of cross-linked fractions in sorghum than in corn, and the sorghum kafirin proteins are more hydrophobic than zein proteins in corn (Wong et al., 2009). Although hydrophobic zein proteins are insoluble in rumen fluid, they are soluble in lactic and acetic acid produced as a product of fermentation. Proteolysis by active plant enzymes or bacterial proteases also induces

degradation of proteins (Hoffman et al., 2011). Data from the current experiment suggests that CP degradation in SD silage increases in response to kernel processing, which agrees with data presented by Ebling and Kung (2004). Dissimilar results were observed by Smith et al. (1986) with sorghum silage and Eun et al. (2004) with barley silage; these authors found no differences in CP digestibility between processed and unprocessed silage. The hydrophobic proteins are more pronounced in sorghum than for barley, which may explain the lack of differences found in CP degradation among the barley silage treatments used by Eun et al. (2004).

Starch Degradability. Starch degradation results are presented in Table 2.4a and Table 2.4b. A maturity × ensiling interaction ($P < 0.01$) was observed for effective ruminal starch degradability (ERD). Effective ruminal starch degradability was greater for SD silage than for HD silage. The ERD of starch in HD silage significantly increased as ensiling duration increased; however, minimal increases in the ERD of SD silage were observed beyond 30 d of ensiling. A trend ($P = 0.06$) for processing to increase ERD of starch was observed for both HD and SD silages. All two-way interactions for the immediately soluble starch fraction *a* were significant ($P \leq 0.02$). The immediately soluble starch fraction was greatest for SD-C silage and was greater for SD-C and HD-C than for SD-W and HD-W silage, respectively. Similar interactions were found for the potentially degradable starch fraction *b*. A more pronounced reduction in the potentially degradable starch fraction was seen between HD-C and HD-W silage than for SD silage treatments in response to ensiling. No interactions ($P > 0.24$) were present for the undegradable starch fraction *c*. Lag time before starch digestion *L* was affected by an interaction ($P < 0.01$) between maturity and ensiling. The fractional rate of starch

digestion was greater ($P < 0.01$) for SD silage than for HD silage. To achieve optimum digestibility of the grain, sorghum must be vigorously processed (Rooney and Pflugfelder, 1986). Difficulty to achieve optimal starch digestion of sorghum grain can be mostly attributed to the hydrophobic protein matrix composed of kafirin proteins and the hardness of the peripheral layer surrounding the embedded starch molecules. Mechanically processing grain in silage has shown to improve digestibility values of starch. Grain processing increases the area for microbial attachment and digestion of the starch. Johnson et al (2002) noted an increase of starch apparently digested in the rumen in response to a significant processing effect, similar to the results of the current experiment. This response to processing was also observed by Smith et al. (1986) with corn silage harvested at late dough and hard grain maturity stages. The effect of mechanical processing on *in situ* degradability requires that samples should not be ground to maintain potential treatment effects (Johnson et al., 1999). Therefore, for this experiment, it can be assumed that effects observed due to a processing interaction may be attributed to chemical changes undergone during the ensiling process. One potential explanation for the increase in ERD of starch observed as ensiling duration increased could be that hydrophobic kafirin proteins were either solubilized in lactic acid during fermentation, or that the kafirin proteins were degraded by proteolytic mechanisms such as plant enzymes or bacterial proteases as mentioned by Hoffman et al. (2011) in regards to zein proteins in corn.

Conclusions

Sorghum silage digestibility is greater for early matured sorghum than for later matured sorghum, with the most pronounced increases in digestibility seen for CP and starch digestion. The results from this experiment suggest that sorghum silage digestibility is enhanced by kernel processing and is generally increased with a prolonged ensiling duration. Drastic increases in CP and starch degradation due to both processing and ensiling were most likely a function of the disruption and degradation of the starch-protein matrix. Results from this research conclude that silage quality is optimized for forage sorghum harvested at soft-dough. For forage sorghum harvested at soft dough, the greatest starch digestibility values were observed at a 60 d ensiling duration. Conversely, for forage sorghums not harvested until hard dough a greater ensiling duration is necessary. Because of substantial differences that exist between sorghum varieties, further research should evaluate the effect of ensiling duration across various varieties.

Literature Cited

Der Bedrosian, M. C., K. E. Nestor, and L. Kung. 2012. The Effects of Hybrid, Maturity, and Length of Storage on the Composition and Nutritive Value of Corn Silage. J. Dairy Sci. 95:5115-5126.

Ebling, T. L., and L. Kung. 2004. A comparison of processed conventional corn silage to unprocessed and processed brown midrib corn silage on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 87: 2519-2527.

Eun, J. S, K. A. Beauchemin, S. H. Hong, and W. Z. Yang. 2004. Effects of mechanical processing on the nutritive value of barley silage for lactating dairy cows. J. Dairy Sci. 87: 4170-4177.

Ferraretto, L. F., S. M. Fredin, and R. D. Shaver. 2015. Influence of ensiling, exogenous protease addition, and bacterial inoculation on fermentation profile, nitrogen factors, and ruminal in vitro starch digestibility in rehydrated and high-moisture corn. J. Dairy Sci. 98:7318-7327.

Hibberd, C. A., D. G. Wagner, R. L. Schemm, E. D. Mitchell, R. L. Hintz, and D. E. Weibel. 1982. Nutritive characteristics of different varieties of sorghum and corn grains. J. Anim. Sci. 55: 665-672.

Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblentz, M. P. Scott, A. L. Bodnar, R. J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on

alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465-2474.

Johnson, L. M., J. H. Harrison, D. Davidson, W. C. Mahanna, K. Shinners, and D. Linder. 2002. Corn silage management: effects of maturity, inoculation, and mechanical processing on pack density and aerobic stability. J. Dairy Sci. 85:434-444.

Johnson, L. M., J. H. Harrison, C. Hunt, K. Shinners, C. G. Doggett, and D. Sapienzas. 1999. Nutritive value of corn silage as affected by maturity and mechanical processing: a contemporary review. J. Dairy Sci. 82: 2813-2825.

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

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 for incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499-503.

Rooney, W. L., and R. L. Pflugfelder. 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. J. Anim. Sci. 63:1607-1623.

Smith, R., K. Bolsen, H. Ilg, J. Hoover, and J. Dickerson. 1985. Whole-plant forage, grain, or nonheading sorghum silages for growing cattle. Kansas Agricultural Experiment Station Research Reports:71–76.

Thomas, M. E., J. L. Foster, K. C. McCuistion, L. A. Redmon, and R. W. Jessup. 2013. Nutritive value, fermentation characteristics, and in situ disappearance kinetics of sorghum silage treated with inoculants. J. Dairy Sci. 96:7120-7131.

Vanzant, E. S., R. C. Cochran, and E. C. Titgemeyer. 1998. Standardization of *in situ* techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76:2717-2729.

Wong, J. H., T. Lau, N. Cai, J. Singh, J. F. Pedersen, W. H. Vensel, W. J. Hurkman, J. D. Wilson, P. G. Lemaux, and B. B. Buchanan. 2009. Digestibility of protein and starch from sorghum (sorghum bicolor) is linked to biochemical and structural features of grain endosperm. J. Cereal Sci. 49:73-82

Table 2.1a Ruminal *in situ* DM degradability characteristics of sorghum silage harvested at soft and hard dough maturity with varying ensiling durations and processing methods in Exp. 1.

	Treatment[1]							
	SD							
	C				**W**			
Item[2]	**0**	**30**	**60**	**120**	**0**	**30**	**60**	**120**
a, %	33.7[d]	39.3[a]	40.5[a]	39.7[a]	27.9[g]	32.4[e]	34.9[c]	36.0[cb]
b, %	29.4	26.6	26.2	27.4	32.0	28.5	29.5	30.0
c, %	36.9	34.0	33.3	33.0	40.2	39.1	35.6	34.1
L, h	4.20	4.14	4.13	4.14	4.22	4.21	4.16	4.15
kd, h	0.0127	0.0122	0.0126	0.0124	0.0120	0.0115	0.0124	0.0131
ERD, %	39.7	44.6	45.8	45.1	34.1	37.8	40.8	42.2
	HD							
	C				**W**			
Item[2]	**0**	**30**	**60**	**120**	**0**	**30**	**60**	**120**
a, %	28.2[gf]	35.0[c]	36.3[b]	39.6[a]	29.1[f]	33.2[ed]	33.6[d]	36.6[b]
b, %	34.8	32.8	29.7	24.3	32.0	32.5	32.5	31.1
c, %	37.0	32.2	34.1	36.1	35.2	34.4	34.0	32.3
L, h	4.30	4.21	4.16	4.10	4.31	4.24	4.20	4.15
kd, h	0.0145	0.0139	0.0128	0.0105	0.0155	0.0146	0.0141	0.0140
ERD, %	36.0	42.1	42.3	43.9	37.6	40.5	40.8	43.5

[1]SD = sorghum in the soft dough stage, HD = sorghum in the hard dough stage, C = cracked sorghum grain, W = unprocessed sorghum grain, 0, 30, 60, and 120 = days of ensiling, respectively.

[2] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

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

Table 2.1b Statistical analysis of ruminal *in situ* DM degradability characteristics in Exp. 1.

		***P*-value[2]**						
Item[1]	**SEM[3]**	**M**	**E**	**P**	**M × E**	**M × P**	**E × P**	**M × E × P**
a, %	0.58	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
b, %	1.58	<0.01	<0.01	<0.01	0.05	0.96	0.34	0.49
c, %	1.65	0.11	0.03	0.22	0.33	0.03	0.23	0.91
L, h	0.014	<0.01	<0.01	<0.01	<0.01	0.86	0.63	0.43
kd, h	0.0005	0.01	0.46	0.19	0.15	0.09	0.50	0.96
ERD, %	0.63	0.36	<0.01	<0.01	0.42	<0.01	0.18	0.28

[1]*a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.
[2]M = main interaction of maturity, P = main interaction of processing method, E = main interaction of ensiling duration, M x E = the interaction of maturity and ensiling duration, M x P = the main interaction of maturity and processing method, E x P = the interaction of ensiling duration and processing method, and M x E x P = the interaction of maturity, ensiling duration, and processing method.
[3]SEM = standard error of mean

Table 2.1a Ruminal *in situ* OM degradability characteristics of sorghum silage harvested at soft and hard dough maturity with varying ensiling durations and processing methods in Exp. 1.

	Treatment[1]							
	SD							
	C				W			
Item[2]	0	30	60	120	0	30	60	120
a, %	32.7^{de}	37.8^{a}	39.0^{a}	38.2^{a}	25.4^{h}	30.0^{f}	33.2^{cd}	34.4^{cb}
b, %	30.5	28.2	27.6	28.4	33.8	30.5	31.2	32.1
c, %	36.8	34.0	33.4	33.4	40.8	39.6	35.7	33.6
L, h	4.22	4.17	4.15	4.16	4.25	4.25	4.18	4.17
kd, h	0.0132	0.0127	0.0131	0.0130	0.0125	0.0120	0.0129	0.0136
ERD, %	39.1	43.5	44.8	44.0	32.3	35.9	39.6	41.2
	HD							
	C				W			
Item[2]	0	30	60	120	0	30	60	120
a, %	26.2^{hg}	32.9^{cde}	35.0^{b}	37.6^{a}	27.2^{g}	31.5^{fe}	31.7^{de}	35.2^{b}
b, %	37.0	35.2	30.7	26.2	37.9	34.4	34.3	32.7
c, %	36.9	31.9	34.3	36.2	34.9	34.1	34.0	32.0
L, h	4.33	4.24	4.18	4.11	4.33	4.27	4.22	4.17
kd, h	0.0153	0.0147	0.0134	0.0113	0.0163	0.0153	0.0145	0.0147
ERD, %	34.7	40.9	41.5	42.5	36.6	39.6	39.5	42.7

[1]SD = sorghum in the soft dough stage, HD = sorghum in the hard dough stage, C = cracked sorghum grain, W = unprocessed sorghum grain, 0, 30, 60, and 120 = days of ensiling, respectively.

[2] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

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

Table 2.2b Statistical analysis of ruminal *in situ* OM degradability characteristics in Exp. 1.

		***P*-value[2]**						
Item[1]	**SEM[3]**	**M**	**E**	**P**	**M × E**	**M × P**	**E × P**	**M × E × P**
a, %	0.80	<0.01	<0.01	<0.01	0.01	<0.01	0.10	<0.01
b, %	1.69	<0.01	<0.01	<0.01	0.05	0.70	0.31	0.60
c, %	1.24	0.07	0.04	0.28	0.25	0.03	0.15	0.92
L, h	0.021	<0.01	<0.01	<0.01	<0.01	0.71	0.71	0.29
kd, h	0.0006	0.01	0.51	0.25	0.15	0.12	0.55	0.96
ERD, %	0.73	0.58	<0.01	<0.01	0.46	<0.01	0.16	0.20

[1] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.
[2]M = main interaction of maturity, P = main interaction of processing method, E = main interaction of ensiling duration, M x E = the interaction of maturity and ensiling duration, M x P = the main interaction of maturity and processing method, E x P = the interaction of ensiling duration and processing method, and M x E x P = the interaction of maturity, ensiling duration, and processing method.
[3]SEM = standard error of mean

Table 2.1a Ruminal *in situ* CP degradability characteristics of sorghum silage harvested at soft and hard dough maturity with varying ensiling durations and processing methods in Exp. 1.

	Treatment[1]							
	SD							
	C				**W**			
Item[2]	**0**	**30**	**60**	**120**	**0**	**30**	**60**	**120**
a, %	43.3	57.7	58.1	58.0	36.8	50.7	51.0	52.5
b, %	50.5	42.3	42.0	42.0	54.3	68.9	49.0	47.5
c, %	6.3	0.0	0.0	0.0	8.9	10.1	0.0	0.0
L, h	4.20	4.14	4.13	4.14	4.22	4.21	4.16	4.15
kd, h	0.0152	0.0096	0.0093	0.0094	0.0208	0.0214	0.0140	0.0138
ERD, %	54.9^{c}	64.5^{a}	64.6^{a}	64.6^{a}	52.6^{de}	61.4^{b}	61.8^{b}	62.8^{ab}
	HD							
	C				**W**			
Item[2]	**0**	**30**	**60**	**120**	**0**	**30**	**60**	**120**
a, %	22.4	31.1	36.8	40.9	18.7	31.1	36.8	34.8
b, %	77.6	68.9	62.5	59.1	81.3	68.9	63.2	65.2
c, %	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0
L, h	4.15	4.15	4.15	4.15	4.15	4.15	4.15	4.15
kd, h	0.0147	0.0129	0.0139	0.0143	0.0159	0.0153	0.0146	0.0170
ERD, %	40.0^{i}	45.2^{h}	50.4^{f}	54.0^{cd}	38.3^{j}	47.2^{g}	51.0^{ef}	51.3^{ef}

[1]SD = sorghum in the soft dough stage, HD = sorghum in the hard dough stage, C = cracked sorghum grain, W = unprocessed sorghum grain, 0, 30, 60, and 120 = days of ensiling, respectively.

[2] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

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

Table 2.3b Statistical analysis of ruminal *in situ* CP degradability characteristics in Exp. 1.

		***P*-value[2]**						
Item[1]	**SEM[3]**	**M**	**E**	**P**	**M × E**	**M × P**	**E × P**	**M × E × P**
a, %	1.58	<0.01	<0.01	<0.01	0.04	0.01	0.62	0.23
b, %	3.00	<0.01	<0.01	0.05	0.01	0.81	0.30	0.68
c, %	1.25	0.02	0.12	0.24	0.09	0.19	0.40	0.46
L, h	0.014	<0.01	<0.01	<0.01	<0.01	0.86	0.63	0.43
kd, h	0.0019	0.49	0.04	<0.01	0.04	0.01	0.31	0.49
ERD, %	0.99	<0.01	<0.01	<0.01	<0.01	<0.01	0.20	<0.01

[1] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

[2]M = main interaction of maturity, P = main interaction of processing method, E = main interaction of ensiling duration, M x E = the interaction of maturity and ensiling duration, M x P = the main interaction of maturity and processing method, E x P = the interaction of ensiling duration and processing method, and M x E x P = the interaction of maturity, ensiling duration, and processing method.

[3]SEM = standard error of mean

Table 2.1a Ruminal *in situ* starch degradability characteristics of sorghum silage harvested at soft and hard dough maturity with varying ensiling durations and processing methods in Exp. 1.

	Treatment[1]							
	SD							
	C				**W**			
Item[2]	**0**	**30**	**60**	**120**	**0**	**30**	**60**	**120**
a, %	68.9	76.1	79.9	78.2	34.6	63.6	51.9	51.2
b, %	31.1	23.1	20.1	21.2	64.2	36.4	48.1	48.8
c, %	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0
L, h	4.20	4.14	4.13	4.14	4.22	4.21	4.16	4.15
kd, h	0.0665	0.0691	0.0539	0.0525	0.0397	0.0637	0.0727	0.0940
ERD, %	86.7	89.2	90.3	89.6	79.5	83.9	80.4	82.3
	HD							
	C				**W**			
Item[2]	**0**	**30**	**60**	**120**	**0**	**30**	**60**	**120**
a, %	22.2	39.0	46.4	51.1	14.8	41.9	40.9	37.8
b, %	77.8	61.0	53.6	48.9	82.3	58.1	59.1	62.2
c, %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L, h	4.15	4.15	4.15	4.15	4.15	4.15	4.15	4.15
kd, h	0.0443	0.0366	0.0461	0.0516	0.0361	0.0393	0.0460	0.0497
ERD, %	58.9	64.8	72.0	75.9	50.5	67.4	69.1	68.6

[1]SD = sorghum in the soft dough stage, HD = sorghum in the hard dough stage, C = cracked sorghum grain, W = unprocessed sorghum grain, 0, 30, 60, and 120 = days of ensiling, respectively.

[2] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

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

Table 2.4b Statistical analysis of ruminal *in situ* starch degradability characteristics in Exp. 1.

		***P*-value[2]**						
Item[1]	**SEM[3]**	**M**	**E**	**P**	**M × E**	**M × P**	**E × P**	**M × E × P**
a, %	3.33	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	0.39
b, %	3.19	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.50
c, %	0.63	0.24	0.67	0.82	0.67	0.82	0.39	0.39
L, h	0.014	<0.01	<0.01	<0.01	<0.01	0.86	0.63	0.43
kd, h	0.0090	<0.01	0.41	0.10	0.28	0.07	0.57	0.34
ERD, %	1.92	<0.01	<0.01	<0.01	<0.01	0.06	0.06	0.19

[1]*a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

[2]M = main interaction of maturity, P = main interaction of processing method, E = main interaction of ensiling duration, M x E = the interaction of maturity and ensiling duration, M x P = the main interaction of maturity and processing method, E x P = the interaction of ensiling duration and processing method, and M x E x P = the interaction of maturity, ensiling duration, and processing method.

[3]SEM = standard error of mean

CHAPTER III

EFFECT OF ENSILING TIME AND HYBRID ON DIGESTIBILITY CHARACTERISTICS OF SORGHUM SILAGE

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) has increasingly become a major crop grown for silage production in semi-arid regions (Di Marco et al., 2009). Frequent variation in the nutritive value of sorghum has been observed, deeming sorghum to be of lesser value than corn (*Zea mays* L.). However, some trials have demonstrated greater digestibility values for sorghum grain than for corn grain (Hibberd et al., 1982). Selectively seeking superior sorghum varieties may play an important role in optimizing ration digestibility. Large differences in digestibility among sorghum varieties have been reported (Hibberd et al., 1982). Oliver et al. (2004) found a significant increase in DM digestibility for BMR sorghum than for conventional sorghum due to an increase in lignin concentration of the conventional sorghum. Digestibility of sorghum silage may also increase with an increased ensiling time, which was demonstrated in corn silage by Der Bedrosian et al. (2012) and in the previous study. Varietal differences in endosperm type such as waxy or normal endosperms, or differences in lignin concentration, grain yield, and berry color have adverse effects on sorghum digestibility. Further research is

warranted to evaluate digestibility differences between sorghum hybrids and to determine an optimal ensiling duration to maximize sorghum silage digestibility. Therefore, the objective of this experiment is to evaluate an optimal ensiling time across three different hybrids: a white food-grade grain sorghum, a BMR forage sorghum, and non-BMR conventional sorghum.

Materials and Methods

All procedures involving live animals were approved by the West Texas A&M University/Cooperative Research, and Education, and Extension Team (CREET) Institutional Animal Care and Use Committee (approval # 02-06-17).

Animals

This experiment was conducted at the Texas A&M AgriLife Research/USDA-ARS Feedlot in Bushland, TX. Three ruminally cannulated beef steers (BW = 570 ± 17.9 kg) were used to calculate *in situ* ruminal digestibility of six sorghum silage treatments. Throughout this experiment, steers consumed a 54% forage based diet consisting of sorghum-sudan grass and corn stalks at 44 and 31.65% (DM), respectively. Steers were individually housed in 2 × 18 m partially covered pens throughout the sampling period. Steers were fed once (0700 h) at maintenance level intake (BCNRM, 2016) with ad libitum access to water. Individual BW was collected before each adaptation period.

Silage Treatments

Long season, brachytic dwarf forage sorghum with a bronze grain (FS; Alta Seed AF7401), medium maturity forage sorghum with brown grain (FS; Sorghum Partners NK300), and a medium maturity grain sorghum with a cream grain (GS; Sorghum Partners SP33S40) were planted in replicated plots located under center pivot irrigation

(Michael Menke Farm, Bushland, Texas). Forage biomass was sampled from a 2.3 m^2 area (0.76-m row × 3.0 m) in each plot at the SD stage. The forage samples were chopped using a chipper shredder (model CS3310, Cub Cadet, Cleveland, OH) to stimulate mechanical kernel processing. Using the chipper shredder, a forage chop length of 2.0 ± 0.5-cm was achieved. Immediately after chopping and processing, DM was obtained by drying a sample at 105°C for 24 h in a forced air oven. The remaining silage samples were vacuum-sealed in polyethylene bags (FoodSaver, series v4840, Sunbeam Products, Inc., Boca Raton, FL). Treatments were arranged in a 2 × 3 factorial arrangement with ensiling duration and hybrid as factors. Ensiling durations for treatments were 0 and 30 d post-harvest. Silage pH was evaluated post-ensiling as a control measure of silage preservation and quality. The *in situ* procedures in this experiment were adapted from Vanzant et al. (1998). Samples were dried for 48 h in a forced-air oven at 55°C (Despatch model LBB2-18-1, Minneapolis, MN) and ground (Wiley Mill, model 4, Thomas Scientific, Swedesboro, NJ) to pass a 2-mm screen. *In situ* bags (Ankom Technology, Macedon Ny; 10 x 20 cm, 53 ± 15 μm pore size) were individually labeled with a solvent resistant marker, and weights of the bags were recorded. Five grams of ground sample were weighed in duplicate into *in situ* bags and then sealed using an impulse sealer (American International Electric, model AIE-200). Duplicate *in situ* bags containing each treatment were then placed into mesh laundry bags; therefore, there were two *in situ* bags per treatment, per designated *in situ* incubation time-point, per steer. Prior to ruminal incubation for each sampling period, *in situ* bags were soaked in 39°C water for 20 min to decrease lag time of microbial attachment. Laundry bags for each *in situ* sample time point were placed in the ventral rumen space (excluding 0 h) of three ruminally

cannulated steers. *In situ* bags were removed at 0, 6, 12, 24, and 48 h of incubation. Following the removal of the rumen, *in situ* bags were thoroughly rinsed to remove the soluble fraction, which was achieved when the rinsing water remained clear. All *in situ* bags were dried for 72 h in a forced air oven at 55°C before weighing. Duplicate bags were composited within animal for subsequent analysis of DM, OM, NDF, ADF, N, and Starch. Dry matter analysis was conducted by drying the sample in an oven (Precision Thelco, model 17, Precision Scientific, Chennai, India) at 105°C for 12 h. Organic matter was determined by ashing samples in a muffle furnace (Thermolyne, model F-A730, Dubuque, IA) at 500°C for 6 h. Neutral detergent fiber content was analyzed using an Ankom fiber analyzer with sodium sulfite and amylase (model 200, Ankom Technologies, Fairport, NY). Total nitrogen and starch of samples was analyzed at a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI).

Statistical Analysis

Compositing the *in situ* bags within animal resulted in two observations per treatment per incubation time-point. *In situ* rumen CP and starch degradation data were fitted to the first-order exponential model without discrete lag (Mertens, 1997) using the iterative Marquardt method and the NLIN procedure of SAS (SAS Institute, Cary, NC). The model is as follows:

$$R_{(t)} = b \times \left(e^{-k_d (t-L)} \right) + c$$

Variables included: $R_{(t)}$ = total undigested residue at any time, t = time incubated in the rumen in h, b = potentially degradable fraction, c = fraction not digested after 48 h ruminal incubation, L = lag time, and k_d = fractional rate of digestion. In situ rumen DM, OM, NDF, and ADF degradation data were not able to meet the convergence criterion;

therefore, did not fit the first-order exponential model, thus discrete lag time (*L*) and the fractional rate of digestion (k_d) were calculated using a logarithmic regression generated by the REG procedure of SAS. The potentially degradable fraction (*b*) was calculated by the difference in residue at 0 h of incubation and 48 h of incubation. Effective ruminal degradability (extent of digestion, **ERD**) was calculated using the model of Ørskov and McDonald (1979):

$$\text{ERD} = a + \{b \times \left[\frac{k_d}{k_d + k_p}\right]\}$$

Where k_p = assumed ruminal passage rate of 0.05, and *a* = immediately soluble fraction (percentage of substrate washed out of the bag at 0 h; *b* and k_d described above). The GLIMMIX procedure of SAS was utilized to compare *a*, *b*, *c*, *L*, *k*, and ERD. Treatment means of each explanatory variables were separated by the LSMEANS statement with PDIFF option with a protected F-test ($P \leq 0.05$), $P \leq 0.05$ was considered significant. Main effects for this research were variety type and ensiling time, and interactions between variables were included in the statistical model.

Results and Discussion

Dry Matter Degradability. Dry matter degradation data is presented in Table 3.1. There was an effect ($P < 0.01$) of hybrid type on the effective ruminal degradability (ERD) of DM for the silage treatments. SP33S40 displayed the greatest ERD values as compared to NK300 and AF7401. An effect ($P < 0.01$) of sorghum hybrid was found for the immediately soluble DM fraction *a*. Means for the immediately soluble DM fraction *a* of SP33S40, AF7401, and NK300 were 49.3, 39.9, and 38.8, respectively, and was greatest ($P < 0.01$) for SP33S40. The potentially degradable DM fraction *b* was less ($P =$

0.02) for all varieties evaluated when ensiled at 60 d than for 0 d. There were no differences in the undegradable DM fraction *c* ($P \geq 0.57$) due to either hybrid or ensiling. Lag time before DM degradation *L* was affected by both hybrid ($P = 0.03$) and ensiling ($P < 0.01$). There were no differences ($P \geq 0.09$) found between silage treatments for the rate of DM degradation k_d. Similar to the results of this trial, Oliver et al. (2004) observed differences in total tract DM digestibility between BMR and non-BMR sorghum silage, which is presumably attributed to the increased lignin levels in the non-BMR sorghum. *In situ* and *in vitro* degradation results by Di Marco et al. (2009) also noted hybrid differences. Similar to the results of the current experiment, the authors also reported that DM disappearance was greater for grain sorghum than for BMR sorghum silage. However, results from the *in vivo* portion of their trial using 15 lambs disagreed with the data of the current experiment, suggesting that there were no differences in DM digestibility between grain and BMR sorghum silage (Di Marco et al., 2009). Bean et al. (2013) compared multiple sorghum classes in relation to their digestibility characteristics and found an increase in *in vitro*-true-digestibility (IVTD) for BMR forage sorghum compared to non-BMR forage sorghum. Bean et al. (2013) observed an increase in IVTD for grain sorghum compared to forage sorghum, but the authors suggested that due to a weak correlation between grain yield and IVTD, high grain producing sorghum cultivars should not be considered when selecting for high digestibility.

Organic Matter Degradability. Organic matter degradation data is presented in Table 2.2. Effective ruminal degradability (ERD) of OM was affected ($P < 0.01$) by hybrid, which significantly increased for SP33S40-0 and SP33S40-60 silage compared to the remaining silage treatments. A hybrid × ensiling interaction ($P < 0.01$) for the

immediately soluble OM fraction *a* was observed. The immediately soluble OM fraction *a* increased as ensiling duration increased from 0 to 60 d for all sorghum varieties, and was greatest for SP33S40-60 silage. The potentially degradable OM fraction *b* decreased ($P = 0.01$) as a result of an increase in ensiling duration from 0 to 60 d. No interactions ($P \geq 0.51$) were found between hybrids and ensiling duration for the undegradable OM fraction *c*. Main effects of ensiling ($P = 0.01$) and variety type ($P = 0.04$) were found for the lag time before OM degradation *L*, with the NK300 variety and 60 d of ensiling being statistically greater. There were no differences ($P = 0.05$) in the rate of OM degradation between hybrids, and no other effects of ensiling were observed. Organic matter digestibility values for the *in vivo* portion of the trial conducted by Di Marco et al. (2009) previously mentioned, remained inconsistent with the values observed in the current experiment in that they found no differences in OM digestibility between grain, BMR, and non-BMR sorghum silages. Similar to the OM degradation data presented in the current trial, Johnson et al. (2002) found an effect of corn hybrid type for on the silage OM truly digested in the rumen. Overall, the majority of data reviewed suggest that the hybrid may affect both the digestibility of DM and OM in sorghum silages (Johnson et al., 2002).

Crude Protein Degradability. Crude protein degradation data is presented in Table 3.3. There were differences in ERD of CP ($P < 0.01$) due to hybrid and ensiling duration. Increasing the ensiling duration from 0 d to 60 d increased the ERD of CP for all hybrids, with SP33S40 displaying the greatest amounts of digestibility. No interaction ($P \geq 0.81$) existed between hybrid and ensiling for *in situ* CP disappearance parameters. Differences were observed for the immediately soluble CP fraction *a* and the potentially

degradable CP fraction *b* because of the main effects of hybrid ($P < 0.01$) and ensiling duration ($P < 0.01$). SP33S40 and 60 d ensiling time displayed the greatest immediately soluble CP fraction *a*, with mean values of 58.5 and 53.8, respectively. The potentially degradable CP fraction *b* was greater for both AF7401 and NK300 than for SPSP33S40, which was greatest when ensiled for 0 d because fermentation did not occur. There was no undegradable CP residue *c* remaining for the silage treatments following the 48 h ruminal incubation. The rate of CP degradation k_d was unaffected ($P \geq 0.19$) by hybrid and ensiling duration. It is well understood that proteolytic mechanisms alter both the composition and degradability of CP throughout the ensiling process. Differences in the ERD of CP between 0 and 60 d of ensiling in the current trial suggest that proteolytic activity during the ensiling process improved CP digestibility, which is in agreement with a study conducted by Hoffman et al. (2011). An experiment conducted by Der Bedrosian et al. (2012) observed a significant interaction of both hybrid and ensiling duration on the percent of CP, soluble protein, and Ammonia-N % in corn silage. Der Bedrosian et al. (2012) attributed the increase of these variables as ensiling time increased to protein degradation during the fermentation process. Wilkinson et al. (1976) observed no effects of ensiling on the apparent digestibility of N; however, the ensiling duration for this experiment was not reported. Oliver et al. (2004) observed no differences in the total tract digestibility of CP between BMR and non-BMR sorghum silage, which disagrees with the results of the current trial. Oliver et al. (2004) most likely saw no differences in protein degradation due to a similar protein profile between the BMR and non-BMR sorghum. The reason for the difference in results between experiments may be due to evaluation of grain sorghum in the current experiment.

Starch Degradability. Starch degradation data is presented in Table 3.4. There were differences in the ERD of starch attributable to main effects ($P \leq 0.01$) for hybrid and ensiling duration. Starch digestibility increased in response to ensiling the forages for 60 d. SP33S40 was superior in starch digestibility compared to the remaining varieties. An interaction between hybrid and ensiling duration was observed for both the immediately soluble starch fraction *a* ($P < 0.01$) and the potentially degradable starch fraction *b* ($P < 0.01$). The immediately soluble starch fraction increased within each hybrid as ensiling time increased from 0 to 60 d, and the immediately soluble starch fraction was greatest ($P \leq 0.01$) for SP33S40 silage at both time points due to the larger fraction of grain with this hybrid. The potentially degradable starch fraction was greater ($P < 0.01$) for SP33S40-0 than for SP33S40-60 and was greater ($P = 0.01$) for AF7401-0 than for AF7401-60. No differences in the undegradable starch fraction *c* ($P \geq 0.34$) were observed due to variety type or ensiling duration. Differences (P = 0.01) were observed for the rate of starch degradation k_d due to variety type, which was greatest SP33S40 silage. The disruption of the protein matrix in sorghum grain is essential to improve starch digestion (Theurer, 1986). Similarly, increasing the ensiling duration improved starch digestion due to the increased solubilization of the zein protein matrix in corn (Ferraretto et al., 2015). Der Bedrosian et al. (2012) also concluded that as ensiling length progressed, *in vitro* starch digestion increased in corn. Very few studies have assessed the relationship between sorghum variety type and starch digestion. Ebling and Kung (2004) found no improvements in starch digestion between conventional and BMR corn silage, and Oliver et al. (2004) reported no improvements in starch digestibility between conventional and BMR sorghum silage. The differences observed in starch digestibility

due to a hybrid effect in the current trial were most likely a result of the increased grain fraction in the grain sorghum silage compared to the BMR and non-BMR forage sorghum hybrids.

Conclusions

The results from this experiment suggest that the grain sorghum used in the current study is superior in digestibility characteristics compared to the BMR and non-BMR forage sorghum silages used. Grain sorghum displayed the greatest digestibility for all variables, which is primarily a function of an increased starch concentration as a result of a greater grain fraction compared to the forage sorghums. Consideration towards feeding a grain sorghum silage should be taken, assuming yield is adequate. Ensiling did not affect *in situ* DM or OM degradation for all hybrids, however it did substantially increase CP and starch degradation, that may be a result of protein solubilization. Furthermore, ensiling sorghum forages for 60 d, regardless of variety, has shown to improve digestibility characteristics of protein and starch, and should be a management decision considered by producers who desire increasing overall forage quality.

Literature Cited

Bean, B.W, R. L. Baumhardt, F. T. McCollum, and K. C. Mccuistion. 2013. Comparison of sorghum classes for grain and forage yield and forage nutritive value. Field Crops Res. 142:20–26.

Der Bedrosian, M. C., K. E. Nestor, and L. Kung. 2012. The Effects of Hybrid, Maturity, and Length of Storage on the Composition and Nutritive Value of Corn Silage. J. Dairy Sci. 95:5115-5126.

Di Marco, O. N., M. S. Aello, M. Nomedeu, S. Van Houtte. 2002. Effect of maize crop maturity on silage chemical composition and digestibility (in vivo, in situ, and in vitro). Anim. Feed Sci. Technol. 99:37-43.

Ebling, T. L., and L. Kung. 2004. A comparison of processed conventional corn silage to unprocessed and processed brown midrib corn silage on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 87: 2519-2527.

Ferraretto, L. F., S. M. Fredin, and R. D. Shaver. 2015. Influence of ensiling, exogenous protease addition, and bacterial inoculation on fermentation profile, nitrogen factors, and ruminal in vitro starch digestibility in rehydrated and high-moisture corn. J. Dairy Sci. 98:7318-7327.

Hibberd, C. A., D. G. Wagner, R. L. Schemm, E. D. Mitchell, R. L. Hintz, and D. E. Weibel. 1982. Nutritive characteristics of different varieties of sorghum and corn grains. J. Anim. Sci. 55: 665-672.

Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblentz, M. P. Scott, A. L. Bodnar, R. J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465-2474.

Johnson, L. M., J. H. Harrison, D. Davidson, W. C. Mahanna, K. Shinners, and D. Linder. 2002. Corn silage management: effects of maturity, inoculation, and mechanical processing on pack density and aerobic stability. J. Dairy Sci. 85:434-444.

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

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 for incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499-503.

Theurer, C. B. Grain processing effects on starch utilization by ruminants. 1986. J. Anim. Sci. 63:1649-1662.

Vanzant, E. S., R. C. Cochran, and E. C. Titgemeyer. 1998. Standardization of *in situ* techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76:2717-2729.

Wilkinson, J. M., J. T. Huber, and H. E. Henderson. 1976. Acidity and proteolysis as factors affecting the nutritive value of corn silage. J. Anim. Sci. 42: 208-218.

Table 3.1 Ruminal *in situ* DM degradability characteristics of three sorghum hybrids[1] when ensiled for either 0 or 60 d in Exp. 2.

	Treatment									
	SP33S40		NK300		AF7401			*P*-value[3]		
Item[2]	0	60	0	60	0	60	SEM[4]	V	E	V × E
a, %	44.6	54.0	36.2	41.4	36.9	42.8	1.26	<0.01	<0.01	0.07
b, %	33.3	18.2	34.5	33.8	37.9	28.2	5.96	0.13	0.02	0.23
c, %	22.1	27.8	29.3	24.9	25.2	29.0	5.41	0.80	0.57	0.35
L, h	2.41	4.85	4.54	6.12	3.68	4.61	0.832	0.03	<0.01	0.43
k_d, h	0.0405	0.0536	0.0395	0.0273	0.0403	0.0200	0.0106	0.09	0.28	0.10
ERD, %	59.3	63.3	50.8	53.2	52.7	50.8	2.72	<0.01	0.32	0.28

[1]SP = SP33S40 (Sorghum Partners, Lubbock, TX); NK = NK300 (Sorghum Partners, Lubbock, TX); AF = AF7401 (Alta Seeds, Irving, TX)

[2] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

[3]V = main interaction of variety type, E = main interaction of ensiling duration, and V x E = the interaction of variety type and ensiling duration.

[4]SEM = standard error of mean

Table 3.2 Ruminal *in situ* OM degradability characteristics of three sorghum hybrids[1] when ensiled for either 0 or 60 d in Exp. 2.

	Treatment									
	SP33S40		NK300		AF7401			*P*-value[3]		
Item[2]	0	60	0	60	0	60	SEM[4]	V	E	V × E
a, %	42.8[b]	53.4[a]	34.5[d]	39.6[c]	35.4[d]	41.4[b]	0.85	<0.01	<0.01	<0.01
b, %	34.3	18.8	35.8	33.5	38.8	29.4	5.47	0.09	0.01	0.24
c, %	22.9	27.8	29.7	27.0	25.8	29.3	5.17	0.69	0.51	0.51
L, h	3.22	4.95	4.67	6.45	3.97	4.67	0.729	0.04	0.01	0.52
k_d, h	0.0456	0.0580	0.0415	0.0319	0.0428	0.0213	0.0110	0.05	0.31	0.11
ERD, %	58.9	63.4	50.3	52.7	52.3	49.9	2.85	<0.01	0.34	0.21

[1]SP = SP33S40 (Sorghum Partners, Lubbock, TX); NK = NK300 (Sorghum Partners, Lubbock, TX); AF = AF7401 (Alta Seeds, Irving, TX)

[2] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

[3]V = main interaction of variety type, E = main interaction of ensiling duration, and V x E = the interaction of variety type and ensiling duration.

[a-d]Within a row, means without a common superscript differ ($P \leq 0.05$).

[4]SEM = standard error of mean

Table 3.3 Ruminal *in situ* CP degradability characteristics of three sorghum hybrids[1] when ensiled for either 0 or 60 d in Exp. 2.

	Treatment									
	SP33S40		NK300		AF7401			*P*-value[3]		
Item[2]	0	60	0	60	0	60	SEM[4]	V	E	V × E
a, %	51.6	65.3	37.9	47.9	36.6	48.3	4.43	<0.01	<0.01	0.82
b, %	48.4	34.7	62.2	52.2	63.4	51.7	4.04	<0.01	<0.01	0.82
c, %	0.0	0.0	0.0	0.0	0.0	0.0	0.00	-	-	-
k_d, h	0.0200	0.0161	0.0136	0.0126	0.0160	0.0125	0.0040	0.19	0.22	0.83
ERD, %	65.6	73.7	51.1	58.4	51.9	58.7	1.69	<0.01	<0.01	0.81

[1]SP = SP33S40 (Sorghum Partners, Lubbock, TX); NK = NK300 (Sorghum Partners, Lubbock, TX); AF = AF7401 (Alta Seeds, Irving, TX)

[2] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

[3]V = main interaction of variety type, E = main interaction of ensiling duration, and V x E = the interaction of variety type and ensiling duration.

[4]SEM = standard error of mean

Table 3.4 Ruminal *in situ* starch degradability characteristics of three sorghum hybrids[1] when ensiled for either 0 or 60 d in Exp. 2.

	Treatment									
	SP33S40		NK300		AF7401			*P*-value[3]		
Item[2]	0	60	0	60	0	60	SEM[4]	V	E	V × E
a, %	58.7^{c}	84.5^{a}	46.9^{d}	52.9^{cd}	56.0^{cd}	70.1^{b}	4.46	<0.01	<0.01	0.03
b, %	40.9^{b}	15.5^{d}	53.1^{a}	47.1^{ab}	44.0^{ab}	29.9^{c}	4.41	<0.01	<0.01	0.03
c, %	0.3	0.0	0.0	0.0	0.0	0.0	0.19	0.40	0.34	0.40
k_d, h	0.0850	0.0669	0.0474	0.0503	0.0540	0.0547	0.0113	0.01	0.47	0.38
ERD, %	84.4	93.4	72.6	76.5	78.9	85.8	1.89	<0.01	<0.01	0.19

[1]SP = SP33S40 (Sorghum Partners, Lubbock, TX); NK = NK300 (Sorghum Partners, Lubbock, TX); AF = AF7401 (Alta Seeds, Irving, TX)

[2] *a*=immediately soluble fraction, *b*=potentially degradable fraction, *c*=undegradable fraction, *L*=discrete lag time, k_d= fractional rate of digestion, and ERD = effective ruminal degradability.

[3]V = main interaction of variety type, E = main interaction of ensiling duration, and V x E = the interaction of variety type and ensiling duration.

[a-d]Within a row, means without a common superscript differ ($P \leq 0.05$).

[4]SEM = standard error of mean