

CATTLE, CORN STOVER AND BIOENERGY: A SUSTAINABLE INTEGRATED SYSTEM

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ABSTRACT

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Stover is an abundant commodity that can be sold as a feedstock for cattle and ethanol production. The objective of this project was to evaluate storage, harvest and feedstock uses of corn stover. High moisture (HM) bales averaging 45% moisture were stored either under a plastic cover or left uncovered exposed to the elements. Lower moisture (LM) bales averaging 35% moisture were stored either under a roof or left uncovered exposed to the elements. Approximately 50-100 g samples were taken from each of the three areas (core, rind and total) of the bale with a forage probe after 0, 30, 120, 240, and 365 days in storage. Samples were then analyzed for ethanol yield, ash content, dry matter, energy, digestibility and mineral content. Bales with less than 35% moisture maintained recoverability of nutrients, structural integrity and dry matter. Corn stover bales were processed and fed to cattle in a high concentrate diet. Treatments were 0, 10 and 20 percent of a dry matter percentage in the total mixed ration. Cattle consumed more DM when corn stover was added to the diet. Average daily gain and carcass characteristics were similar among treatments. Improving feedstock yield and quality was investigated by interseeding a winter annual cereal in corn. The mixed biomass feedstocks resulting from the incorporation of a winter annual cereal with corn stover improved feedstock quality and quantity relative to stover-only feedstocks.

In dedication to my Grandpa:

Tom Bollman

One of my biggest supporters and an amazing farmer who shared his love of agriculture with everyone.

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KEY TO ABBREVIATIONS

ADF	acid detergent fiber
ADG	average daily gain
Ca	calcium
CF	crude fiber
cm	centimeter (s)
CP	crude protein
Cu	copper
DDGS	dry distiller's grain with solubles
DM	dry matter
DMR	dry matter recovery
DMI	dry matter intake
FBW	final body weight
Fe	iron
g	gram (s)
G:F	kg of weight gain per kg feed consumed
HM	high moisture
HMC	high moisture corn
IBW	initial body weight
K	potassium
kg	kilogram (s)
KPH	kidney, pelvic and heart fat
LM	low moisture
MDGS	modified distillers grains with solubles

Mg	magnesium
Mn	manganese
MT	metric ton
Na	sodium
NDF	neutral detergent fiber
NE _g	net energy for gain
NE _m	net energy for maintenance
N	nitrogen
P	phosphorus
QG	USDA quality grade
RFV	relative feed value
TMR	total mixed ration
TDN	total digestible nutrients
YG	USDA yield grade
Zn	zinc

CHAPTER 1

INTRODUCTION

Corn stover (*Zea mays*) is the non-grain portion of the corn plant, including the husk, cob, stalk and leaf. After harvesting corn grain, stover is the remainder of the crop often referred to as residue. Residue management options include tilling, harvesting, burning and leaving it on top of the soil. From 2010 to 2015, corn production in Michigan has increased 763.6 kg ha⁻¹ (USDA, 2015) leaving an additional 183.7 kg ha⁻¹ of stover in the field. As a general rule, the amount of stover produced is about the same as the amount of grain produced (Tollenaar et. al. 2006). Increased residue production causes management difficulties, especially for no-till farmers, creating a planting barrier. Persistent residue decreases soil warming in the spring, can serve as a host for disease, and decreases seed-soil contact at the time of planting (Kravchenko and Thelen, 2007). Sustainable harvest of stover can assist with residue management without substantially decreasing the soil organic matter. Harvest at a rate of 2.23 mg ha⁻¹ has been shown to have minimal effect on grain yield, stover composition and soil quality factors (Birrell et al., 2014). Corn stover is an additional commodity for the producer that can be marketed.

Stover is an abundant commodity that can be used as a biomass for paper production, the pharmaceutical industry and the agricultural industry. In the agricultural sectors, stover can be used as bedding, as a feedstock for cattle, and ethanol production. An increase in corn prices over the past decade resulted in acreage being converted to corn production instead of traditional forages, forcing cattle producers to adopt alternative forages (Watson et al. 2015). Stover is typically low in crude protein and high in fiber (Fuller, 2004) and although this

nutrition is available in other feeds, stover is unique because it is readily available and relatively inexpensive.

Cellulosic biomass, such as corn stover, does not directly compete with the food supply and offers an alternative sustainable energy source. Cellulosic ethanol also addresses our over reliance on imported fuels by supplying an “American made” fuel. Corn stover is thought to have the greatest biomass feedstock potential in North America when compared to other feedstock, with potential annual yields of 38.4 GL of bioethanol (Dale, 2003). Three ethanol plants in the central Corn Belt are currently contracting with farms to supply approximately 275,000 tons of corn stover per plant (Dedecker and Gould, 2014). Michigan farmers could also benefit from this market, selling their corn stover biomass to ethanol refineries. A two year, four location study across Michigan showed that location had a significant effect on the stover quality while the presence of Bt transgene did not. Hybrid type was found to have a significant effect on the glucose and lignin levels indicating hybrid selection to be an area where optimization of ethanol yield could be maximized (Tumbalam et al., 2015). Ethanol, xylose and glucose yield ranged from 0.17-0.20, 0.18-0.19 and 0.33-0.36 g g⁻¹, respectively (Tumbalam et al., 2015).

The objective of our research was to evaluate corn stover quality and yield in four experiments. The first experiment evaluated the effects of time, moisture and storage method on corn stover quality. Quality was determined as ash content, ethanol yield and the ability to maintain dry matter over time. The second experiment also evaluated stover quality while in storage, but as energy content, metabolic digestibility, crude protein and dry matter. In

experiment three, the stover bales were processed and fed to cattle, allowing cattle performance and carcass characteristics to be evaluated for diets containing 0, 10 and 20% stover on a dry matter basis. The fourth experiment evaluated the effect of interseeding a winter cereal cover crop into corn on yield and quality of stover. Stover-only and mixed biomass feedstock was also harvested in the spring and fall to see how the yield and quality was affected by delayed harvest. The overall objective of this research was to develop the best management practices for Michigan farmers for producing corn stover. Recommendations for storage, harvest time, use of cover crop and rate for cattle feed were investigated.

CHAPTER 2

REVIEW OF LITERATURE

Environmental implications of harvesting corn stover

Removal of corn stover has been found to have short term and long term impacts on crop yield. When corn stover remains on a cool, moist, soil with reduced tillage, a delay in soil warming and drying occurs due to the excess crop residue (Kravchenko and Thelen, 2009). Corn residue also impedes stand establishment and reduced nitrogen availability (Kravchenko and Thelen, 2009). Under cool, moist, soil with reduced tillage, stover harvest aided in residue management and potentially increased grain yield (Swan et al., 1994). In contrast, other research studies have shown long-term a significant reduction in corn grain yield when stover from previous corn crops was removed (Blanco-Canqui and Lal, 2007). Previous research has shown corn stover removal can have a negative long-term effect on soil organic carbon, nutrient cycling, water holding capacity and soil compaction (Blanco-Canqui, 2013).

Besides contributing carbon to soil organic matter, stover also contains nutrients utilized by plants. Corn stover contains 9.97 kg of nitrogen, 3.63 kg phosphorus and 14.5 potassium of nutrient per ton of stover (Brechbill and Tyner, 2008). In 2008, the estimated nutrient replacement cost for stover's nutrient content was \$15.64 per ton of stover removed (Brechbill and Tyner, 2008). As expected, harvesting greater amounts of stover resulted in an increase N, P and K removal. A 239 site-years meta-analysis was completed that included 36 sites and 7 states (Karlen et al., 2014). With a low rate of 3.9 MT ha⁻¹ (46% of above ground biomass) harvest, nutrient removal was 24, 2.7 and 31 kg ha⁻¹ of N, P and K, respectfully. A high rate of

7.2 MT ha⁻¹ (85% of above ground biomass) harvest removed 47, 5.5 and 62 kg ha⁻¹ of N, P and K respectfully (Karlen et al., 2014). Non-removal of stover also showed a significant decrease of 1.8 Mg ha⁻¹ in yields when comparing conventional tillage to no-tillage practices (Karlen et al., 2014). Harvest method had no effect on the nutrient removal or stover yield. Harvesting the corn stover also resulted in a slight increase in corn yield. These results indicate that harvesting stover is an important practice for residue management, decreasing the need for aggressive tillage. When the residue was not harvested in a no till system corn grain yield decreased. However, harvesting stover removes valuable nutrients which may result in increased fertilizer application.

A study harvesting stover at rates of 0, 25, 50, 75 and 100 percent found that a removal rate of greater than 25 percent reduced soil organic carbon and soil productivity with the magnitude of impact dependent on the soil type and topography (Blanco-Canqui and Lal, 2007). Water available to plants and earthworms were reduced by 50% and soil compaction was moderate when removal was greater than 25% (Blanco-Canqui and Lal, 2007). Other studies suggest that 30-50% of crop residue can be removed without causing a severe negative impact on soil quality (Graham et al. 2007, Kim and Dale, 2004, and Nelson, 2004). With such potential negative impact on the soil organic matter and microbiome of the soil, a solution to counteract or reduce the degradation would be crucial to make stover harvest environmentally neutral. Pratt et al. (2014) study found that the addition of a cover crop allowed for an increase of 4 metric tons/ha of stover to be removed sustainably. Cover crops were also reported to improve environmental and soil quality benefits (Kaspar and Bakker, 2015) and help control weeds (De Bruin et al, 2005).

Cereal cover crops

Use of cover crops has been found to off-set the loss of soil organic carbon (Blanco-Canqui, 2013). Osborn et. al (2014) found when corn stover residue was removed, the soil was less protected and soil organic matter decreased. Implications of reduced soil organic matter were decreased water holding capacity and nutrient supply. The use of cover crops was found to reduce the impact of erosion and loss of soil organic matter (Osborn et al., 2014).

Bonner et al. (2014) evaluated cover crops and vegetative barriers with a landscape planning process to ensure sustainable stover harvest. They emphasized the importance of soil capability class and slope evaluation to determine the effectiveness of conservation practices and feasibility of sustainable stover harvest. A winter rye cover crop was used and a vegetative barrier was defined as a 3 m wide, single native perennial grass barrier located in the middle of each slope profile (Bonner et al., 2014). Soils of capability class 4 and slopes greater than 4 %, particularly with crop rotation that included soybeans, were less favorable. The use of cover crop, reduced tillage and vegetative barriers was found to significantly increase the sustainable corn stover availability for harvest. Cover crop integration was found to have the greatest effect on sustainable residue removal when continuous corn and slopes up to 6 % were harvested. These results further emphasize the importance of soil conservation methods specifically the integration of cover crops into corn rotations.

The main limitations of cover crops are the cost of establishment, termination and the immobilization of N reducing availability for subsequent crop growth (De Bruin et al., 2015). There is also a concern that removing stover reduces corn grain yield the following year, but

this seems to be an inconsistent result (Kaspar and Bakker, 2015). The possible causes of yield reduction are increased disease pressure, planter performance, cooler soil temperatures and increased water usage (Kaspar and Bakker, 2015). A study conducted in Illinois with cereal rye planted into corn, found that with optimal nitrogen and cover crop termination management practices, the likelihood of N losses from the cropping system decreased (Crandall et al., 2005). Optimal management practices consisted of the cereal winter cover crop being killed off with glyphosate two to three weeks prior optimal corn planting with an application of nitrogen at corn planting. This resulted in a non-significant grain yield reduction and improved the soil $\text{NO}_3\text{-N}$ content at planting (Crandall et al., 2005). These results are not to optimize cover crop yield, but to prevent detrimental effects on corn grain yield from interseeding a cover crop. To address the concern of additional cost of optimal management practices Pratt et al. (2014) evaluated the use of annual rye, cereal rye, crimson clover, hairy vetch, oats and oil seed radish interseeded into corn. This research found that the benefits of using a cover crop, such as decreased erosion and contribution to soil organic matter, were greater than the cost (Pratt et al., 2014).

Several studies assessing the nutritive value of a winter cereal cover crop as replacement forage for cattle defined best planting and harvesting dates. It is recommended to plant winter rye in September with potential harvest in mid to late spring. Triticale is also planted in September but matures in early June. Harvesting at vegetative or boot stage is recommended for winter annual small grains (Edmisten et al., 2008). Rye, when compared to barley, oat and wheat, was found to be highly digestible and greater protein content when green chopped or grazed at vegetative to boot stage. A three year study in Wisconsin

(Undersander, 2013) compared winter rye and triticale as cover crops for forage use. Potential dry matter yield of 6.7-7.8 MT ha⁻¹ are possible with 11- 12% CP when planted in September and harvested at boot stage. Relative feed-value decreased by half for winter rye and triticale when harvested in the spring instead of the fall due to less nutrient content (Oplinger et al., 1997). Harvest time has been found to have significant effect on both the nutritive value and yield.

In another study to determine potential yields, winter rye and triticale were seeded in mid-August at a planting rate of 186.7 kg ha⁻¹ at Arlington and Marshfield, WI (Oplinger et al., 1997). The Hancock variety was planted for cereal rye and the variety Enduro was planted for triticale. Harvesting in October resulted in a 0.84 MT ha⁻¹ yield of winter rye with 18.3% CP, 17.5 % ADF and 32.2 % NDF nutritive profile (Oplinger et al., 1997). Winter rye harvested in the spring averaged 6.2 MT ha⁻¹ yield, with a nutritive profile of 10.2% CP, 38.3% ADF and 66.8% NDF (Oplinger et al., 1997). Harvesting winter triticale in the fall resulted in a 0.60 MT ha⁻¹ yield with a nutritive value of 20.2 % CP, 15.7 % ADF and 32 % NDF (Oplinger et al., 1997). Triticale harvested in the spring yielded 5.7 MT ha⁻¹ with a nutritive value of 10.1 CP, 33.2 ADF and 61.4% NDF (Oplinger et al., 1997). Spring harvest resulted in a greater yield but less CP and digestible fiber. Small grain forages like rye and triticale decrease in DM, CP and increase in NDF, ADF, and lignin as growth stages progress (Edmisten et al., 2008).

Cereal cover crops have greater crude protein than stover, providing a valuable nutritive addition when harvested together. Stover quality decreased with successive harvesting days post grain harvest, similar to cover crops (Hung et al., 2012). A fall timed harvest would allow both stover and cereal cover crop to be harvested together. Feeding trials

in a confined feedlot setting that incorporate a winter cover crop into the ration seem to be novel, but one grazing study was found. Franslebbbers and Stuedemann (2014) found dry beef cows gained well grazing on fields with corn stover and cereal cover crops.

Harvest and storage of corn stover

Stover is an abundant commodity that can be used as a biomass for paper production, pharmaceutical industry and the agricultural industry. In the agricultural sector stover can be used as bedding, as a feedstock for cattle and ethanol production. An increase in corn prices over the past decade resulted in acreage being converted to corn production instead of traditional forages forcing cattle producers to adopt alternative forages (Watson et al. 2015). The quality and quantity of stover produced each crop year depends on weather, soil type and management practices like fertilizer and pest control applications (Pennington, 2013). Harvest date can also have a significant effect on stover quality.

Harvest date significantly affects the dry matter yield, composition, and nutritive value of corn stover. In the harvest window from late August to late November lignin increased with successive harvest dates, but DM and crude protein decreased (Hung et al., 2012). Dry matter yield and total digestible nutrient concentration was greater when corn just reached physiological maturity (Hung et al., 2012). Harvest of stover and corn soon after physiologic maturity is the best strategy for maximizing the corn stover value. However, if the stover and grain are harvested earlier, before dry down, then the corn stover has less acid detergent fiber (ADF) and lignin and higher in vitro dry matter digestibility (IVDMD; Russell, 1986). Early harvest, before dry down, could affect the quality of the corn and require the farmers to dry the

corn, increasing input costs. This difference in dry matter is also what allows bales with varied moisture levels to be harvested. This variation can affect the quality and storage of the corn stover bales.

The type of harvest affects the quality of the stover and the cost efficiency of the system. There are several options to harvest stover including single-pass, two-pass and three-pass systems. Single-pass chopping or baling systems allow for stover to be harvested at the same time the grain portion is harvested reducing harvest cost and time. Two-pass systems involved windrowing the stover during grain harvest and baling the stover later. This requires less equipment modification, gives the option of drying and requires less combine power. Three-pass systems include a first pass with a combine with an ear-snap header to harvest the grain followed by a second pass shredding with a flail shredder that forms a windrow and a third pass that harvests the stover with a round baler or forage harvester. Three-pass systems have been found to be 25-42% more expensive than the one or two pass system. For collection of the stover, baling compared to chopping is more cost effective (33-45% cheaper). (Vadas, 2013)

The method of harvest significantly impacts ash content; suggesting equipment that minimizes soil disturbance is optimal (Bonner et al., 2014) Specifically, shred flailed fields were found to be superior compared to mowed and non-mowed fields when evaluating ash content and bale uniformity (Bonner et al.2014). Raking the stover into a row so it can be baled is also not desirable due to additional field operations and soil contamination (Shinner et al., 2007). There are chemical treatments such as sodium citrate (Reza et al., 2015) that can be used to

reduce structural ash, but they add an additional step and cost to processing. In a study that looked at the harvest and storage methods of switch grass, another biomass used for ethanol production, ash content decreased when biomass was left in the field overwinter and storage technique did not have a significant effect on ash content (Adkins and Thelen, 2015). To prevent or minimize the amount of ash in the feedstock, proper harvest could provide an advantage. Ash is a component of stover, and may inhibit the conversion process to ethanol. For biochemical conversion increased mineral content, like ash, reduces the carbohydrate availability causing a reduction in sugar content and ethanol yield (Reza et al., 2015). Ash also increases cost and logistics of cellulosic ethanol bio-waste disposal. A 5% increase of ash in 2,000-T/day biomass refinery would cause an increase biomass waste of 32,000 tons annually at a cost of \$28.86 per ton for disposal (Humbird et al., 2011). Therefore harvest techniques that minimize ash content have significant economic benefit.

Once harvested, farmers will need to store the corn stover without diminished nutritional content. Corn stover has a short harvest window, so preservation of the bales to maximize use is necessary (Shah et. al, 2011). Michigan's fall weather conditions can complicate the harvest window. Baled corn stover can be stored wrapped in plastic, under a tarp, under a roof or left outdoors. Shinnars et. al., (2007) found that corn stover lost 3.3% dry matter when stored indoors and 18.1% dry matter outdoors. If the bales are ensiled at higher moisture greater dry matter yield is realized compared to dry stover (Shinnars et. al, 2007). Once stored, high moisture stover produced more uniform moisture content with less moisture loss (Shinnars et. al, 2007). Vadas and Digman (2013) found that outdoor storage of wrapped bales was the most

cost efficient way to store corn stover compared to indoor storage and outdoor uncovered storage.

The cause for degradation of the bales stored uncovered comes from repetitive addition of moisture, triggering microbial activity and heating. Microbial activity is reduced at less than 22 percent moisture content and becomes stagnant at 18 percent (Shah and Darr, 2014). Storage of large square bales with greater than 25 percent moisture and uncovered resulted in a complete loss of structural integrity. Overall recommendation for optimal corn stover harvest made by Iowa State University Extension was to store bales with less than 25 percent moisture under tarps; bales with greater than 25 percent moisture should be wrapped in plastic to prevent aerobic deterioration and dry matter losses. Bales with less than 25 percent moisture could be stored under roof, but a relatively small difference was observed when compared to storage under tarp. Shah and Darr (2014) also reported heat producing periods for bales in large stacks at less than 25 percent moisture content will occur until 2-3 month into storage. After that, bales showed internal temperatures similar to the ambient temperature unless storage allowed the bales to be exposed to water again causing internal bale temperatures to rise and reactivate microbial activity (Shah and Darr, 2014).

Shinner et al. (2010) evaluated moist corn stover stored for 237 days in piles covered, uncovered and in anaerobic silo bags. Stover stored uncovered was subject to precipitation and resulted in a 21.5 percent DM loss. Stover above 36 percent moisture under tarp averaged 11.9 percent DM loss. Stover bales with 45 percent moisture and exposed to air (uncovered) were found to reach temperatures (greater than 70° C) that could lead to spontaneous combustion.

When anaerobic conditions were met, wet stover averaged a 0.7 percent DM loss. When anaerobic conditions were not maintained, DM losses increased to 6.1 percent. The best way to conserve dry matter of moist corn stover is by providing an anaerobic storage method.

Wet storage (stover greater than 45% moisture) has been recognized recently as a more favorable storage method due to its immediate harvest advantage and improved feedstock digestibility after storage (Cui et al, 2012). Wet stover ensiles due to microorganisms exhausting oxygen and converting sugar into acids creating an anaerobic environment. Pretreatment of these bales with lignin-degrading fungus has found to further reduce loss of carbohydrates (Cui et al, 2012). Other chemical treatments have also been used including ammoniation and the addition of hydrated lime. Rations with greater than 30% stover (Keys and Smith, 1984) on a DM basis, has been found to be inadequate for growth.

Nutritive value of corn stover

Common nutritive value for corn stover has been outlined in Table 2.1. A ration can be composed of concentrate and/or roughages that can be physically and/or chemically treated to enhance the feed value. With such a variety of feed, determining the nutrient content to maximize the energy and digestibility determines the feed's value. Lab studies and feeding studies are two ways that the feed values can be accessed. Corn stover is a plant consisting of leaves, stalks, husk and cob. These different sections of the plant have different dry matter, ADF, NDF, chemical composition and morphology (Li, H.Y. et al. 2014). Leaf blade, ear husk and stem pith have the highest nutrient value than the other fractions due to higher CP and DM degradability (Li, H.Y. et al. 2014).

Table 2.1 Composition (DM basis) of corn stover from two sources.		
	NRC 2016	Eastridge 2007
DM %	85.0	85.0
CP %	6.1	5.0
TDN %	52.7	49.0
ME Mcal/kg	1.90	1.74
NE_L Mcal/kg	.	1.08
NE_m Mcal/kg	1.06	1.10
NE_g Mcal/kg	0.51	0.42
NDF %	70.8	65.0
ADF %	46.7	42.4
Lignin %	6.3	10.0
Ash %	11.1	7.2
DM = Dry matter, CP = crude protein, TDN = total digestible nutrients, ME = metabolizable energy, NE _m = net energy for maintenance NE _g = net energy for gain, NDF = neutral detergent fiber and ADF = acid detergent fiber		

Conducting invitro lab studies is one way to determine ruminal degradability. Essays found that leaf blade, ear husk, and stem pith have higher degradability than the other portions of corn stover (Li, H.Y. et al. 2014). Identification of portions of corn stover with digestibility would allow for harvest of plant portions to be used as feed or ethanol production. A feeding trial is the most effective method to determine the value as a feedstock. Beside the nutritive value, palatability can have a profound effect on whether the cattle will consume the feed. Taste, smell and moisture are just a few variables that affect the palatability of a feed. It is recommended that stover be ground prior to incorporation into a ration (Lardy, 2011). This decreases the nutrient density and requires more supplementation, but the palatability of the stover is improved. Utilizing high moisture stover (greater than 20% moisture) to increase

palatability and ensiling potential similar to corn silage may be another favorable option (Meeter, 2014).

A study conducted by Shreck et al. (2012) compared 5 and 20% corn stover in a high concentrate diet and observed reduced performance and quality grade with the 20% corn stover diet. Final weight, ADG and HCW were significantly lower in the treatment fed 20% corn stover. DMI, back fat, yield grade and marbling were similar amongst treatments. Johnson et al. (2015) compared steers averaging 315 kg for a similar set of treatments and reported similar DMI but lower ADG when fed the higher rate of corn stover. Feed efficiency was also significantly lower in the 20% stover diet compared to the 5%. Final BW, HCW, back fat and marbling scores were lower for cattle fed 20% stover. These results are similar to Shreck's results.

Corn stover can be utilized in corn calf operations as well. Calves nursing cows fed a corn stover/distiller's grain diet were found to have a higher rate of gain than calves nursing cows fed corn silage, wheat middling's, barley hull and straw (Anderson et. al, 2013). The condition scores of the cows were similar among the two diets and the daily feed costs were \$1.71 and \$2.22 per head for the corn stover/distiller's grain and corn silage based diets, respectively. In 2011 the state of Michigan accounted for 99,000 head of beef cows (National Agricultural Statistics Service, 2011). Extrapolations of the reduced feeding cost from the Anderson study to all cow calf operations in Michigan would have saved \$50,500 in daily feed costs. Gunn et al. (2014) found that feeding corn stover with a preexisting feed program resulted in greater

pregnancy rates and higher weight progeny. Keys and Smith (1984) found that digestion of dry matter did not differ between the 60% corn stover, 30% corn stover and 100% alfalfa diets.

Stover is typically low in crude protein and high in fiber (Fuller, 2004). This provides ruminants with fiber essential for efficient rumination and rate of passage. Although this nutrition is available in other feeds, stover is unique because of its high availability. To improve the low crude protein content, physical and chemical treatment can be done (Fuller, 2004). Sewell et al. (2009) found that when thermochemically treated corn stover was mixed with distiller's dried grains with solubles, the feed value could be as nutritious as corn in ruminant diets.

Treating with an alkali-like hydroxide was found to improve intake, and treatment with 3% NH_3 was found to improve digestibility (Oli, U.I. et al. 1977). Increases of in vitro dry matter digestibility (IVDMD) of 9 to 14 percentage units resulted from treatment with NH_3 or urea. Another study using urease found that IVDMD was not improved (Iftikhar, 1991). Treating with nitrogen resulted in a substantial increase in the crude protein of corn stover. Lactic acid was detected in substantial levels when stover was mixed with 50% poultry litter. Ammonia and urea treatments decreased neutral detergent fiber content of stover from 4 to 7 percent. (Iftikhar, 1991) Stover can also be pelleted allowing for it to be shipped and stored like a grain. Alkaline-treated pelleted stover with DDG and solubles resulted in greater ADG and total-tract digestibility but the authors reported concerns of bloat and cost (Gramkow et al., 2016).

When treating stover with lime it is recommended that the stover is coarsely ground and hydrated by at least fifty percent; this costs approximately twenty dollars per ton (Combs,

2012; Rust, 2013). After being treated the stover must be stored for at least seven days to allow for decomposition of fiber-lignin bonds (Rust, 2013). If not fed within ten days, anaerobic storage is required to prevent dry matter loss (ADM, 2015). Ammoniation of stover requires storage under plastic, use of chemical for 2-4 weeks and an additional cost of twenty five to thirty dollars. Since the cost of treatment may outweigh the nutritive value of corn stover, treatments may not be feasible.

Grazing the corn stover is the simplest utilization, eliminating the shipping and harvesting costs (Welshans, 2014). Grazing stover also allows the non-eaten stover portions to be returned to the soil. Different sections of the plant have been found to have different dry matter, ADF, NDF, chemical composition and morphology (Li, H.Y. et al. 2014). Watson et al (2015) reported lower digestibility in the stalk and cob portions of the stover which account for sixty percent of the plant. If cattle are stocked to consume 3.6 kg forage/25.5 kg of grain cattle will selectively graze the husk and leaves improving performance when compared to higher stocked treatments (Watson et al, 2015). Often producers do not have the resources such as fencing and water supply to make grazing a practical option (Welshans, 2014). Grazing has also been found to cause compaction, potentially decreasing yield of the following crop (Clark et al. 2004). Grazing was also found to have a negative impact on stover and cover crop production following harvest (Franslebbbers and Stuedemann, 2014). To prevent compaction, cattle must be grazed only if soil temperatures are below freezing or the farmer plans to till before planting next year's crop (Clark et al. 2004). Although grazing offers an economical advantage to harvesting the stover, the disadvantages of soil compaction and resources allocation may limit its use. For some farmers, mechanically harvesting the corn stover is a more viable option.

Corn stover is relatively inexpensive, readily available and contains nutritional value that renders it a viable alternative forage for cattle.

Irrigation and hybrid selection are common practices used to protect the corn plant and increase plant growth. Cattle grazing on a corn rootworm-protected hybrid corn residue had similar performance as cattle grazing corn residue that was nontransgenic (Vander Pol et al., 2005). Folmer et al. (2002) study found that cattle did not exhibit preferential grazing and performed similarly when grazing Bt compared to non-Bt corn residue.

Another common practice to increase corn yield is irrigation. One study found that stover in irrigated fields had greater amounts of residue but lower amounts of leaf and husk proportions (Fernandez-Rivera and Klopfenstein, 1989). Gardine et al. (2016) assumed cattle consume 1/3 husk and 2/3 leaf when grazing. This produces nutritive value of 4.25% CP, 14% ash and 45% TDN on irrigated corn fields. The irrigated plant parts have been found to contain less CP and less nutritive dense proportions (husk and leaf) than the plant parts from non-irrigated corn (Fernandez-Rivera and Klopfenstein, 1989). These results show that transgenic varieties appear to have no effect on the performance of cattle consuming stover but irrigation seems to decrease the availability of the more nutritive dense proportions of the plant.

Corn stover as a feedstock for the ethanol industry

Cellulosic biomass, such as corn stover, does not directly compete with the food supply and offers an alternative sustainable energy. Cellulosic ethanol also addresses our overreliance on imported fuels by supplying an “American made” fuel. In 2013, three ethanol plants in the central Corn Belt contracted with farms to supply approximately 275,000 tons of corn stover per plant (Dedecker and Gould, 2014). Michigan farmers could also benefit from this market,

selling their corn stover biomass to ethanol refineries. Corn stover is thought to have the greatest biomass feedstock potential in North America when compared to other feedstock, with potential annual yields of 38.4 GL of bioethanol (Dale, 2003). Ethanol, xylose and glucose yield ranged from 0.17-0.20, 0.18-0.19 and 0.33-0.36 g g⁻¹, respectfully (Tumbalam et al., 2015).

Liquid fuels can be produced from biomass in 5 ways: gasification of biomass to syngas which is then converted to diesel, pyrolysis of biomass to oil, direct liquefaction, conversion of plant oil to biodiesel and the release of sugars from fermentation to ethanol (Yang and Wyman, 2008). The biological platform of fermentation creates ethanol and consists of a two step process: pretreatment and fermentation. Hemicellulose and cellulose are two long chained polymers of monosaccharides that make up the cell wall. Lignin is the non-digestible portion of the plant cell wall which limits the enzymes ability to gain accesses to the digestible portion, is toxic to microorganisms and absorbs enzymes (Yang and Wyman, 2008). Digestion is maximized by exposing and deconstructing the fermentable carbohydrate components. Once the feedstock is pretreated and goes through hydrolysis, the slurry is then further treated with an enzyme. The enzyme and saccharification reaction creates a fermentation broth that recovers ethanol, a syrup and a solid residue. Sludge that is produced from the pretreatment step, along with the syrup and solid residue, are used to produce electricity (Luo et al., 2009).

Many pretreatments have been developed to maximize the amount of available sugars. Forty percent of the cost to produce fuel is associated with the pretreatment process, enzyme production and enzymatic hydrolysis (Yang and Wyman, 2008). The pretreatment process is responsible for freeing up the cellulose and hemicelluloses portions. A pretreatment (Table 2.2)

process should be chosen based on its ability to work without having to reduce biomass particle size, inexpensive, minimal chemicals, result in high yields, require low power and produce a compatible distribution of sugars for the enzymatic hydrolysis that follows (Yang and Wyman, 2008).

Table 2.2 Pros and cons of the most common pretreatments used for cellulosic biomass conversion (Yang and Wyman, 2008).		
Pretreatment Type	+	-
Physical	No chemicals	High cost, poor performance
Steam	Simple, effective at recovering hemicelluloses sugars	High water and energy
Na/K hydroxide	Good yields, delignify	High cost
Ethanol/methanol	Delignify with organosolv	High cost
Carbon dioxide	Improves cellulose digestibility	Mixed results, high pressure
Ionic liquids	Nonflammable, recyclable, dissolves carbohydrate and lignin	Cost unknown, impurity concerns
Dilute acid	High hemicellulose recovered, disrupts lignin	Very corrosive, long reaction time, treatment of reaction degradation products
AFEX	Recycle ammonia, improves cellulose digestion, high conversion	Fermentability of oligameric hemicelluloses, cost
Sulfur dioxide	Lower pH, rapid penetration	Safety concerns, cost
ARP	Recycles ammonia, completely fractionates biomass	High liquid loading, cost, half of xylose is oligamer
Lime	Low cost, safe, high availability, recyclable	Slow, less effective on woody biomass, low xylose yield-oligamer
Controlled pH	Prevents hydrolytic reaction and degradation	Lower xylose yields-oligamer

Composition of stover has been found to be highly variable with harvest year, environment and variety, affecting the glucose, lignin and/or xylose concentrations (Templeton et al., 2010). Average ethanol yield over 6 studies was found to be 0.3 L/kg stover and a net energy value of 5.54 MJ/kg (Templeton et al., 2010). The main disadvantage of using corn stover as a cellulosic ethanol feedstock is that the corn plant requires intensive inputs and agricultural practices. Specifically nitrogen fertilizer was found to consume 90% of the total fertilizer energy (Lou et al. 2009). Incineration and gasification of corn stover is also a valuable way to produce energy with a net energy of 4.42 and 6.30 MJ/kg stover produced, respectfully (Lou et al. 2009). Corn stover as a feedstock supplies diverse products including electricity, fuel and pharmaceutical precursors (Lou et al. 2009).

Corn stover is also being utilized in the pharmaceutical industry as a feedstock that produces a pharmaceutical precursor called succinic acid. Succinic acid is commonly accepted as the most important platform chemical used in the chemical, food and pharmaceutical industry (Zheng et al. 2010). Corn stover is an economically renewable resource used in succinic acid production and is becoming more attractive due to its relative abundance, low cost and availability (Zheng et al., 2010).

Corn stover can also be converted to electricity by using microbial fuel cells. In Zuo et al. (2006) study, stover was found to produce 933 mW/m² when a neutral pretreatment was used and 971 mW/m² when an acidic pretreatment was used. A modest yield of 150 million tons per year of stover produced 4.6x10¹⁰ kWh/yr of electricity (Zuo et al. 2006). This amount of energy is equal to 52 power plants generating 100 MW each (Zuo et al. 2006).

Other types of energy production that can utilize stover are anaerobic digestion, pyrolysis and torrefaction. Anaerobic digestion, like the fermentation process that produces ethanol, it uses a biological platform that, when coupled with combustion, is only 25-35% efficient compared to ethanol conversion at 47% efficiency (Zuo et al. 2006). Torrefaction of stover is a thermochemical process conducted at temperatures around 200-300 °C under an inert atmosphere, and is used to produce a biofuel (Medic et al., 2012). Temperature, residence time, feedstock particle size and purge gas residence time are all important parameters used to access the quality and quantity of the solid product (lignin and cellulose) permanent gases and condensable (liquid) (Medic et al., 2012). Pyrolysis is a similar process to torrefecation using a thermo-chemical platform to convert biomass into a biofuel (Medic et al., 2012). Production of transportation fuels from lignocellulosic biomass is gaining attention due to their positive effects on fossil fuel displacement, reduction in greenhouse gas emissions, additional revenue for farmers and national security enhancement (Medic et al., 2012). Other parameters are also being assessed to understand the effects of agronomic practices on quantity and quality of ethanol.

Transgenic Bt corn currently accounts for 76% of the corn planted in the U.S. and could be a possible source of the variation seen in stover quality (USDA, 2013). *Bacillus thuringiensis* is a soil bacterium that naturally occurs and produces proteins that are toxic to specific insects. Plants genetically modified to produce the Bt protein kill lepidopteron pests like the European corn borer (Tumbalam et al., 2015). A two year, four location study across Michigan showed that location had a significant effect on the stover quality while the presence of the Bt trangene did not. The quantity of the stover produced was found to be more critical than the quality

when evaluating yield on a land area basis (Tumbalam et al., 2015). The corn hybrid had a significant effect on the glucose and lignin levels indicating hybrid selection to be an area where optimization of ethanol yield could be maximized. Ethanol, xylose and glucose yield ranged from 0.17-0.20, 0.18-0.19 and 0.33-0.36 g g⁻¹, respectfully (Tumbalam et al., 2015).

It is important to increase the quality of the stover without compromising the grain yield. Elite germplasm was used as the hybrid (B73xMo17) which is already available for sale and found to not adversely affect grain yield or agronomic traits (lodging, plant height) (Lewis et al. 2010). Glucose, (concentration in the cell wall) glucose release (release of cell wall glucose through pretreatment and saccharification) and lignin (concentration of lignin in the cell wall) were the traits measured to determine stover quality. With selective breeding, an increase of 25% in total glucose yields were realized (Lewis et al. 2010). By selecting hybrids that do not affect the yield of corn but increase the ethanol yields, an increase in ethanol production can be realized. The hybrid that was utilized in Lewis et al. (2010) study already exists in the market allowing for a simple and inexpensive production change.

The cut of the stover during harvest also has an effect on the stover quality, directly affecting the ethanol yield. Hoskinson et al. (2007) evaluated different harvest heights of low, medium and high cut rates and determined a medium height was the best. To determine ethanol yield, samples were treated with dilute sulfuric acid pretreatment and simultaneous saccharification and fermentation was used. Stover cut at 40 cm height was found to yield 2258 L ha⁻¹ on a DM basis with 339 mg g⁻¹ of glucan and 215 mg g⁻¹ of xylan (Hoskinson et al., 2007). Harvesting at a lower height did produce more ethanol, 3002 L ha⁻¹, but increased

nutrient replacement costs, decreased surface cover and increased water content of the stover which could cause storage issues and ash contamination (Hoskinson et al., 2007).

Transportation and storage cost increase as moisture level increases. Nutrient removal at 40 cm consisted of 42, 4.0 and 34.3 kg ha⁻¹ of N, P and K , respectfully (Hoskinson et al., 2007). Macro-nutrient replacement values were 411.27 mg-1 costing \$57.36 ha⁻¹ (Hoskinson et al., 2007).

Nutrient concentrations in the corn stover were 8.0, 0.79 and 6.74 mg g⁻¹ of N, P and K, respectfully (Hoskinson et al., 2007). Harvesting at the height of 40 cm also allowed for a faster harvest time and resulted in a higher quality stover. Ash content and percent moisture were found to be the major discriminators in energy yield with gasification when stover was used as the feedstock (Hoskinson et al., 2007). In summary, a medium harvest height provides optimal feedstock whether it was being used for ethanol or energy production.

Chapter 3

EFFECTS OF STORAGE ON CORN STOVER QUALITY FOR CELLULOSIC ETHANOL PRODUCTION

Abstract

Stover is a commodity that can be sold as a feedstock for cattle and ethanol production. By using Michigan annual corn yields, it can be estimated that 9.03 billion kg of stover was produced in 2014 (USDA, 2015). This abundant commodity could address foreign oil concerns and the need for alternative energy, while not directly competing with the food supply. The objective of this study was to evaluate the effects of time and storage method on corn stover quality. High moisture (HM) bales, averaging 39% moisture, were stored either under a plastic cover or left uncovered exposed to the elements. Low moisture (LM) bales, averaging 24% moisture, were stored either under a roof or left uncovered exposed to the elements. Approximately 50 to 100 gram samples were taken from each of the three areas (core and rind) of the bale with a forage probe on 0, 30, 120, 240, and 365 days in storage. Samples were then analyzed for ash content, ethanol yield and dry matter. The low moisture bales under cover were the driest bales ($P<0.01$) and had 92% dry matter recovery after one year in storage. HM bales uncovered had the greatest DM loss of 20% ($P<0.01$). Yield of ethanol (EtOH recovery), from HM covered bales had the greatest loss with 74% recovery, compared to the other treatments that recovered 86% of initial ethanol yield ($P<0.01$). Ash content was also the greatest in HM, covered bales at 8.6% ($P<0.01$). Results indicated that low moisture bales kept structural integrity and had the best results for ethanol production including higher sugar content, higher ethanol yields and lower ash.

Introduction

Corn stover is the non-grain portion of the corn plant, including the husk, cob, stalk and leaf. After harvesting corn grain, stover is the remainder of the crop often referred to as residue. Residue management options include tilling, harvesting, burning and leaving it on top of the soil. From 2010 to 2015, corn production in Michigan has increased 763.6 kg ha^{-1} (USDA, 2015) leaving an additional 183.7 kg ha^{-1} of stover in the field. As a general rule, the amount of stover produced is about the same as the amount of grain produced (Tollenaar et. al. 2006). Increased residue production causes management difficulties, especially for no-till farmers, creating a planting barrier. Persistent residue decreases soil warming in the spring, can serve as a host for disease and decreases seed-soil contact at the time of planting (Kravchenko and Thelen, 2007). Sustainable harvesting of stover can assist with residue management without substantially decreasing soil organic matter. Harvesting stover at a rate of 1 ton/acre has been shown to have minimal effect on grain yield, stover composition and soil quality factors (Birrell et al., 2014). Stover is also an additional commodity for the producer that can be marketed.

Stover is an abundant commodity that can be used as a biomass for paper production, pharmaceutical industry and the agricultural industry. In the agricultural sectors stover can be used as bedding and as a feedstock for cattle and ethanol production. Cellulosic biomass, such as corn stover, does not directly compete with the food supply and offers an alternative renewable energy source. Ethanol is derived from carbohydrate sugar components in the plant wall. Glucose and xylose are the primary sugars that are fermented to produce ethanol. To determine the quality of a feedstock for ethanol production, the sugar content is analyzed and

then used to estimate the potential ethanol yield. Corn stover is thought to have the greatest biomass feedstock potential in North America when compared to other feedstock, with potential annual yields of 38.4 GL of bioethanol (Dale, 2003). To develop a sustainable and profitable market for corn stover best agronomic practices for harvest and storage need to be developed in Michigan. The quality and quantity of stover produced each crop year depends on weather, soil type and management practices like fertilizer and pest control applications (Pennington, 2013). Harvest date significantly affects the dry matter yield, composition, and nutritive value of corn stover (Hung et al., 2012). The objective of this study was to evaluate the effects of time, moisture and storage method on corn stover quality.

Materials & Methods

Site Description

Research was conducted at the Michigan State University Beef Cattle Teaching and Research Center (BCTRC) located at Lat: 42°69'87.28"N Lon: 84°47'01.97"W. Monthly averages of temperature and precipitation during the storage study are shown in Table 3.1. Weather data was retrieved from the National Center for Environmental Information (NOAA, 2014-2016).

Table 3.1 Average precipitation and temperature for month-year.		
	Avg Percip (cm)	Avg Temp (°C)
Dec-14	3.96	-0.22
Jan-15	3.43	-6.39
Feb-15	2.29	-11.4
Mar-15	1.88	0.5
Apr-15	3.25	8.61
May-15	9.57	16.2
Jun-15	23.0	19.1
Jul-15	6.07	21.2
Aug-15	17.3	20.9
Sep-15	3.40	19.2
Oct-15	5.59	11.1
Nov-15	4.93	7.0
Dec-15	6.91	3.89
Jan-16	3.68	-3.39

Harvest Method

Bale moisture averages were taken with a moisture probe in the field after harvest. Field moisture levels were 45% for HM and 22% for LM. Higher moisture bales (HM) were harvested

in East Lansing, MI located at Lat: 42°40'10.32"N, Lon: 84°28'16.42"W on December 1st, 2014 immediately after grain harvest. Stover was windrowed, baled with 4 ft round baler and net wrapped. This is considered a one pass baling system. Low moisture (LM) bales were harvested in Portland, MI; field located at Lat: 42°48'12.49"N, Lon: 84°57'21.76"W on December 5th, 2014, one week after the grain was harvested. Stover was cut, windrowed with an 8' stalk chopper then baled and net wrapped with a John Deere (John Deere, Corp., Moline, IL) 4 ft round baler. The corn variety was Golden Harvest G05T82-3122A. This is considered a two pass baling system.

Storage Method

The storage study for the HM stover bales (averaging 39% moisture) was started on 12/5/14 at the BCTRC. Twenty four bales were stored under tarp (C) and the other twenty four were stored uncovered outside (NC). The LM bale trial (averaging 24% moisture) was started on 1/5/15 at the BCTRC. Forty eight bales were used with twenty four stored under roof (C) and twenty four uncovered (NC). Bales that were placed outdoors were surrounded by buildings, except for the northern side. Outdoor bales were placed on top of wooden pallets.

Experimental Design

The comparison between storage treatments was conducted in a split plot design with cover, moisture, and time being main effects and bale area as the split.

Sampling Method

Bales from both moisture levels were sampled on 0, 30, 120, 240 and 365 days in storage. Six bales from the two moisture levels were bore sampled on day 0, then 12 bales were bore sampled on the remaining sampling days. Methods for bore sampling were modeled after Shinnars et al. (2010) study. All bales were weighed on day 0 and on their assigned sampling day. If bales were unable to be weighed due to lack of structural integrity, replacement bales were used that were under same treatment conditions. Sampling created a portal for oxygen infiltration increasing the rate of spoilage; therefore, bales were sampled once and then removed from the storage study. Sampling included 3 to 4 bore samples per site to collect approximately 50 to 100 g of forage material with a hay probe bale sampler (Best Harvest, Bay City, MI) that was 61 cm in length and 2.5 cm in diameter. Areas sampled were the core (middle portion) and rind located 13 cm from the exterior (Figure 1). The rind was a composite sample taken from the North, South, East and West areas of the bale. High moisture bales sampled at 120, 240 and 365 days were in poor condition. Modified sample techniques

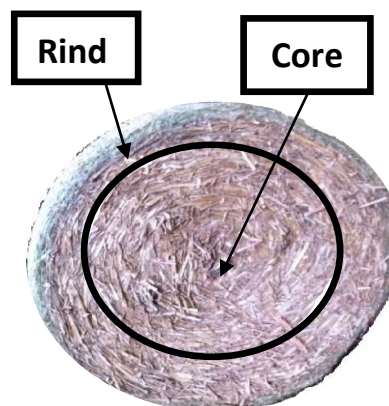


Figure 3.1 Illustration of the sampling areas used to collect bore samples.

were used when bales were unable to be drilled due to loss of structural integrity. The modified technique was drilling into the bale as previously described but then reaching into the designated areas to retrieve the samples. Core and rind samples were then split with a half portion used for nutrient analysis and moisture determination (Litchfield Analytical Services, Litchfield, MI). A 50 mg subsample of each dried sample was sent to the Great Lakes Biological Research Center's Cell Wall Facility for ethanol yield determination.

Ethanol Production

Fermentable Sugar Determination

Biomass sample grinding, feeding, and weighing were performed by a custom-designed robot (Labman Automation Ltd., United Kingdom). Samples of dried plant material (20 to 40 mg) were loaded manually into Sarstedt 2-mL screw-cap microtubes along with three, 5.56 mm stainless steel balls (Salem Specialty Ball Co, Canton, CT). The tubes were placed into racks and positioned in the robot, and pulverization of the biomass was accomplished by ball milling. The length of the grind time was adjusted sufficient to reduce the sample to a fine powder. A 1.5 mg subsample of biomass was transferred to a barcoded 1.4 ml polypropylene microtube (Micronic brand) sealed with a thermoplastic elastomer cap mat (Micronic brand) and 750 μ L of pretreatment solution (NaOH 62.5 mM). Pretreatment solution was pipetted into each tube and then placed in a 90°C water bath for 3 h. As needed, reactions were neutralized with ~7.5 μ L 6N hydrochloric acid. Next, 50 μ L of a solution containing 0.5 μ L Accellerase 1000 (Genencor, Rochester, NY), 33.3 μ L 1 M citrate buffer (pH 4.5) plus 10 μ L 1% w/v sodium azide; 72 η L C-Tec2 and 8 η L H-tec2 enzymes were added to all tubes. Enzymatic hydrolysis was done in a final

volume of 0.8 mL using an enzyme concentration of 50 mg protein/g glucan. Tubes were placed in racks and incubated for 20 h in a rotisserie oven at 50°C. Racks were centrifuged and supernatants were transferred to 0.8 mL deep-well plates. The glucose and xylose content of samples were determined using enzyme-based assay kits (Megazyme, Ireland). Glucose was assayed with the glucose oxidase/oxidase (GOPOD) method (K-GLUC, Megazyme, Ireland) using 4 µL of the supernatant of the digestion reaction mixture and 64 µL of the GOPOD assay reagent. Xylose was assayed enzymatically (K-XYLOSE, Megazyme) using 8 µL sample and 62 µL K-XYLOSE assay reagent. Further details on the analyses used to determine fermentable glucose and xylose content of biomass are outlined by Santoro et al., 2010.

Ethanol Yield Estimation

Ethanol yield was calculated based on the empirically derived fermentable glucose and xylose levels using equation: $([Glc] + [Xyl]) * 51.1\% * \text{metabolic yield} = (\text{EtOH mg/kg})$

Where [Glc] is the glucose concentration of the biomass following pretreatment and enzymatic hydrolysis (mg/kg) and [Xyl] is the xylose concentration of the biomass following pretreatment and enzymatic hydrolysis (mg/kg). The mass conversion of fermentable sugars to ethanol is 51.1%, and metabolic yield equals the ratio of ethanol to the consumed sugars in the fermentation process divided by 51.1% (Lau and Dale, 2009). Metabolic yield values were determined using a separate hydrolysis and fermentation (SHF) process and are derived from Jin et al., (2012) for corn stover (93.1%). Concentration of ethanol yield was multiplied by the dry matter content of each bale to determine the harvest system yield.

Ethanol Recovery Calculation

EtOH loss: $(A-B)/A$

A: initial bale weight* 100% DM content*initial EtOH yield

B: weight from sampling day * dry matter content* sampling day EtOH yield

Ash Content Determination

Samples were analyzed at Litchlab Analytical Services (Litchfield, MI). Ash content percentage was determined by burning off the organic matter and weighing the residue.

Dry Matter Determination

Dry matter (DM) is the non-water portion of the stover. Samples were dried at 65° C for a minimum of 72 hr. The dried weight was then divided by the original weight to get the DM. The dry matter is then used to calculate the DM recovered over time. DM loss (%) was calculated by subtracting the DM recovery from 100 %.

$$\text{DM (\%)} = (\text{dry weight/wet weight}) * 100$$

$$\text{DM recovery (\%)} = ((\text{final bale weight} * (\text{bale DM\%/100}))/(\text{initial bale weight} * (\text{day 0 DM\%/100}))) * 100$$

$$\text{DM loss (\%)} = 100 - \text{DM recovery (\%)}$$

Statistical Analysis

The experimental unit was the bale with bale within cover*moisture*time as the random variable to account for the random variation between bales. Data was analyzed for variance and normality. If unequal variance was detected data was re-analyzed using the Kenward Rogers model. The bale average data was calculated and run as a separate model with cover, moisture and time as the fixed effects. Data was analyzed using the Proc Mixed procedure in SAS Inc. 9.4 (SAS, 2012). The results of ash content, ethanol yield, DM recovery and ethanol recovery were summarized over time using Proc Reg in SAS. Pairwise comparison

was used with the LSMEANS statement of Proc Mixed in SAS 9.4 to determine mean separation when mean square was significant (SAS Inc., 2012). Results were reported as statistically significant at $\alpha = 0.05$ and a trend at $\alpha = 0.10$.

Results & Discussion

Bales were analyzed by area (core and rind) and by a bale weighted mean where the variable concentration was multiplied by a percentage of area established for the core and rind

Table 3.2 Summary of the areas and weighted mean averages for DM, glucose (Glu), xylose (Xyl), ethanol (EtOH) yield (gg⁻¹) and ash.			
	Core	Rind	Weighted mean
DM, %	75.0	67.0	73.0
Glu, mg kg⁻¹	0.231	0.225	0.226
Xyl, mg kg⁻¹	0.119	0.117	0.116
EtOH, gg⁻¹	0.166	0.163	0.163
Ash, %	5.99	6.17	6.15

then averaged. Results by area compared to the results by bale average were generally the same when accessing the cover, moisture and time interaction (Table 3.2). Extensive mold growth was visually apparent with the HM stover bales under cover. The HM bales exposed also had some visual mold, but not as extensive as the bales under cover. Higher moisture bales were also very difficult to handle and move after 120 days of storage due to decomposition, unlike LM bales which stayed intact throughout the study. Storing large square bales uncovered with greater than 25 percent moisture also resulted in a complete loss due to loss of structural integrity in previous research (Shah and Darr, 2014).

Ethanol Production

When comparing HM bales to LM bales, HM were found to have lower sugar concentration and ethanol yield from d-30 to one year in storage. Ethanol yield and sugar content decreased over time ($P<0.01$) regardless of storage method (Figure 3.2). Cover,

moisture and time for all bale types decreased in ethanol yield after 120d of storage ($P<0.01$). Average ethanol yields began at 0.186 gg^{-1} and ended at 0.137 gg^{-1} . Previous research reported ethanol yields ranging from $0.17 - 0.20 \text{ gg}^{-1}$ (Thumbalam et al., 2015).

Ethanol recovery of HM bales under cover was similar to HM bales uncovered at 49% and 48% respectfully (Figure 3.3). LM bale under cover lost 31% and LM bales uncovered lost 27% of its recoverable ethanol yield (Figure 3.3). Bales having a higher ethanol recovery value indicated better bale preservation since it is a measurement of yield and DM recovery. These results indicate that low moisture bales are a better option for ethanol production due to greater retention of sugar content and subsequent ethanol yield. Hybrid type has been found to have a significant effect on glucose and lignin levels indicating differences in ethanol yield could be due to hybrid type (Thumbalam et al., 2015). Bales used in this experiment both had the Bt transgene but were not the same variety.

Ash Content

High moisture bales averaged 2.7% more ash content than LM bales on 0-d ($P<0.01$). This result may be confounded with the harvest method used, because HM bales and LM bales were harvested with different equipment. Cover, moisture and time had a significant effect on ash content (Figure 3.4), especially for covered HM bales with 10% ash content on day 365 ($P=0.02$). Results show greater decomposition of DM in HM bales indicated by the increase in ash, because that fraction is indigestible. High moisture bales uncovered maintained ash content indicating little change in the volume of the bale. Ash content of LM bales increased

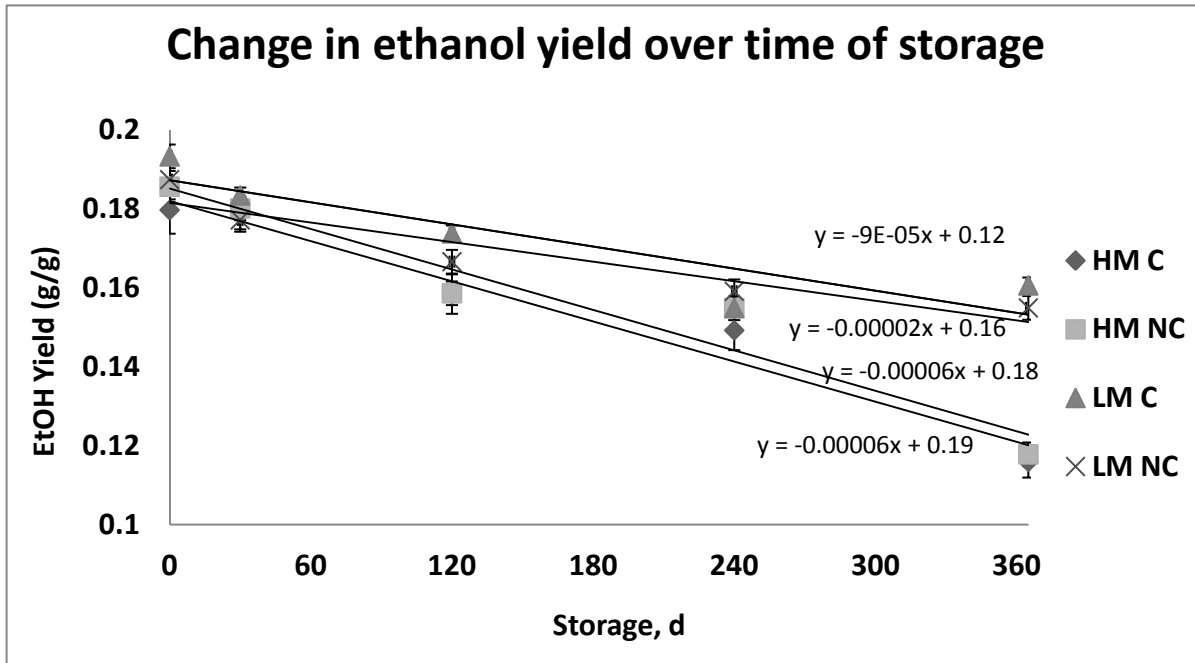


Figure 3.2 Regression analysis of ethanol yield over time for the main effects. High moisture covered (HM C) $P < 0.01$, high moisture uncovered (HM NC) $P < 0.01$, low moisture covered (LM C) $P < 0.01$, and low moisture (LM NC) $P = 0.15$.

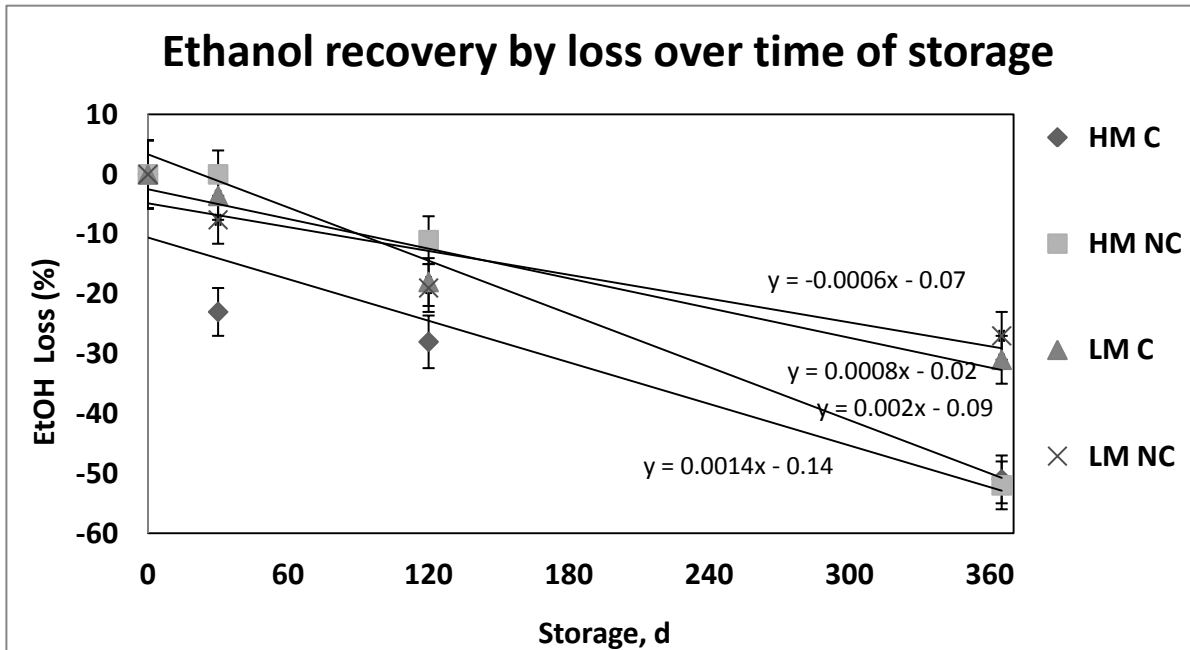


Figure 3.3 Regression analysis of ethanol recovery by loss over time for the main effects. High moisture covered (HM C) $p < 0.01$, high moisture uncovered (HM NC) $p < 0.01$, low moisture covered (LM C) $p < 0.01$, and low moisture uncovered (LM NC) $p < 0.01$.

over time, but not as drastically as the HM covered bales ($P < 0.01$). Bales with 3.5% ash are acceptable for feedstock use since that is a standard amount of structural ash in the corn stover (Schon and Darr, 2014). The remainder could be contamination from harvest, diluting the valuable cellulosic component (Schon and Darr, 2014). Ash results could be confounded with the type of bale harvest method and harvest time, which could be the source of the lower ash content exhibited by LM bales on day 0. Harvest date significantly affects the dry matter yield, composition and nutritive value of corn stover (Hung et al., 2012).

Dry Matter Recovery

As time passed, all of the bales became drier ($P < 0.01$) and exhibited dry matter loss ($P < 0.01$). At the end of the study, HM bales covered, HM bale uncovered, LM bales covered and LM bales uncovered dry matter recovered was 71%, 66%, 80%, and 85% (Figure 3.5), respectively ($P < 0.01$). Outdoor unwrapped bale storage, even with 12% DM loss was cheaper than indoor storage according to a study by Vadas et al. (2013). This makes uncovered low moisture Dry matter content by bale area was different regardless of cover type with the core being significantly drier than the rind (Table 3.2).

In a previous study by Arthmananthan et al. (2014) found stover samples with 34% moisture had a 10% dry matter loss, lignin mass fraction increased and sugar levels of glucan and xylan levels decreased by 10% and 7%, respectively. Our results concur suggesting that greater moisture levels facilitate microbial activity resulting in dry matter and ethanol yield loss. The dry matter losses exhibited by the HM bales, especially when covered, indicate poor preservation while in storage. The decrease in the degradation of the uncovered HM bales may

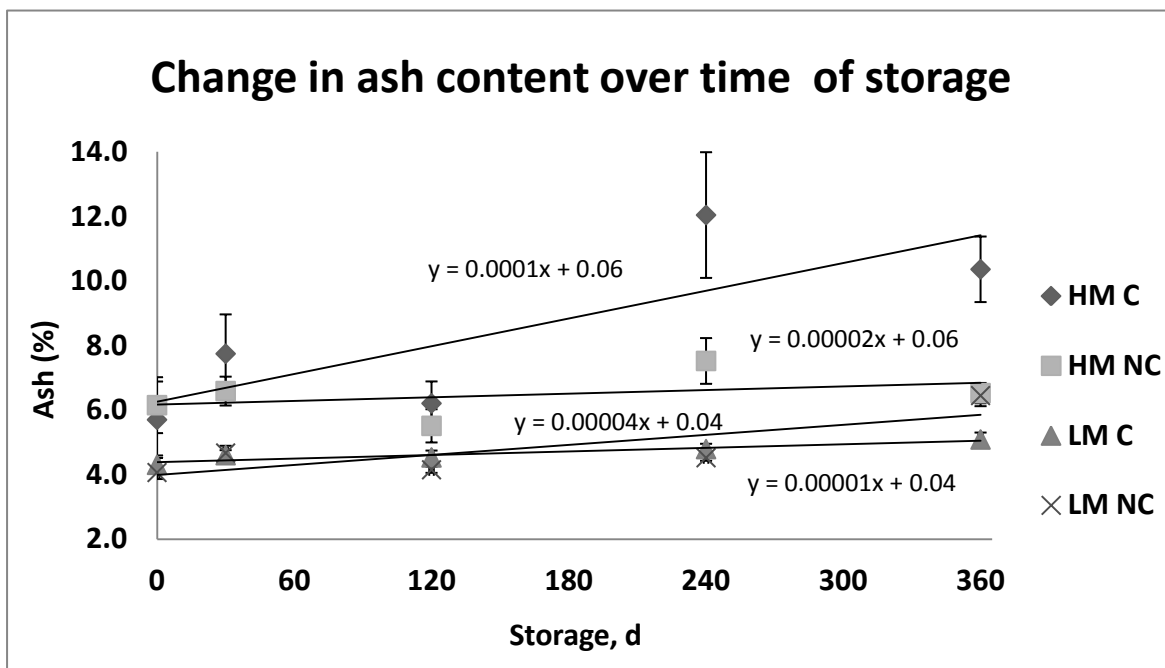


Figure 3.4 Regression analysis of ash content over time for the main effects. Higher moisture covered (HM C) $P < 0.01$, high moisture uncovered (HM NC) $P = 0.35$, low moisture covered (LM C) $P < 0.01$ and low moisture uncovered (LM NC) $P < 0.01$

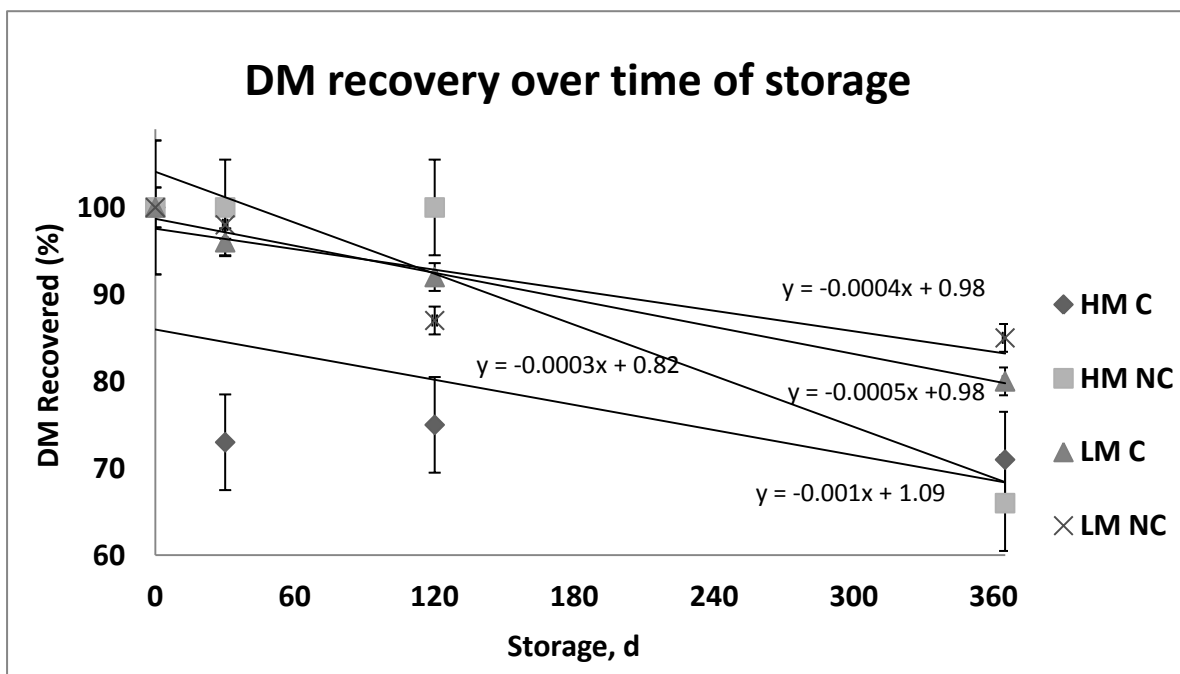


Figure 3.5 Regression analysis DM recoveries over time for the main effects. High moisture covered (HM C) $P = 0.21$, high moisture uncovered (HM NC) $P < 0.01$, low moisture covered (LM C) $P < 0.01$ and low moisture uncovered (LM NC) $P < 0.01$

be due to the fact that the bales were able to dry. The HM bales under cover may have held moisture and had higher temperatures allowing for higher microbial activity and degradation. Shinner et al. (2011) found that stover piles, with less than 30% moisture, stored covered for 8 months, had minimal DM loss, mold growth and change in chemical composition. In contrast, an uncovered aerobic pile had significant DM loss and mold growth due to repetitive rehydration from precipitation. Piles of stover at 57% moisture whether covered or uncovered exhibited degradation and mold growth. Shinner's study also reported variable moisture levels between core at 21.5% and rind at 49.7%.

Conclusions

Uncovered LM bales lost 27% of initial ethanol yield after a year of storage with a significant decline by 120 days. They averaged 4.6% ash content and 85% dry matter recovery. LM bales uncovered had similar results to covered LM bales, showing significantly higher dry matter recovery. Low moisture bales exhibited higher ethanol yield, dry matter recovery and lower ash content compared to HM bales. This makes uncovered low moisture bales an attractive option from both an economical and product quality standpoint. Results also indicated an increase in degradation of HM bales when stored under a tarp, compared to uncovered bales, making the best storage method for high moisture bales an uncovered system. Dry matter degradation may have also been influenced by cover type used, indicating further research into better cover types for HM bales. Our results may also have implications for harvesting, since a farmer may choose to harvest HM bales as opposed to allowing stover to field dry before baling. Overall the findings of this study give producers flexibility when choosing storage type and harvest time for stover with the intended purpose of biomass use.

Chapter 4

EFFECT OF CORN STOVER NUTRIENT QUALITY ON STORAGE AND CATTLE PERFORMANCE

Abstract

Corn stover is an abundant commodity that can be sold as a feedstock for cattle and ethanol production. The objective of this study was to determine the best storage technique to maintain quality (Experiment 1) and to evaluate the use of stover as an alternative forage for cattle (Experiment 2). Stover round bales were stored at 35 or 45 % moisture and stored either covered or uncovered. Approximately 50-100 g samples were taken from each of the two areas (core and circumference) of the bale with a forage probe after 0, 30, 120, 240, and 365 d of storage. Samples were analyzed for DM, energy, digestibility, mineral content and nutrient recovery. Low moisture (LM) bales had greater dry matter content and DM recovery but lower digestibility and energy content than high moisture (HM) bales on 0 d. Storing bales under cover improved dry matter and nutrient content when bales had 45% moisture. Corn stover bales were processed and fed as a percentage in the total mixed ration. Treatments were 0, 10 and 20% stover on a DM basis. Feeding stover increased DMI intake, but ADG and carcass characteristics were similar among treatments. Overall, LM bales had better preservation in storage but less nutrient content compared to HM bales. The calculated NE_g value for corn stover, derived from the feeding trial, was 0.425 Mcal/kg.

Introduction

Corn stover is the non-grain portion of the corn plant, including the husk, cob, stalk and leaf. Stover is a commodity that can be used as a biomass for paper production, pharmaceutical industry and the agricultural industry. In the agricultural sectors stover can be used as bedding and as a feedstock for cattle and ethanol production. From 2010 to 2015, corn production in Michigan has increased 763.6 kg ha^{-1} (USDA, 2015) leaving an additional 183.7 kg ha^{-1} of stover in the field. As a general rule, the amount of stover produced is about the same as the amount of grain produced (Tollenaar et. al. 2006). Persistent crop residue decreases soil warming in the spring, can serve as a host for disease and decreases seed-soil contact at the time of planting (Kravchenko and Thelen, 2007). Harvesting stover provides residue management and an additional crop with several markets.

Corn stover has a short harvest window, so preservation of the bales to maximize use is necessary (Shah et. al, 2011). Shinnars et. al., (2007) found stover stored indoors lost 3.3% DM and 18.1% DM when stored outdoors. Vadas and Digman (2013) found that outdoor storage with wrapped bales was the most cost efficient way to store corn stover compared to indoor storage. Stover is typically low in crude protein and high in fiber (Fuller, 2004). Although nutrients are available in other feeds, stover is unique because of its high availability and low cost. Replacing 20% of the corn with alkaline treated corn stover in a high concentrate feedlot diet increased fiber digestibility without affecting performance (Chapple et al., 2015). Much research has been done on the replacement of corn grain with treated stover, but little literature can be found comparing non-treated stover to a high concentrate control diet. The

objective of this study was twofold. Experiment 1 evaluated two different storage methods and two moisture levels. In Experiment 2, corn stover was fed to evaluate the effects on cattle performance in a confined feedlot system.

Materials & Methods

Site Description

Research was conducted at the Michigan State University Beef Cattle Teaching and Research Center (BCTRC) located at Lat: 42°69'87.28"N Lon: 84°47'01.97"W. Monthly averages of temperature and precipitation during the storage are shown in Table 3.1. Weather data was retrieved from the National Center for Environmental Information (NOAA, 2014-2016). The research was approved by the Institutional Animal Care and Use Committee AUF#: 9/14-171-99.

Table 4.1 Monthly averages of precipitation and temperature during month-year.		
	Avg Percip (cm)	Avg Temp (°C)
Dec-14	3.96	-0.22
Jan-15	3.43	-6.39
Feb-15	2.29	-11.4
Mar-15	1.88	0.5
Apr-15	3.25	8.61
May-15	9.57	16.2
Jun-15	23.0	19.1
Jul-15	6.07	21.2
Aug-15	17.3	20.9
Sep-15	3.40	19.2
Oct-15	5.59	11.1
Nov-15	4.93	7.0
Dec-15	6.91	3.89
Jan-16	3.68	-3.39

Experiment 1

Harvest Method

Bale moisture averages were taken with a moisture probe in the field after harvest. Field moisture levels were 45% for HM and 22% for LM. Higher moisture bales (HM) were harvested in East Lansing, MI located at Lat: 42°40'10.32"N, Lon: 84°28'16.42"W on December 1st, 2014 immediately after grain harvest. Stover was windrowed, baled with 4 ft round baler and netwrapped. This is considered a one pass baling system. Low moisture (LM) bales were harvested in Portland, MI; field located at Lat: 42°48'12.49"N, Lon: 84°57'21.76"W on December 5th, 2014, one week after the grain was harvested. Stover was cut, windrowed with an 8' stalk chopper then baled and netwrapped with a John Deere (John Deere, Corp., Moline, IL) 4 ft round baler. The corn variety was Golden Harvest G05T82-3122A. This is considered a two pass baling system.

Storage Method

The storage study for the HM stover bales (averaging 46.5% moisture) was started on 12/5/14 at the BCTRC. Twenty four bales were stored under tarp (C) and twenty four were stored uncovered outdoors (NC). The LM bale trial started at BCTRC on 1/5/15. Forty eight bales (averaging 36.1% moisture) were used with twenty four stored under roof cover (C) and twenty four stored outside without cover (NC). Bales that were placed outdoors were surrounded by buildings, except for the northern side. Outdoor bales were placed on top of wooden pallets.

Bale Sampling Method

Bales from both moisture levels were sampled on 0, 30, 120, 240 and 365 days in storage. Six bales from each moisture level were bore sampled on day 0, then 12 bales were bore sampled on each of the remaining sampling days. Bales were sampled on assigned storage

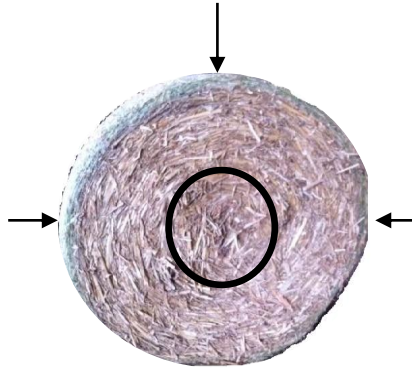


Figure 4.1 Illustration of the sampling areas used to collect bore samples.

days and then removed from Experiment 1. All bales were weighed on day 0 and on their assigned sampling day. If bales were unable to be weighed due to lack of structural integrity, replacement bales were used that were under same treatment conditions. Sampling creates a portal for oxygen infiltration that increases the rate of spoilage. Therefore, bales were sampled once and then removed from the study. Sampling included 3 to 4 bore samples per site to collect approximately 50 to 100 g of forage material with a hay probe bale sampler (Best Harvest, Bay City, MI) that was 61 cm in length and 2.5 cm wide. Sampling sites are shown in Figure 4.1. Samples were composited by area and analyzed for nutrient analysis and moisture determination (Litchfield Analytical Services, Litchfield, MI).

Experimental Design

Comparison between storage treatments were conducted as a randomized design with a 2x2x5 factorial arrangement of treatments with storage type (C/NC), moisture (HM/LM), and time (0, 30, 120, 240, 365) being main effects.

Experiment 2

Feeding Method

One hundred and forty-four Holstein yearling steers averaging 432 kg were blocked into six weight groups and assigned to three pens to equalize weight within pens in a block. There were 8 head of cattle in every pen. Subsamples of all feedstuffs were taken weekly, composited monthly and sent to Litchfield Analytical Services (Litchfield, MI) for nutrient analysis.

Table 4.2 Total ration components (DM %).			
	Control	10 %	20 %
Corn stover	0	10	20
Dry rolled corn	26	26	25
High moisture corn	20	20	20
Corn silage	20	10	0
Dry distiller's grains w/ solubles	30	30	31
Supplement¹	4	4	4
¹ Contained monensin (667 g/ton), 15% crude protein, 3% crude fat, 16% crude fiber, 14-16.8% Ca, 0.3% P, 6.5-7.8% salt, 0.1% K, 200,000 IU/kg vitamin A, 20,000 IU/kg vitamin D3 and 57.8 IU/kg vitamin E.			

Nutrient composition and feedstuff composition of the rations are shown in Tables 4.2 and 4.3. The distiller's grains with solubles contained 35.2% crude protein and 9.82% fat. Corn

stover bales to be fed were processed once each week with a Hay Buster 2564 (DuraTech Industries International, Jamestown, ND).

Table 4.3 Nutrient composition of rations.			
	Control	10 %	20 %
Dry matter, %	61±0.78 ¹	64±2.9	67.0±3.5
NE_g², Mcal/kg	1.27±0.017	1.16±0.02	1.11±0.15
	% of DM		
Crude protein	15.9±0.95	14.9±2.4	14.0±2.8
TDN	79.1±1.4	79.9±7.9	79.5±9.2
ADF	11.1±0.79	13.8±1.6	17.0±1.9
Crude fiber	8.9±0.63	11±1.3	13.4±1.5
Calcium	0.61±0.15	0.63±0.16	0.70±0.17
Phosphorus	0.53±0.06	0.49±0.09	0.46±0.1
Potassium	0.84±0.16	0.83±0.22	0.70±0.17
Magnesium	0.26±0.1	0.25±0.11	0.25±0.1
Sodium	0.25±0.03	0.24±0.04	0.24±0.04
	ppm		
Copper	18.1±2	18.0±2.1	18.2±2.1
Iron	114±37	208±80.6	304±131
Manganese	53.0±5.4	79.2±3.7	63.5±7.3
Zinc	81.8±4.6	57.7±6.1	78.5±3.3
¹ mean±SD			
² Net energy for gain (NE _g)			

Cattle Performance

Cattle initial and final weights were the average of weights taken on two consecutive days. Interim weights were measured every 28 days. Cattle were fed once daily with feed orts recorded on each weigh day. Total corn intake was the sum of the dietary contribution of the dry rolled corn, high moisture corn and corn silage (assuming 50% of silage is corn). Cattle were harvested at JBS, Plainwell, MI. At harvest, liver abscess incidence and hot carcass weight

(HCW) were measured. After a 24-48 hour chill, routine carcass evaluation of the 12th rib backfat thickness, ribeye area, and marbling were measured. The plant provided kidney, heart and pelvic fat (KPH) and muscle grade. Quality grade was estimated using the marbling score (USDA, 2013). Yield grade was calculated with USDA derived equation (USDA, 2013). Carcass adjusted final live weights were obtained by division of HCW by the overall average dressing percent. Final shrunk weight and carcass adjusted final live weights were used to calculate ADG and feed efficiency. Dressing percentage was calculated from full and shrunk live weights. Dietary NE_m and NE_g were calculated from maintenance energy and growth performance using the equations of Zinn et al. (2003).

Experimental Design

Comparisons between ration treatments were conducted in a completely randomized design with treatment as the main effect.

Nutritive Value

Samples were analyzed at Litchlab Analytical Services (Litchfield, MI). Feed analysis definitions can be obtained from the appendix Table B.4. Crude protein (CP) and acid detergent fiber (ADF) were determined using wet chemistry. Net energy for gain (NE_g), NE_m , TDN and CF were estimated using calculations based off the determined components. Mineral content determined included ash, phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn). Calculations for nutrient recovery, ash and weighted mean are summarized below. The weighted mean of each bale's nutrient concentration was determined by multiplying the area's percentage by the nutrient

concentration. Percentage of area (96% circumference; 4% core) was determined by taking the total area and dividing by the designated area proportion.

$$\begin{aligned}\text{DM recovery (\%)} &= ((\text{Final bale wt} * (\text{DM\%/100})) / ((\text{initial bale DM \%/100}) * \text{initial bale wt})) * 100 \\ \text{OM (\%)} &= 100\% - \text{ash content} \\ \text{Nutrient recovery (\%)} &= (\text{DM bale wt} * \text{weighted nutrient concentration on sampling day}) / (\text{initial DM bale wt} * \text{DM initial weighted nutrient concentration}) * 100\end{aligned}$$

Statistical Analysis

Experimental unit in Experiment 1 was the bale. The bale nutrient concentrations were calculated and analyzed as a 2 x 2 x 5 factorial arrangement of treatments with cover, moisture, and time as main effects. Data was analyzed for variance and normality. If unequal variance was detected, data was re-analyzed using the Kenward Rogers procedure. Data was analyzed using the Proc Mixed procedure in SAS 9.4 (SAS, 2012). Pairwise comparisons were used with the LSMEANS statement of Proc Mixed in SAS 9.4 to determine mean separation when the mean square was significant (SAS Inc., 2012). Results were reported as statistically significant at $\alpha=0.05$ and a trend at $\alpha=0.10$.

The experimental unit in Experiment 2 was pen and the random variable was pen. Data was analyzed for variance and normality. If unequal variance was detected data was re-analyzed using the Kenward Rogers procedure. Data was analyzed using the Proc Mixed procedure in SAS 9.4 (SAS Inc., 2012). Interim data was analyzed with a repeated procedure. Pairwise comparison was used with the LSMEANS statement of Proc Mixed in SAS 9.4 to determine mean separation when mean square was significant (SAS Inc., 2012). Results were reported as

statistically significant at $\alpha = 0.05$ and a trend at $\alpha = 0.10$.

Results & Discussions

Experiment 1

Extensive mold growth was visually apparent on d-120 with the HM stover bales stored under cover. The HM bales stored uncovered also had some visual mold on d-120, but not as extensive as the bales under cover. Higher moisture bales were also very difficult to handle and move after 120 d of storage due to decomposition, unlike LM bales which stayed intact throughout the study. Density of the 6 control bales HM and LM on day 0 were 2.62 and 2.55 kg/m³, respectfully.

Dry Matter

Storage type, moisture and time significantly affected the DM ($P=0.01$; Figure 4.2) and showed a trend in OM content of the bales ($P=0.08$; Figure 4.3). Bales that were covered compared to uncovered were found to be 20% drier after 365 days in storage ($P<0.01$). The HM bales exposed to the elements had the lowest DM content ($P<0.01$) amongst the bale treatments across time. Shinner et al. (2011) found that stover piles with less than 30% moisture stored covered for 8 months had minimal DM loss, mold growth and chemical composition change. In contrast, an uncovered aerobic pile had significant DM loss and mold growth due to repetitive rehydration from precipitation. Piles of stover at 57% moisture whether covered or uncovered had significant degradation and mold growth. The LM bales in this experiment, originally averaged 22% moisture when harvested, but did not begin the study until one month later. The LM bales sat on the end of the field they were harvested from. The moisture level on arrival to BCTRC was 14.1 percentage units greater than the harvested

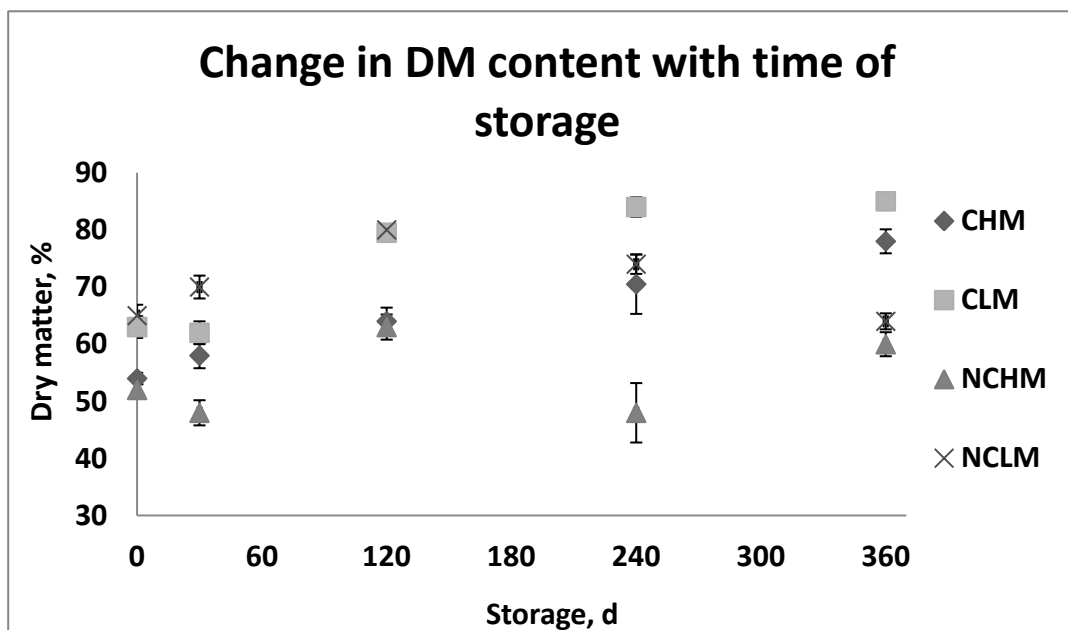


Figure 4.2 Interaction of cover, moisture and time on dry matter content ($P=0.01$). High moisture cover (CHM), low moisture cover (CLM), high moisture uncovered (NCHM) and low moisture uncovered (NCLM)

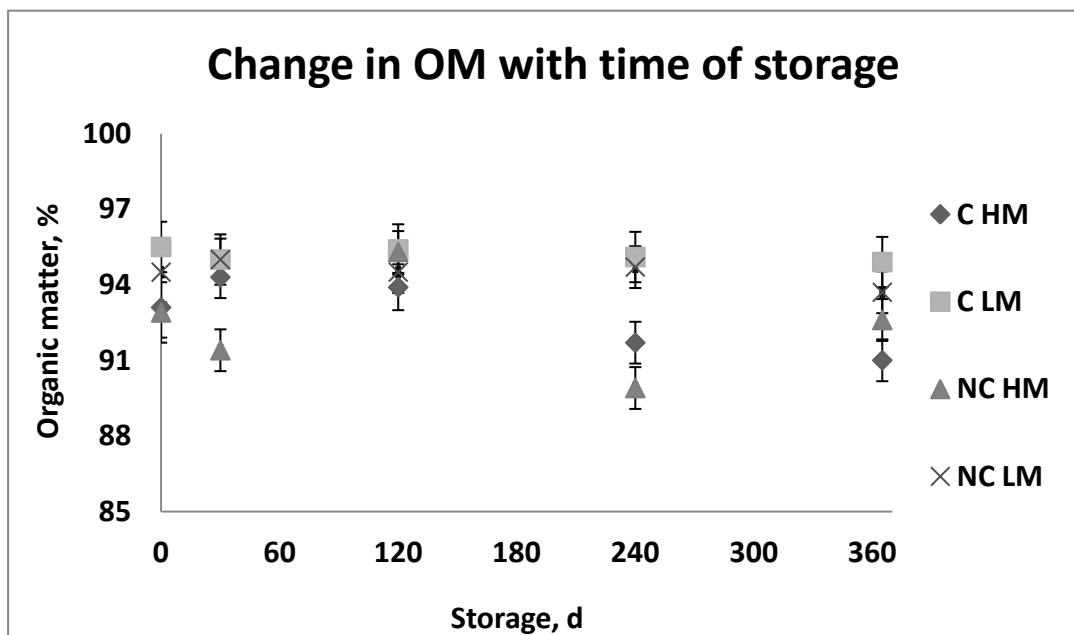


Figure 4.3 Interaction of cover, moisture and time on organic matter content ($P=0.08$). High moisture covered (CHM), low moisture covered (CLM), high moisture uncovered (NCHM) and low moisture uncovered (NCLM)

moisture. Low moisture bales in this study have similar results as the less than 30% moisture stover piles in Shinner et al. (2011) study. High moisture bales started the trial at 46.5% moisture and were found to have mold growth and significant DM loss, also similar to the Shinner et al. (2011) study. The effect of cover on dry matter was found to provide bales with the opportunity to dry while in storage (Figure 4.2) for both the HM and LM bales.

Nutritive Value

On day 0, CP was numerically greater in the HM compared to LM by 1.0 percentage unit (Table 4.4). The ADF was found to be 5.2 percentage units greater in LM bales on day 0 ($P<0.01$). In a previous study, Watson et al. (1993) reported high quality bales had 7% CP and were 55% digestible whereas low quality had 4.5% CP and 40% digestible. Bales in this study had higher digestibility but lower concentrations of protein than the Watson et al. (1993) study. Crude fiber (CF) was also found to be greater in LM bales than HM bales ($P<0.01$). Total digestible nutrients (TDN) was found to be greater in HM bales by 3.4 percentage units compared to the LM bales on day 0 ($P<0.01$). Similar results were also found for NE_m and NE_g as the TDN results. High moisture and LM bales had different corn varieties used and could be a possible influence on digestibility. Cattle grazing on a corn rootworm-protected hybrid corn residue had similar performance as cattle grazing corn residue that was nontransgenic (Vander Pol et al., 2005). Folmer et al. (2002) study found that cattle did not exhibit preferential grazing and performed similarly when grazing Bt compared to non-Bt corn residue.

The method of harvest of the stover bales, one versus two pass, can influence the nutrient content as different proportions of the corn plant are harvested. This is particularly

evident with ash content. Ash content was 1.98 percentage units greater in HM ($P<0.01$) than LM bales (Table 4.4). Soil contamination during harvest increased ash content and DM loss during storage also increase ash content. Bales with 3.5% ash are acceptable for feedstock use since that is a standard amount of structural ash (Schon and Darr, 2014). The remainder could be contamination during harvest, which dilutes the DM (Schon and Darr, 2014). Ash had a trend to increase over time ($P=0.09$, results not shown). Phosphorus was 0.01 percentage units greater in the LM bales on day 0 than the HM bales ($P=0.02$). Calcium, Na, and Mn concentrations were similar between treatments. The remaining minerals, Cu, Mg, Cu, Fe and Zn were all significantly greater in HM bales than LM ($P<0.05$). Nutrient Requirements of Beef Cattle (1984) reported the composition of stover and is presented in Table 4.4 for comparison. This study's reported nutrient composition appeared to have higher energy, lower protein and lower ash content than the NRC's values. Hybrid, field history, harvest method and location could be sources of variation from NRC reported values.

Table 4.4 Protein, fiber, energy and mineral content of corn stover on day 0 by high moisture (HM) and low moisture (LM)				
	HM	LM	Prob.	NRC, 1984
DM, %	53.5±2.5	63.9±4.4	<0.01	40.7
NE_g, Mcal/kg	0.62±0.009	0.51±0.07	<0.01	0.54
NE_m, Mcal/kg	1.17±0.009	1.06±0.07	<0.01	1.09
	% DM			
OM	93.0±0.024	95.0±0.012	0.04	87.9
CP	5.11±0.35	4.11±0.29	0.28	6.81
CF	37.5±0.35	41.7±2.0	<0.01	.
ADF	46.9±0.44	52.1±2.6	<0.01	45.6
TDN	55.9±0.29	52.5±1.7	<0.01	53.6
Ash	6.97±2.4	4.99±1.1	0.05	12.1
P	0.06±0.004	0.07±0.004	0.02	0.16
Ca	0.32±0.02	0.48±0.04	0.50	1.76
K	0.69±0.1	0.58±0.3	0.05	1.62
Mg	0.17±0.02	0.14±0.02	0.02	0.22
Na	0.01±0	0.01±0	0.39	0.24
	ppm			
Cu	8.5±0.84	6.5±0.55	0.01	7.8
Fe	920.1±386.2	464.1±188.8	0.04	1,021
Zn	20.1±2.64	11.9±1.79	0.04	30.0
Mn	86.7±22.4	55.9±15.6	0.42	63.9
Dry matter (DM), net energy for gain (NE _g), net energy for maintenance (NE _m), organic matter (OM), crude protein (CP), acid detergent fiber (ADF), crude fiber (CF), total digestible nutrient (TDN), crude fiber (CF), phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), copper (Cu), iron (Fe), zinc (Zn) and manganese(Mn)				

Nutrient Recovery

Nutrient recovery was not determined on d-240 as an error in weights occurred. A three-way interaction between cover type, moisture and time influenced ($P<0.05$) all measurements of nutrient recovery (Table 4.5). Since the source of bales was influenced by the method of harvest, the age of the bale at trial initiation and agronomic factors, the effects of time and storage will be discussed within each moisture level. Recoveries of CF, CP, DM and OM were similar regardless of storage length for all covered bales. The LM bales had similar recoveries in both storage treatments. Crude protein recovery in HM was similar between cover and uncovered. High moisture bales maintained crude protein. The HM bales had decreased DM recovery as length of storage increased. Uncovered HM bales stored for 365 d had decreased recoveries of DM, ADF, TDN, NE_g and NE_m ($P<0.04$). Ash percentage was found to increase in HM covered bales and decrease in uncovered bales ($P=0.02$). The recovery of OM, crude protein and TDN with cover and no cover were 95.8% and 91.4%; 104.3% and 107.1%; 92.4% and 94.6%, respectively. Similarly, recoveries of OM, crude protein and TDN with time of storage were 95.4%, 97.3% and 88.2%; 97.7%, 118.2% and 101.4%; 95.6%, 96.9% and 87.9%; respectively. Even though storage of HM bales uncovered resulted in lower nutrient recoveries, the economic cost of storage should be considered. Outdoor unwrapped bale storage, even with 12% DM, was cheaper than indoor storage dependent on building cost and storage value according to Vadas et al. (2013).

Table 4.5 Effects of storage method, time and moisture on nutrient recovery ² (%).														
	LM						HM						3-way	
	Cover			No Cover			Cover			No Cover				
	30	120	365	30	120	365	30	120	365	30	120	365	SEM	Prob.
DMR ¹	94.3 ^B	103.6 ^A	95.3 ^B	98.8 ^B	99.1 ^A	96.0 ^B	100 ^{AB}	89 ^B	96.8 ^B	88.0 ^B	99.4 ^A	67.2 ^C	4.5	<0.01
OMR	94.7 ^{AB}	104.3 ^A	95.6 ^{AB}	99.1 ^A	98.9 ^A	95.2 ^A	101.5 ^A	84.1 ^B	94.9 ^{AB}	86.4 ^B	101.9 ^A	67 ^C	4.0	<0.01
CPR	89.1 ^B	117.1 ^A	100.3 ^{AB}	100.7 ^{AB}	109.3 ^A	99.8 ^{AB}	104.8 ^A	102.9 ^A	111.7 ^A	88.6 ^B	121.4 ^A	85.3 ^B	7.5	0.03
ADFR	91.2 ^{AB}	101.2 ^A	94.6 ^{AB}	86.6 ^B	98.7 ^A	81.5 ^B	113.5 ^A	96.8 ^{BC}	113.0 ^A	101.1 ^{AB}	112 ^A	79.9 ^C	5.1	0.04
CFR	91.2 ^{AB}	101.2 ^A	94.6 ^{AB}	86.6 ^B	98.7 ^A	81.5 ^B	113.5 ^A	96.8 ^{BC}	113.0 ^A	101.1 ^{AB}	112 ^A	80 ^C	5.5	0.04
TDNR	96.5 ^A	104.5 ^A	95.8 ^A	106.7 ^A	99.5 ^A	105.5 ^A	92.6 ^A	75.3 ^{BC}	87.8 ^A	80.8 ^{AB}	89.3 ^A	60.1 ^C	4.3	<0.01
NE _M R	97.9 ^B	105.3 ^A	96.2 ^B	112.3 ^A	99.7 ^A	111.4 ^A	87.9 ^A	70.7 ^{BC}	82.1 ^{AB}	76.2 ^{AB}	84.9 ^{AB}	55.5 ^C	5.3	<0.01
NE _G R	101.3 ^B	107 ^{AB}	97 ^B	124.9 ^A	100.2 ^B	125.1 ^A	78.4 ^A	61.1 ^A	70.6 ^A	67 ^A	76.2 ^A	46.4 ^B	7.2	0.01
AshR	94.8 ^{AB}	95.8 ^{AB}	96.5 ^{AB}	98.7 ^{AB}	109.8 ^A	117.9 ^A	80.6 ^B	70.7 ^{BC}	122.6 ^A	109 ^A	66.5 ^C	69.2 ^C	11.5	0.02
^{ABC} Means in a row and moisture heading with unlike superscripts differ														
¹ Dry matter recovery (DMR), organic matter recovery (OMR), crude protein recovery (CPR), acid detergent fiber recovery (ADFR), crude fiber recovery (CFR), net energy for gain recovery (NE _g R), net energy for maintenance recovery (NE _m R), total digestible nutrient recovery (TDNR), crude fiber recovery (CFR), and ash recovery (AshR)														
² Nutrient recovery is the weight of nutrient on sampling day divided by the day-0 nutrient weight														

Experiment 2

Initial weight, final weight, and ADG were similar among feeding treatments. Dry matter intakes were greater for cattle fed both corn stover treatments ($P<0.01$; Table 4.6) as compared to the cattle fed the control diet. Cattle fed the 10 and 20% corn stover diets consumed 10 and 11.4 percentage units more feed than the control cattle, respectively. Cattle fed the 20% stover diet consumed 2.07% of their body weight (14.2 kg/d). The increased intake was consistent across weigh periods ($P<0.01$). Total corn intake was equal for the control and 10% corn stover diets which indicated cattle were able to compensate for the lower corn percentage in the diets with increased intakes. Cattle fed the 20% corn stover diets were unable to fully compensate for corn intake and as a result numerically gained less weight. Overall feed conversion efficiency was greater for the control treatment compared to the corn stover diets, whether calculated from full, shrunk, or carcass adjusted live weights ($P<0.02$). Carcass adjusted feed efficiencies for cattle fed the control, 10% and 20% stover diets were 121.6, 109.6 and 104.9 mg of gain per kg of DMI, respectively. The calculated NE_g content of the diet was 1.55 Mcal/kg for control, 1.40 Mcal/kg for 10% corn stover and 1.31 Mcal/kg for 20% corn stover diets (Table 4.6). Back calculations from the ration calculated NE_g value for corn stover were 0.37 and 0.48 Mcal/kg for the 10% and 20% rations. The recorded values for corn stalkage and corn stalks in the NRC (2016) were 0.54 and 0.51 Mcal/kg, respectively. The values in this study were lower which suggest the NE_g value in high concentration diets are lower than predicted from laboratory analysis (fiber content) or values listed in NRC (2016). Additionally, the NE_g value of corn stover was lower in the total ration with a greater NE_g content.

Table 4.6 Effects of dietary corn stover on cattle performance.					
	Control	10% stover	20% stover	SEM	Prob.
Initial weight, kg	434.2	435.7	435.2	1.95	0.85
Final weight, kg	694.1	695.1	683.6	5.58	0.13
ADG¹, kg					
0-28	1.93	1.92	1.81	0.13	0.94
29-56	1.71	1.82	1.77	0.13	
57-84	1.87	1.76	1.52	0.13	
85-112	1.20	1.34	1.39	0.13	
113-140	1.37	1.30	1.21	0.13	
141-end	1.54	1.55	1.38	0.13	
0-end	1.59	1.60	1.52	0.04	0.39
Carcass Adj ² . 0-end	1.58	1.58	1.53	0.03	0.41
Shrunk ³ 0-end	1.44	1.43	1.38	0.03	0.41
DMI⁴, kg/d					
0-28	12.0 ^A	13.1 ^B	13.6 ^B	0.26	<0.01
29-56	12.9 ^A	14.6 ^B	13.9 ^B	0.36	<0.01
57-84	13.0 ^A	14.0 ^C	13.6 ^B	0.03	<0.01
85-112	13.2 ^A	14.9 ^B	15.0 ^B	0.40	<0.01
113-140	12.6 ^A	13.8 ^B	14.8 ^B	0.24	<0.01
141-end	12.5 ^A	13.6 ^B	14.7 ^B	0.05	<0.01
0-end	12.7 ^A	14.0 ^B	14.2 ^B	0.26	<0.01
Corn intake	7.09 ^A	7.13 ^A	6.39 ^B	0.13	<0.01
Gain/feed, mg gain/kg DMI					
0-28	161.1	146.0	133.7	8.8	0.63
29-56	131.4	124.7	126.7	8.3	
57-84	144.7	126.9	112.0	8.5	
85-112	90.1	90.5	92.9	5.8	
113-141	108.4	94.4	82.8	8.2	
141-end	122.2	113.3	92.7	14.3	
0-end	125.6 ^A	114.5 ^B	107.1 ^C	2.2	<0.01
Carcass Adj. 0-end	121.6 ^A	109.6 ^B	104.7 ^B	3.3	0.01
Shrunk 0-end	110.5 ^A	99.5 ^B	94.9 ^B	3.2	0.02
Ration NE _m ⁵ (Mcal/kg)	2.24 ^A	2.07 ^B	1.96 ^C	0.03	<0.01
Ration NE _g ⁶ (Mcal/kg)	1.55 ^A	1.40 ^B	1.31 ^C	0.03	<0.01
^{ABC} Means in a row with unlike superscripts differ, $\alpha=0.05$ ¹ Average daily gain (ADG) ² Carcass adjusted ADG and gain/feed ³ Shrunk ADG and gain/feed were calculated by using a final live weight multiplied by 0.96 to account for shrunk dressing percentage ⁴ Dry matter intake (DMI) ⁵ Net energy for maintenance (NE _m) ⁶ Net energy for gain (NE _g)					

Dressing percentages calculated from live and shrunk final weights were similar among treatments (Table 4.7). The HCW were similar 395.5, 396.4 and 390 kg for the control, 10 and 20% stover treatments (P=0.35). The quality of the carcass was similar amount treatments as measured by REA, marbling, backfat, muscle grade, calculated yield grade and quality grade. Average Choice was the overall quality grade. Only 6% of cattle had liver abscesses and 7% had

Table 4.7 Effects of corn stover on cattle carcass characteristics.					
	Control	10% stover	20% stover	SEM	Prob.
Dressing percentage	57.1	56.9	57.2	0.32	0.89
Shrunk dressing percentage	59.4	59.3	59.5	0.33	0.89
Hot carc. wt., kg	395.5	396.4	390.0	3.4	0.35
Ribeye area, cm²	83.0	84.4	84.3	1.1	0.55
Marbling^d	616	602	586	12.0	0.22
Backfat, cm	0.72	0.75	0.68	0.04	0.45
Kidney, pelvic & heart fat, %	3.75 ^A	3.50 ^A	2.00 ^B	0.02	<0.01
Muscle grade^f	2.04	1.96	2.01	0.05	0.43
Quality grade^f	19.7	19.6	19.4	0.14	0.34
Calc. yield grade	3.38	3.27	3.07	0.28	0.72
^{ABC} Means in a row with unlike superscripts differ ^d Marbling score: 600=modest; 700=moderate ^e Quality grade: 19=Choice; 20=average Choice ^f Harvest facility derived score					

maturity scores over 30 months (data not shown). Kidney, pelvic and heart fat percentage was greater for the cattle fed the control ration (3.75%), when compared to the cattle being fed 20% stover (2.00 %; $P < 0.01$).

Previous research has contradicted and supported these results. A study conducted by Shreck et al. (2012) fed steers (372.8 kg) diets containing 5 or 20% corn stover and reported reduced quality carcass characteristics, performance, final weight, ADG and HCW with 20% corn stover. Dry matter intakes were similar between treatments, very different from the results concluded in this experiment. Backfat, yield grade and marbling were also similar between treatments, which support the observations in this study. Johnson et al. (2015) compared 5% and 20% corn stover diets and reported DMI was similar for steers, but ADG was lower with the 20% diet. The steers in the current study were heavy, yearlings Holstein steers with large rumen capacities that may have allowed for the greater intake and similar gains as compared to the Shreck and Johnson studies that utilized beef type steers. Holsteins have been reported to consume more than beef type steers (Rust and Abney, 2005). In contrast to the current study, final BW, HCW, backfat and marbling scores were lower for cattle fed 20% stover than the Shreck and Johnson studies. In the current study, corn stover replaced corn silage in the 10% corn stover diet and corn silage and HMC in the 20% corn stover diet. The Shreck and Johnson studies replaced corn only with additional corn stover.

Overall, the results indicated that feeding stover had a significant effect on DMI intake, but the carcass characteristics remained similar among treatments. When looking at the feed efficiency for the entire trial, feed conversion efficiency decreased from 125.6 to 107.1 mg

gain/kg DMI for control and 20% diet, respectfully ($P < 0.01$). Although the HCW and final weight differences were not significant, they were approximately 6 kg and 10 kg less in the 20% stover diets compared to the other diets.

Conclusions

Nutrient recovery for energy, digestibility and dry matter were found to decrease when bales of higher moisture were uncovered. Uncovered HM bales had similar nutrient and dry matter recoveries through 120 d, making it feasible to store bales of 45% moisture outdoors for a limited amount of time. Higher moisture bales became very difficult to move after 120 d in storage due to decreased structural integrity. Storage offers an advantage for bales that have moisture of 45% and greater, preventing degradation of nutrient content and dry matter. There was no advantage to storing bales with moisture content of 34% indoors, since the nutrient and dry matter recovery was similar over time, regardless of storage.

Feeding stover significantly increased DMI intake and reduced feed efficiency, but average daily gain and carcass characteristics were similar among treatments. Cattle were able to compensate for a lower energy diet containing less corn by increased intake when fed a 20% corn stover diet. Corn stover is a viable alternative forage for cattle that could be fed as 10% or 20% portion of the diet without a significant effect on weight gain or carcass characteristics.

Chapter 5

IMPROVING FEEDSTOCK YIELD AND QUALITY BY INTERCROPPING A WINTER CEREAL WITH CORN STOVER

Abstract

Corn stover (*Zea mays L.*) is a commodity that can be sold as a feedstock for the livestock or bioenergy industries. When stover is removed at high rates, studies have shown a decrease in soil organic matter, but the addition of a winter annual cover crop can offset the impact of stover harvest on soil organic matter loss. The objective of this study was to evaluate the yield and quality of biomass feedstocks resulting from the harvest of mixed stands of corn stover interseeded with winter cereals. The experimental design was a randomized complete block with a whole plot factor of interseeding winter annual cereals and a split-plot factor of harvest time. The winter cereal crops interseeded with corn stover consisted of cereal rye (*Secale cereale L.*), and triticale (*Triticale hexaploide Lart.*). A stover-only treatment was used as an experimental control. The harvest times evaluated were a two-harvest system (spring and followed by fall), and a one-harvest system (spring or fall). Regardless of harvest system, the incorporation of a winter cereal crop always yielded greater dry matter, ethanol, crude protein and energy content than the stover-only treatment. Total biomass feedstock harvested from the two-harvest system (spring + fall) had greater dry matter, ethanol, crude protein and energy content on a land area basis compared with the one harvest system. On a land-area basis, the stover-only feedstock had lower dry matter yield, ethanol, energy and crude protein content when it was harvested in the spring compared to the fall. However, spring harvested corn stover had a higher ethanol yield on g g^{-1} basis relative to fall harvested stover. Overall,

the mixed biomass feedstocks resulting from the incorporation of a winter annual cereal with corn stover improved feedstock quality and quantity relative to stover-only feedstocks.

Introduction

Corn stover (*Zea mays L.*) is the non-grain portion of the corn plant, including the husk, cob, stalk and leaf. Stover is an abundant commodity that can be used as a biomass feedstock for paper production, the pharmaceutical industry and the agricultural industry. After harvesting corn grain, stover is the remainder of the crop often referred to as residue. Persistent residue decreases soil warming in the spring, can serve as a host for disease and can decrease seed-soil contact when a rotational crop is planted in the spring (Kravchenko and Thelen, 2007).

Sustainable harvesting of stover can assist with residue management without substantially decreasing soil organic matter. Harvesting stover at a rate of 2.23 MT ha⁻¹ has minimal effect on grain yield, stover composition and soil quality factors (Birrell et al., 2014). To make harvesting stover economical for farmers, harvesting at a rate greater than 2.23 MT ha⁻¹ may be necessary.

Pratt et al. (2014) found that the addition of a cover crop allowed for an increase of 4 MT ha⁻¹ of stover to be removed sustainably. Cover crops are also credited for environmental and soil quality benefits (Kaspar and Bakker, 2015), including weed suppression (De Bruin et al, 2005). Another possible use for cover crops involves their use as a feedstock for livestock or the bioenergy industry. If cover crops could be harvested with stover, there may be an increase in the nutritive value and biomass yield, while contributing root carbon to soil organic matter. This study evaluated the yield and quality of mixed biomass feedstocks resulting from the addition of an inter-seeded winter annual cereal cover crop, cereal rye (*Secale cereale L.*) or triticale (*Triticale hexaploide Lart.*), with corn stover. Additionally, a fall and spring two-harvest system and a spring, one- harvest system, were evaluated across the corn stover plus winter cereal

crop mixed feedstocks. A one-harvest system, fall and spring, was evaluated for the corn stover-only treatments which were not interseeded with winter annual cereal cover crops.

Materials & Methods

Site Description

The research was conducted at two locations: Michigan State University (MSU) located in central Michigan USA (MSU, 42°41' 14" N, 84°29'4" W) and W.K. Kellogg Biological Station in southwest Michigan USA (KBS, 42°23'47" N, 85°22'26" W). Predominant soil series at KBS are the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, semiactive, mesic Typic Hapludalfs) series. The predominant soil series at MSU is a Capac loam (fine-loamy, mixed, mesic, Aeric Ochraqualfs). The mean annual temperature and 30-yr mean annual precipitation at KBS were 9.95 °C and 9.15 cm, respectively (NOAA, 2016) (Figure 5.1). The mean annual temperature and 30-yr mean annual precipitation at MSU were 8.76 °C and 12.72 cm, respectively (NOAA, 2016) (Figure 5.1).

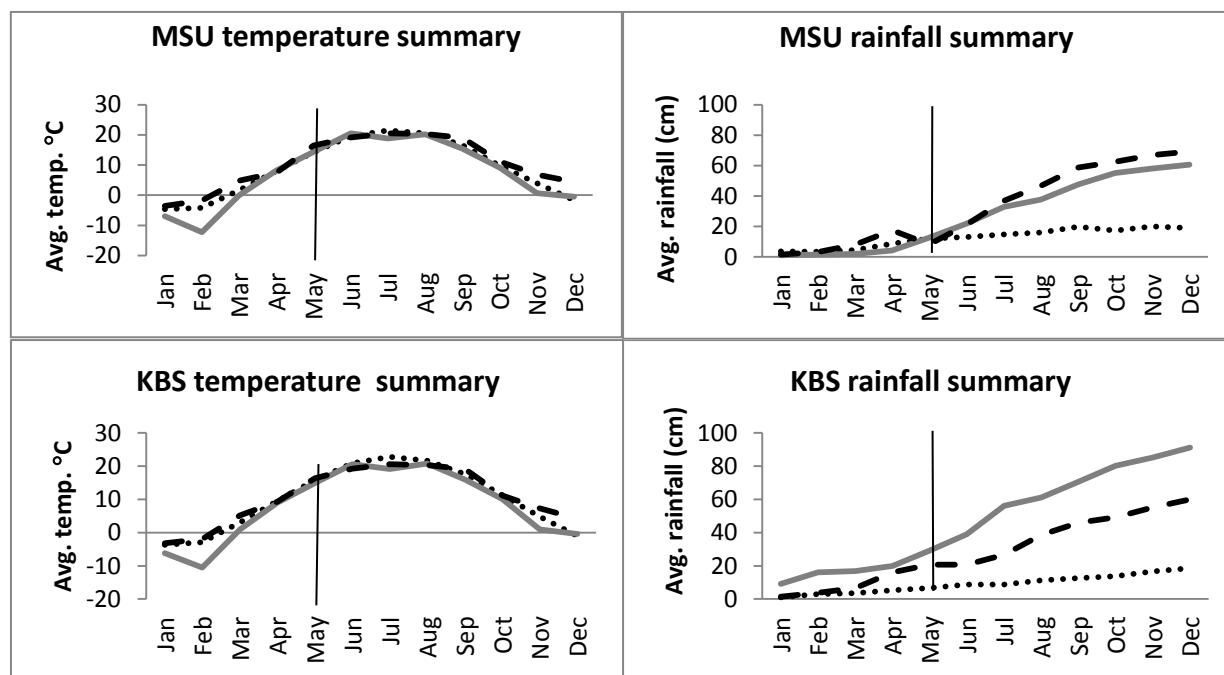


Figure 5.1 Average 30 year (1886-2016) temperature and rainfall for MSU and KBS (dotted line), and annual temperature and rainfall for 2014 (grey line) and 2015 (dashed line). Growing season is defined as May of 2014 thru May of 2015 and May of 2015 thru May of 2016 (black vertical line).

Material Inputs

Table 5.1 Summary of planting, harvest dates and agronomic inputs for both years and locations of the experiment.				
Location	KBS 2014	KBS 2015	EL 2014	EL 2015
Cultivar	¹ DKC39-07RIB	DKC39-07RIB	DKC39-07RIB	DKC39-07RIB
Seed Density	79,012 seeds ha ⁻¹	79,012 seeds ha ⁻¹	79,012 seeds ha ⁻¹	79,012 seeds ha ⁻¹
Planting date	5/12	6/24	5/27	6/4
Harvest date	11/3	11/9	10/22	11/14
Fertilizer app	5/12 potassium (potash) 78.6 kg ha ⁻¹ 6/27 nitrogen (UAN ²) 135 kg ha ⁻¹	6/24 nitrogen (UAN) 190 kg ha ⁻¹	6/22 nitrogen (UAN) with disks 151 kg ha ⁻¹	7/3 nitrogen (UAN) with disks 151 kg ha ⁻¹ 7/6 nitrogen (urea) 56 kg ha ⁻¹
Herbicide app	5/11 glyphosate ³ 2.9 liter ha ⁻¹ 6/9 glyphosate 2.3 liter ha ⁻¹ 6/27 glyphosate and AMS ⁴ 2.3 liter ha ⁻¹	6/6 glyphosate 2.9 liter ha ⁻¹ 7/13 glyphosate and AMS 2.3 liter ha ⁻¹	6/22 glyphosate and AMS 2.3 liter ha ⁻¹	6/6 glyphosate 2.9 liter ha ⁻¹ 7/2 glyphosate 3.2 liter ha ⁻¹
Cover crop planting date	9/8	9/10	9/8	9/10
¹ DEKALB corn variety (Monsanto Company LLC DeKalb, IL) ² Urea ammonium nitrate solution ³ glyphosate (N-(phosphonomethyl) glycine in the potassium-salt form) ⁴ ammonium sulfate(AMS) (alkyl polyglycoside, polydimethylsiloxane)				

Agronomic decisions about planting densities, hybrid selection, nutrient management, and herbicide application followed local best management practices as recommended by Michigan State University (MSU) Extension. Agronomic inputs and decisions are summarized in Table 5.1. Rye and triticale were planted by walking down the center row within the field plot

and hand spreading the seed into the mature corn stand. Cereal rye (*Secale cereale* L.) and triticale (*Triticale hexaploide* Lart.) winter annual cover crops were interseeded at a rate of 106.7 kg ha⁻¹ into corn at the dates indicated in Table 5.1. Soybeans were planted previous to this study and both field sites were cultivated with a chisel plow and finisher before planting corn in 2014. The 2015 corn was no-till planted into the 2014 plots. Corn grain was machine harvested by a JD9410 model combine (John Deere, Corp., Moline, IL).

Experimental Design

To assess the yield and quality of biomass feedstocks produced from mixed stands of corn stover and winter annual cereals, a split plot design was used. Six treatments, in two locations, using a randomized complete block design with four replications totaling 48 plots. The whole plot factor was the treatment of corn stover plus rye, corn stover plus triticale or stover-only crops. Each treatment had a subplot factor of harvest time: spring-only; or fall and spring. The two locations were Hickory Corners, MI (KBS) and East Lansing, MI (MSU).

Harvest Method

Corn stover and corn stover plus winter annual cereal mixed stands were harvested with a mechanical forage plot harvester (Carter Mfg. Co., Brookston, IN). Fall harvest dates were 10/23/14 and 11/4/15 at MSU and 11/3/14 and 11/9/15 at KBS. Spring harvest dates were conducted on 6/2/15 and 5/20/16 for both locations. Prior to harvesting the rows were cut back to 12.2 m. Within a plot the center 2 rows were mechanically harvested using a 1.2 m head. The harvested biomass was then weighed and sub-sampled to assess yield quantity and quality. The samples were weighed with a scientific scale, dried with an oven and ground down

with a mill. Dried sample from the machine harvest was then analyzed for dry matter content, ethanol yield (fermentable sugars) determination and nutritive value. A small subsample, (531 cm linear row length) of the standing corn (stover and grain) was hand harvested in each plot by clipping near ground level (10.2 cm above ground) prior to machine harvest to determine harvest efficiency. Hand harvest was conducted on 10/17/14 and 10/22/15 at MSU and 10/28/14 and 10/19/15 at KBS. Harvest population and total biomass weight were taken. Grain was separated and moisture and dry matter yield were determined. Two stalks from each plot were used to determine plant dry matter and potential dry matter yield (MT ha^{-1}).

Ethanol Production

Fermentable Sugar Determination

Biomass sample grinding, feeding, and weighing were performed by a custom-designed robot (Labman Automation Ltd., United Kingdom). Samples of dried plant material (20–40 mg) were loaded manually into Sarstedt 2-mL screw-cap microtubes along with three 5.56 mm stainless steel balls (Salem Specialty Ball Co, Canton, CT). The tubes were placed into racks and positioned in the robot, and pulverization of the biomass was accomplished by ball milling. The length of the grind time was adjusted sufficient to reduce the sample to a fine powder. A 1.5 mg subsample of biomass was transferred to a barcoded 1.4 ml polypropylene microtube (Micronic brand) sealed with a thermoplastic elastomer cap mat (Micronic brand) and 750 μL of pretreatment solution (NaOH 62.5 mM). Pretreatment solution was pipetted into each tube and then placed in a 90°C water bath for 3 h. As needed, reactions were neutralized with $\sim 7.5 \mu\text{L}$ 6N hydrochloric acid. Next, 50 μL of a solution containing 0.5 μL Accellerase 1000 (Genencor,

Rochester, NY), 33.3 µl 1 M citrate buffer (pH 4.5) plus 10 µl 1% w/v sodium azide; 72 ηL C-Tec2 and 8 ηL H-tec2 enzymes were added to all tubes. Enzymatic hydrolysis was done in a final volume of 0.8 mL using an enzyme concentration of 50 mg protein/g glucan. Tubes were placed in racks and incubated for 20 h in a rotisserie oven at 50°C. Racks were centrifuged and supernatants were transferred to 0.8 mL deep-well plates. The glucose and xylose contents were determined using enzyme-based assay kits (Megazyme, Ireland). Glucose was assayed with the glucose oxidase/peroxidase (GOPOD) method (K-GLUC, Megazyme, Ireland) using 4 µL of the supernatant of the digestion reaction mixture and 64 µL of the GOPOD assay reagent. Xylose was assayed enzymatically (K-XYLOSE, Megazyme) using 8 µL sample and 62 µL K-XYLOSE assay reagent. Further details on the analyses used to determine fermentable glucose and xylose content of biomass are outlined by Santoro et al., 2010.

Ethanol Yield Estimation

Ethanol yield was calculated based on the empirically derived fermentable glucose and xylose levels using the equation: $([Glc] + [Xyl]) * 51.1\% * \text{metabolic yield} = (\text{EtOH mg/kg})$

Where [Glc] is the glucose concentration of the biomass following pretreatment and enzymatic hydrolysis (mg/kg) and [Xyl] is the xylose concentration of the biomass following pretreatment and enzymatic hydrolysis (mg/kg). The mass conversion of fermentable sugars to ethanol is 51.1%, and metabolic yield equals to the ratio of ethanol to the consumed sugars in the fermentation process divided by 51.1% (Lau and Dale, 2009). Metabolic yield values were determined using a separate hydrolysis and fermentation (SHF) process and are derived from

Jin et al., (2012) for corn stover (93.1%). Concentration of ethanol yield was multiplied by the dry matter yield of the plot to determine the harvest system yield.

Nutritive Value

Samples were analyzed at Litchlab Analytical Services (Litchfield, MI). Feed analysis definitions can be found in Appendix Table B.4. Crude protein (CP) and acid detergent fiber (ADF) were determined using wet chemistry. Net energy for gain (NE_g), net energy for maintenance (NE_m), total digestible nutrients (TDN) and crude fiber (CF) were estimated using calculations based off the determined components. Mineral content determined included ash, phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn). Inter-coupled plasma spectrometry (ICPS) was used to determine mineral content. Concentration of CP and TDN were multiplied by the DM yield of the plot to determine the harvest system yield.

Statistical Analysis

The experimental unit was the plot with the location, replication (location) and $trt \times replication$ (location) as the random variable to account for the random variation between the plots. Data was analyzed for variance and normality by examining the Levene's test results and normal probability plots. If unequal variance was detected, data was re-analyzed using the Kenward Rogers model. The data was analyzed as a RCBD with split plot factor of harvest time with a whole plot factor of cover crop. Tukey-Kramer all pairwise comparison was used with the LSMEANS statement of Proc Mixed in SAS 9.4 to determine mean separation when mean square was significant (SAS Inc., 2012). Data was analyzed using the Proc Mixed procedure in

SAS 9.4 (SAS Inc., 2012). Results were reported as statistically significant at $\alpha = 0.05$ and a trend at $\alpha = 0.10$.

Results & Discussion

Figure 5.1 compares the 30-year average temperature history to the 2014 and 2015 growing seasons. Rainfall was much higher during both growing seasons and locations compared to the 30-year average.

Two harvest timings were used in the study, a two-harvest system and a one-harvest system. Two-harvest system consisted of a fall harvest followed by a spring harvest. The one-harvest system for corn stover plots interseeded with winter annual cereal cover crops was spring-only. The one-harvest system for the stover-only treatment had either a fall or spring timing to determine over-winter field loss of stover. The harvested feedstock from the interseeded plots during the fall harvest time consisted primarily of corn stover biomass with very little of the winter annual cereal present due to the limited time frame from growth of the fall-planted winter cereals. Conversely, the subsequent spring harvest in the two-harvest interseeded plots consisted primarily of the winter annual cereal crop biomass since the stover fraction had been effectively removed during the fall harvest. The one-harvest system consisted of a mix of winter cereal crop and stover for the interseeded treatments, or corn stover only in the stover-only control plots. Harvest time has a significant effect on both the nutritive value and yield of winter annual cover crops (Undersander, 2013 and Oplinger et al., 1997).

Nutritive and ethanol analytical values were reported by harvest timing for all three treatments to summarize feedstock composition within each unique harvest time. Dry matter yield, ethanol yield, CP and TDN were also analyzed on a land-area basis and are summarized and compared across harvest time and system.

Hand Harvest

Plant population, harvest index, corn grain and corn stover yield did not differ across the experimental site prior to inter-seeding the winter annual cereal crops (data not shown). Corn grain and corn stover yield were greater in 2014 than 2015 (Table 5.2). Harvest index was significantly lower the second year ($p < 0.01$) which may have been effected by a decrease in grain yield ($p < 0.01$). Corn grain yield was 4.02 MT ha^{-1} greater in 2014 ($p < 0.01$).

Table 5.2 Hand harvested population, harvest index (HI) and corn grain and corn stover yield in the fall before corn grain and stover machine harvest.				
	2014	2015	SEM	Prob.
Population	32.08	33.0	1.87	0.09
Corn Grain yield (MT ha^{-1} @15.5 %)	11.31	7.29	0.75	<0.01
Potential stover yield (dm MT ha^{-1})	9.58	8.78	0.34	0.09
HI², %	50.0	41.5	0.06	<0.01
¹ represents the proportion of corn grain yield to corn stover yield on a DM basis				

Overall, interseeding a winter annual cereal and having a later harvest had no effect on the following year's corn population. A meta-analysis of 26 independent studies concluded winter annual grass cover crops were found to not affect corn grain yields and was not dependent on the use of nitrogen fertilizer (Miguez and Bollero, 2005). Miguez and Bollero (2005) concluded the use of winter annual cover crops improved soil properties and/or reduced nitrate losses but did not change corn yield.

Harvest efficiency (HE) was measured for corn stover and can be compared for treatments that primarily consist of corn stover (fall and stover only). Harvest efficiency is the percentage of harvested stover over the potential amount available. A later, over-winter

Table 5.3 Interaction of harvest time and cover crop on harvest efficiency of corn stover (HE).

	Rye + stover	Triticale + stover	Stover only	
	Fall	Fall	Fall	Spring
HE ¹ , %	50.3 ^A	51.9 ^A	54.6 ^A	21.5 ^B
¹ percentage of stover harvested over the amount available ^{abc} Means with unlike letters differ, $\alpha=0.05$				

harvest of stover, harvest time of spring, caused a significant reduction in HE from 55 to 22% (Table 5.3). This could be due to leaf loss and degradation of stover over-winter causing less stover to be harvested in the spring. Liu et al. (2009) found that later harvest time decreased the yield of stover due to a large decrease in the leaf portion.

Nutrient Composition and Ethanol Yield

Ethanol Yield

Table 5.4 Interaction of harvest time and treatment on glucose (Glu) and xylose (Xyl) concentration and ethanol (EtOH) yield (g g⁻¹).

	Rye + Stover			Triticale + Stover			Stover-only	
	Two-harvest system		One-harvest system	Two-harvest System		One-harvest system	One-harvest system	
	Fall	Spring	Spring	Fall	Spring	Spring	Fall	Spring
Glu, mg kg ⁻¹	0.213 ^B	0.197 ^C	0.242 ^A	0.214 ^B	0.200 ^C	0.251 ^A	0.213 ^B	0.285 ^A
Xyl, mg kg ⁻¹	0.118 ^B	0.116 ^B	0.125 ^A	0.114 ^B	0.113 ^B	0.126 ^A	0.118 ^B	0.136 ^A
EtOH, g g ⁻¹	0.158 ^C	0.149 ^D	0.175 ^B	0.156 ^C	0.149 ^D	0.180 ^B	0.158 ^C	0.201 ^A
^{abc} Means with unlike letters differ, $\alpha=0.05$								

Glucose and xylose concentration and ethanol yield on a g g^{-1} basis was found to be highest in the one-harvest system (spring), regardless of treatment (Table 5.4). This is likely due to partial degradation of the corn stover fraction of the feedstock, during the over-winter period in the field, rendering the cellulose more available to yield fermentable sugars. The stover-only feedstock had the highest concentration of glucose and ethanol. Pentose concentration was similar for stover-only and winter cereal + stover feedstocks. Stover-only feedstocks harvested in the spring yielded the highest ethanol concentration at 0.201 g g^{-1} (Table 5.4) commensurate with the higher sugar yield. Comparable ethanol yields have been reported in previous research using a similar biomass deconstruction pretreatment, ranging from $0.17\text{-}0.20 \text{ g g}^{-1}$ (Tumbalam et al., 2015). Conversely, Liu et al. (2009) found that hemicellulose content decreased and lignin increased with the later harvest time causing a decrease in the ethanol yield.

Nutritive Value

Potassium, which is highly water soluble and subject to over-winter leaching, was lower in the spring-harvested stover-only biomass than the other treatments. Conversely, spring-harvested feedstocks containing winter annual cereal biomass had relatively higher levels of potassium due to active uptake from the growing winter cereals. Dry matter content was found to be much greater for stover-only when left in the field and harvested in the spring. The stover fraction in the fall stover + cover crop mix increased DM relative to the spring harvest, which consisted primarily of green winter cereal biomass. The amount of moisture is an important factor when looking at the storage and transportation costs and options. Microbial activity

slows at less than 22 percent moisture content and becomes stagnant at 18 percent (Shah and Darr, 2014). Having material harvested that is less than 22 percent moisture would be ideal to prevent degradation of the nutrient content and quality during storage. Spring harvested winter cereal feedstocks would require field drying for dry bale or ensiled chop harvest systems.

The crude protein level was greatest in the winter cereal plus stover mixture, especially when harvested in the spring, because the feedstock consists primarily of the winter cereal crop. The ADF and crude fiber (CF), representing lower digestibility, were greatest in the stover only treatment especially when harvested later. The triticale + stover mixture, harvested in the spring, was the most digestible treatment with the lowest ADF and CF. Total digestible nutrients (TDN), NE_g and NE_m were found to be greatest for triticale harvested in the spring at 63.64%, 0.85 Mcal kg^{-1} and 1.44 Mcal kg^{-1} , respectfully (Table 5.5). Stover-only feedstock was found to contain the lowest energy content which decreased with a later harvest time. The spring harvest of triticale + stover mixture is primarily composed of biomass from the winter annual cereal, since the stover component had been harvested in the fall. The spring harvested triticale treatment was the most nutrient dense with the highest digestibility, crude protein content and energy content.

Calcium was lowest in cereal rye plus stover feedstock harvested in the spring at 0.177% of DM. Phosphorus (P), magnesium (Mg), sodium (Na), copper (Cu) and iron (Fe) had no statistical difference when evaluating across treatment and harvest time. Potassium (K) was greatest in triticale + stover harvested in the spring and was significantly lower for feedstocks

Table 5.5 Interaction of harvest time and treatment on feedstock nutrient content.								
	Rye + Stover			Triticale +Stover			Stover- only	
	Two-harvest System		One-harvest system	Two-harvest System		One-harvest system	One-harvest system	
	Fall	Spring	Spring	Fall	Spring	Spring	Fall	Spring
DM, %	68.40 ^B	26.90 ^D	40.20 ^C	68.20 ^B	25.20 ^D	42.10 ^C	68.40 ^B	76.90 ^A
NE_m, Mcal kg⁻¹	1.12 ^C	1.34 ^B	1.04 ^D	1.16 ^C	1.44 ^A	1.01 ^D	1.16 ^C	0.748 ^E
NE_g, Mcal kg⁻¹	0.56 ^C	0.76 ^B	0.49 ^D	0.59 ^C	0.85 ^A	0.46 ^D	0.60 ^C	0.21 ^E
	% of DM							
CP	4.56 ^C	7.62 ^A	5.99 ^{AB}	4.38 ^C	8.54 ^A	6.01 ^{AB}	4.00 ^C	3.84 ^C
ADF¹	48.40 ^B	39.70 ^C	48.56 ^B	47.90 ^B	36.10 ^D	49.10 ^B	47.90 ^B	56.40 ^A
CF¹	38.70 ^B	31.76 ^C	38.85 ^B	38.36 ^B	28.88 ^D	39.27 ^B	38.33 ^B	45.10 ^A
TDN	54.22 ^C	60.47 ^B	51.96 ^C	55.18 ^C	63.64 ^A	51.21 ^C	55.47 ^C	43.87 ^D
P	0.06	0.25	0.17	0.08	0.28	0.15	0.06	0.11
Ca	0.38 ^A	0.18 ^C	0.22 ^{AB}	0.37 ^A	0.22 ^B	0.24 ^{AB}	0.36 ^{AB}	0.26 ^{AB}
K	0.72 ^D	1.79 ^B	1.18 ^C	0.77 ^D	2.01 ^A	1.07 ^C	0.69 ^D	0.44 ^E
Mg	0.21	0.09	0.13	0.22	0.12	0.14	0.21	0.15
Na	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	ppm							
Cu	5.0	3.4	3.9	4.9	4.3	3.4	5.2	2.9
Fe	133.6	252.2	506.5	152	318	585.2	202.8	622.7
Zn	12.1 ^B	12.5 ^B	12.0 ^B	13.5 ^B	17.4 ^A	12.7 ^B	11.1 ^B	9.2 ^B
Mn	38.9 ^A	30.9 ^B	38.7 ^A	38.3 ^A	39.3 ^A	44.6 ^A	42.9 ^A	45.4 ^A
¹ Acid detergent fiber (ADF) and crude fiber (CF) determine fiber fractions								
² Total digestible nutrients (TDN), net energy for maintenance (NE _m) and net energy for gain (NE _g) are calculated from ADF value								
³ Mineral content: phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn)								
^{abc} Means with unlike letters differ, $\alpha=0.05$								

that consisted of primarily of stover. Calcium (Ca) was the greatest in all treatments when harvested in the fall, which would primarily consist of stover. Spring-harvested cereal rye plus stover feedstock had the lowest concentration of Ca at 0.18 %, which consisted primarily of cereal rye. Zinc was greatest in spring-harvested triticale, at 17.4 ppm. Manganese was lowest in rye harvested in the spring at 30.9 ppm. Having a higher nutrient content present in the harvested feedstock, especially P and K, could lead to greater nutrient replacement and fertilizer costs. Corn stover contained 9.97 kg of nitrogen, 3.63 kg of phosphorus and 14.5 kg of potassium of nutrient per ton of stover (Brechbill and Tyner, 2008). Estimated nutrient replacement cost for corn stover's nutrient content is \$15.64 per ton of stover removed (Brechbill and Tyner, 2008). As expected, harvesting greater amounts of stover resulted in an increased N, P and K removal.

Supplying an adequate amount of minerals is important for cattle performance and health affecting claw integrity, fertility, lactation and immune function (Miller et al., 1988).

Trace minerals such as Zn, Mn, Cu and Co are important for protein synthesis, vitamin metabolism, formation of connective tissue and immune function (Miller et al., 1988).

Fall-harvested feedstocks from corn stover inter-seeded with winter cereal crops had comparable nutrient content to the stover-only treatment harvested in the fall. This collaborates our observation that at the time of fall harvest, very little biomass had accumulated from the winter cereals. The CP levels from the spring-harvest of our winter annual mixed feedstocks were less than a previous reported concentration for spring harvested cereal cover crops at 10.2 % (Oplinger et al, 1997). Digestibility (ADF) when compared to

previous research is similar averaging 38.3 % and 33.2 % for rye and triticale, respectfully (Oplinger et al, 1997). The RFV (relative feed value) decreased by half for winter rye and triticale when harvested in the spring instead of the fall (Undersander, 2013). Undersander (2013) concluded that winter cereal rye and triticale had similar nutritive values and yield. Corn stover plus rye and triticale nutritive values harvested in the spring, had similar mineral content, DM and crude protein but differed in energy and digestibility. Triticale had greater metabolic energy content (TDN, NE_g and NE_m) and digestibility (ADF and CF) than the cereal rye.

Harvest System

Harvest systems were divided into a two harvest system or a one harvest system. Machine harvested yield was collected for all treatments on a dry matter basis (Figure 5.2) and was greatest for the two-harvest system. Previous research has reported potential yield of 5.4-7.8 MT ha⁻¹ for cereal cover crops when harvested in the spring (Undersander, 2013 and Oplinger et al., 1997). This magnitude of yield was not realized for the harvest time that consisted of mostly cover crop (spring) but was comparable for the two-harvest system. A significant decrease in stover yield occurred with a later single-harvest in the spring compared to once in the fall. The DM yield reduction in stover-only from fall to spring was 2.70 MT ha⁻¹ and the DM yield reduction in the mixed feedstocks of triticale + stover and rye +stover from fall to spring was 2.99 MT ha⁻¹ and 3.22 MT ha⁻¹, respectfully. Therefore, the yield reduction due to the over-winter biomass loss of the stover fraction appeared to account for the majority of the yield difference observed between the 2x and 1x harvest systems for the mixed feedstocks.

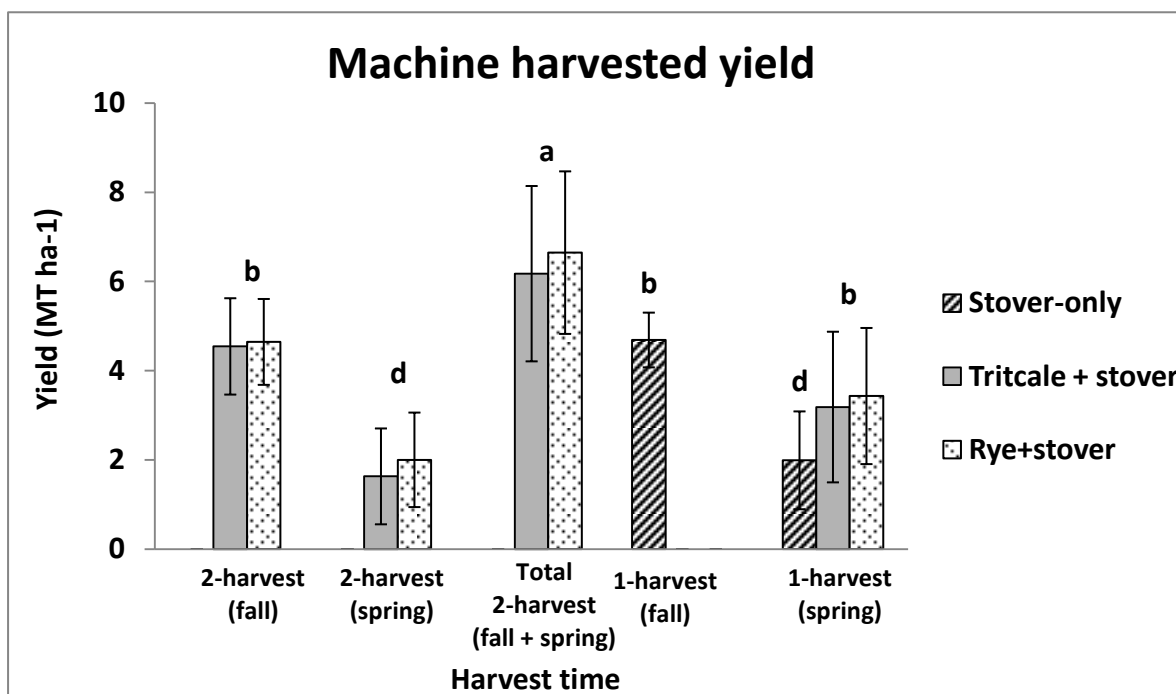


Figure 5.2 Machine harvested (MT ha^{-1}) yield on a dry matter basis for two-harvest system of fall followed by spring harvest or single harvest system (spring or fall only). Data are averaged across year and location. ^{abc}Means with unlike letters differ, $\alpha=0.05$.

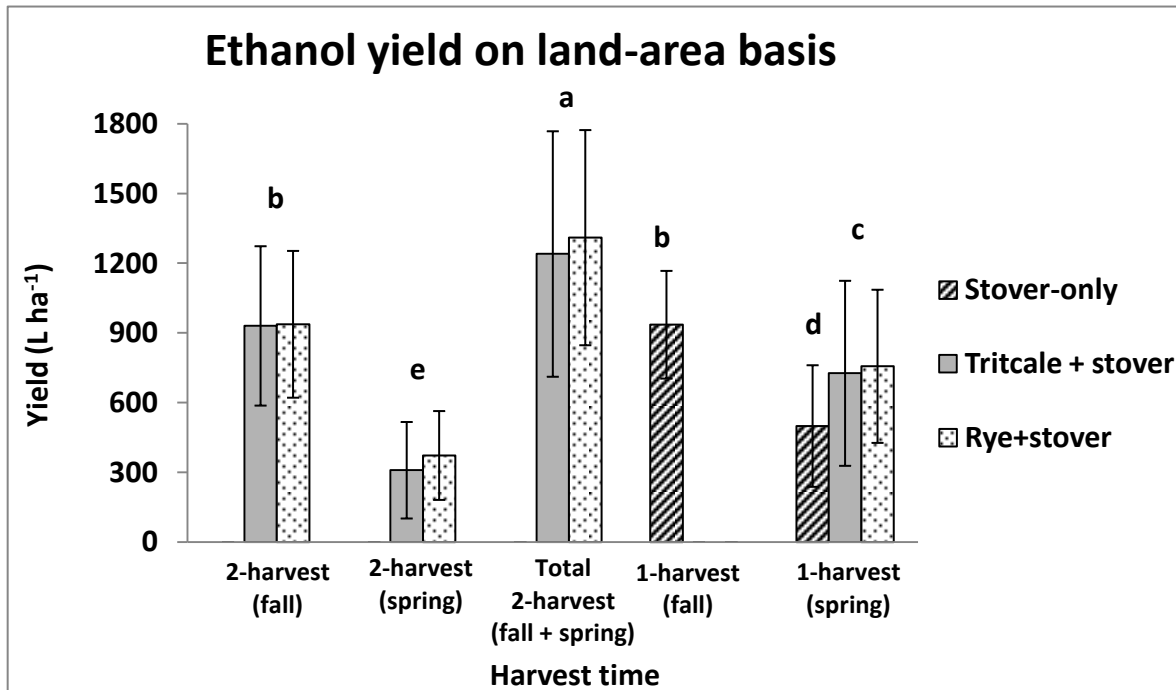


Figure 5.3 Ethanol yields on a land basis (L ha^{-1}) for two-harvest system of fall followed by spring harvest or single harvest system (spring or fall only). Data are averaged across year and location. ^{abc}Means with unlike letters differ, $\alpha=0.05$.

Ethanol and Nutrient Yield on a Land-area Basis

Ethanol yield on a land-area basis (L ha^{-1} ; Figure 5.3) was greatest in the two-harvest system for the mixed biomass feedstocks of triticale + stover and rye + stover. A significant decrease in ethanol yield occurred with spring-harvested corn stover-only relative to a fall harvest. Similarly, a significant decrease of ethanol yield for mixed feedstocks of winter cereal cover crop plus the stover occurred with the single harvest system compared to the double harvest system. This is likely due to over-winter field loss of some of the stover fraction in the mixed feedstock. The one-harvest system yielded significantly higher ethanol with the incorporation of an inter-seeded winter annual cereal crop compared to without.

Crude protein (MT ha^{-1} ; Figure 5.4) was greatest in the two harvest system when a winter annual cereal crop was inter-seeded with the corn stover. For the single harvest systems, the inclusion of the winter cereal yielded significantly higher crude protein than a stover-only treatment ($P < 0.05$). Stover-only feedstock had decreased crude protein content when the stover was over-wintered in the field and harvested in the spring. Total digestible nutrients (MT ha^{-1} ; Figure 5.5) is an estimate of the feedstocks livestock feed value and includes protein, digestibility and energy components. A two harvest system which included inter-seeded winter cereals contained the greatest amount of TDN on a land-area basis. Stover feedstock harvested in the fall contained greater TDN on a land-area basis than spring-harvested feedstock consisting primarily of winter cereal biomass due to a relatively lower harvest yield. A single harvest of winter cereal plus corn stover feedstock also yield greater TDN content on a land-area basis than a winter cereal-only fraction.

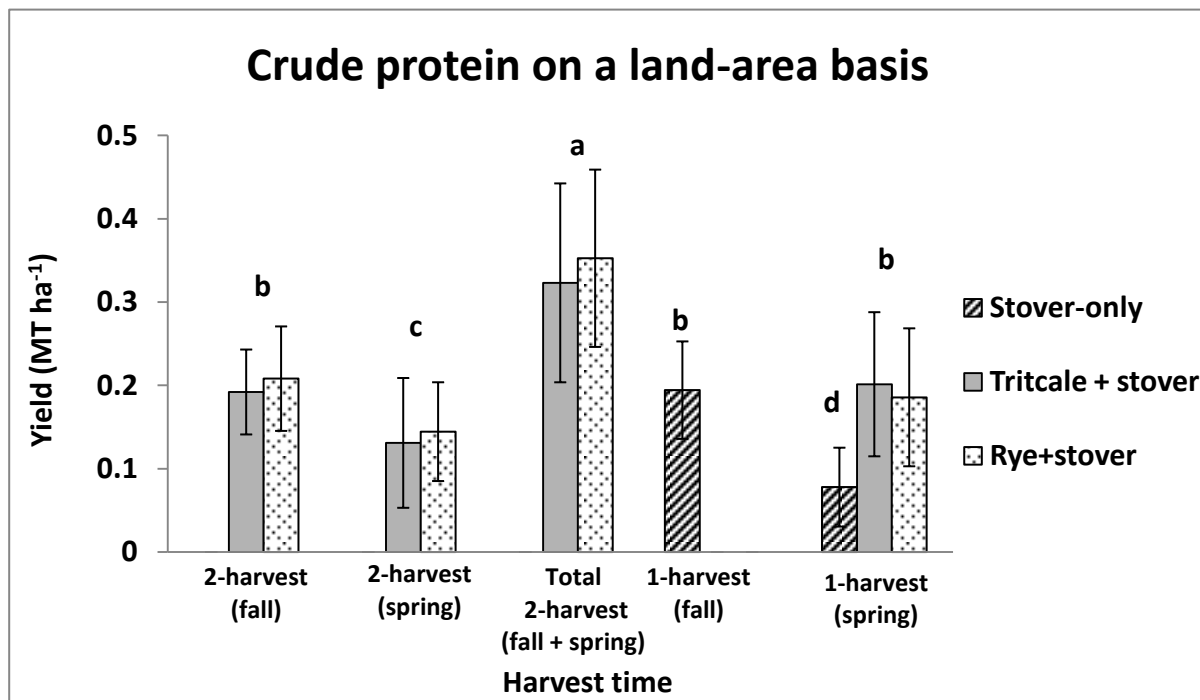


Figure 5.4 Crude protein on a land basis (MT ha^{-1}) for two-harvest system of fall followed by spring harvest or single harvest system (spring or fall only). Data are averaged across year and location. ^{abc}Means with unlike letters differ, $\alpha=0.05$.

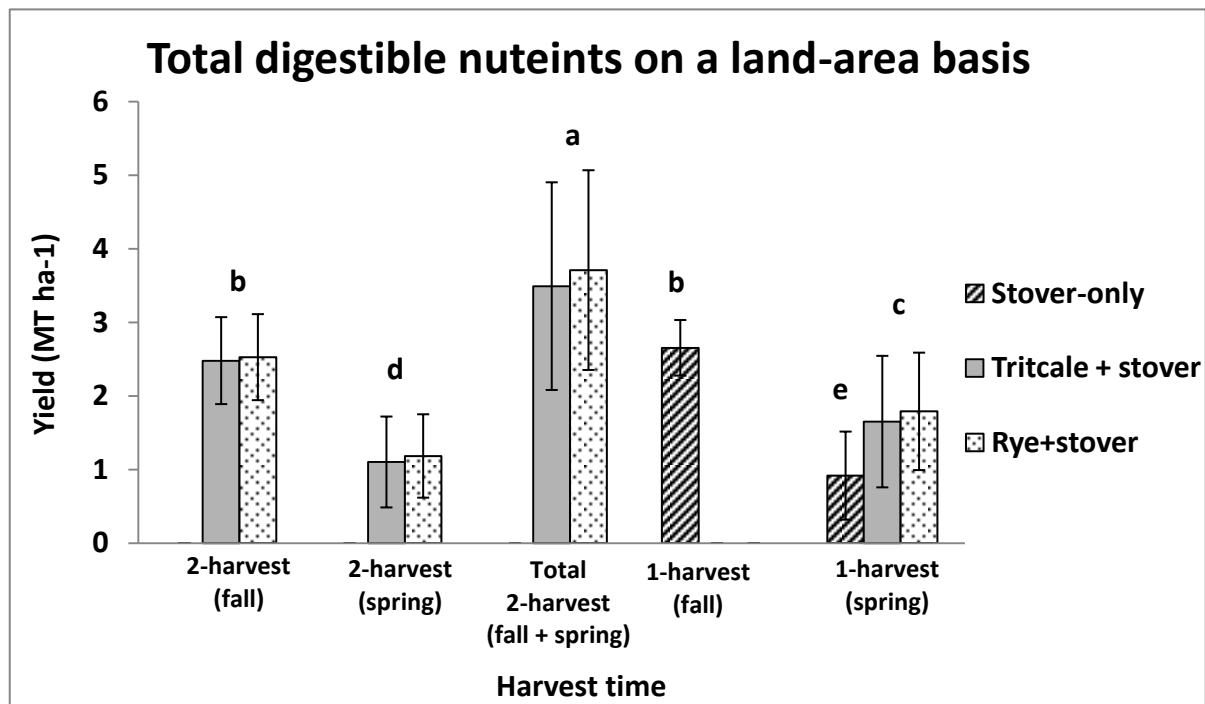


Figure 5.5 Total digestible nutrients on a land basis (MT ha^{-1}) for two-harvest system of fall followed by spring harvest or single harvest system (spring or fall only). Data are averaged across year and location. ^{abc}Means with unlike letters differ, $\alpha=0.05$.

Conclusions

Regardless of harvest system (two-harvest fall followed by spring vs. single spring), on a land-area basis, interseeding a winter annual cereal with corn stover always resulted in a biomass feedstock having greater dry matter and ethanol yield, and higher crude protein and energy content than the stover-only feedstock. The two-harvest system of a fall stover harvest followed by a subsequent spring cereal cover crop harvest, had greater dry matter, ethanol, crude protein and energy content compared with the spring one-harvest system. On a land-area basis, biomass feedstock harvested from the stover-only field plots was lower in dry matter and ethanol yield, metabolic energy, and crude protein content when the stover was over-wintered in the field and harvested in the spring compared to the fall. However, on a concentration basis (g g^{-1}), spring-harvested corn stover was higher in glucose, xylose and ethanol yield than fall-harvested stover. Although the nutrient composition of triticale was significantly greater than any other treatment, biomass yield was similar to rye. Overall, the addition of an inter-seeded winter annual cereal with corn stover increased the quantity and quality of the harvested biomass feedstock on a land-area basis.

Chapter 6

SUMMARY AND CONCLUSIONS

The objective of this research was to develop guidelines for harvest of corn stover as a biomass feedstock for the livestock or bioenergy industries. To offset the loss of carbon when stover is harvested, a cover crop can be integrated into the system. This addition of an inter-seeded winter annual cereal with corn stover increased the quantity and quality of the harvested biomass feedstock on a land-area basis. Optimal harvest time of stover- only was found to be in the fall due to a significant harvestable yield reduction, decreasing nutrient content and lower ethanol yield on a land-area basis when harvest was delayed to spring. If harvesting a mixed feedstock, the two-harvest system of a fall harvest followed by a subsequent spring harvest, had greater dry matter, ethanol, crude protein and energy content than the spring one-harvest system.

Corn stover baled for the bioenergy feedstock industry should contain minimal moisture and ash to optimize ethanol yield and dry matter recovery. There was no perceived advantage to storing bales with moisture content of 34% indoors since the nutrient and dry matter contents were similar over time in storage. Higher moisture bales became very difficult to move after 120 days in storage due to a loss of structural integrity. Corn stover bales with the intended purpose of livestock feedstock should contain less than 34% moisture or be stored indoors to maintain dry matter and nutrient content. Uncovered higher moisture bales maintained nutrient and dry matter through 120 days, making it still feasible to store bales of 45% moisture outdoors for a limited amount of time. Overall bales with lower moisture performed better regardless of intended use or storage method.

Corn stover is a viable alternative forage for cattle and could be used as a 10% or 20% portion of the diet with minimal effect of weight gain or carcass qualities.

Future Work

The storage study conducted does give great insight into the results of storing corn stover, but exhibited several confounding effects and would have benefited from a multi-year replication. A storage study evaluating nutritive value and ethanol parameters of a mixed forage, winter cereal cover crop and corn stover, would need to be conducted to evaluate the feasibility of use. Corn stover and the cereal cover crops had very different moisture content causing a possible storage complication. Research into the impact of incorporating and harvesting the cover crop on biodiversity and soil carbon should also be assessed. A long term study would be necessary to be able to determine if there is an advantage or disadvantage of incorporating and harvesting a cereal cover crop with corn stover harvest on biodiversity and soil carbon. Having an economic impact study on the value of interseeding corn stover with a cereal cover crop would be beneficial. An economic comparison of one-harvest vs two-harvest system would also be beneficial.

Currently, silage is harvested as a feedstuff for cattle production. This gives farmers the option of an earlier harvest with less passes compared to a corn grain harvest with a subsequent corn stover harvest. If technology was developed to produce ethanol from silage, producers would have another market option. Incorporation of a cover crop may also be feasible in a one pass silage system.

Cost and benefit analysis for all of the conducted studies would be imperative to determine what the best management options are. This could include a budget sheet allowing producers to enter the quality or quantity of the stover and determine best storage type, harvest and use in the market. Costs that should be outlined include, but are not limited to, the cost to process the stover bales for feeding, storage of bales, harvest and transportation.

APPENDICES

Appendix A

Chapter 1 Probability Tables

Table A.1 Probabilities for main effects and interactions for ethanol production storage study							
Effect	DM	Glucose	Xylose	EtOH	Ash	DM recov	EtOH loss
Cover	0.95	0.50	0.72	0.71	0.01	<0.01	0.02
Moisture	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
cover*moisture	0.02	0.11	0.04	0.05	0.01	<0.01	<0.01
Time	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
cover*time	<0.01	0.12	0.84	0.27	0.34	0.01	0.10
moisture*time	0.59	<0.01	<0.01	<0.01	0.13	0.13	<0.01
cover*moisture*time	0.58	0.99	0.99	0.99	0.07	<0.01	<0.01
Dry matter (DM), dry matter recovery (DM recov) and loss of recoverable ethanol (EtOH loss)							

Table A.2 DM recovery over time of storage					
	0	30	120	365	Prob.
HM C	100±7.7	73±5.5	75±5.5	71±5.5	0.21
HM NC	100±7.7	100±5.5	100±5.5	66±5.5	<0.01
LM C	100±2.3	96±1.6	92±1.6	80±1.6	<0.01
LM NC	100±2.3	98±1.6	87±1.6	85±1.6	<0.01
High moisture under cover (HM C), high moisture uncovered (HM NC), low moisture under cover (LM C) and low moisture uncovered (LM NC)					

Table A.3 Ethanol recovery by loss over time of storage					
	0	30	120	365	Prob.
HM C	0±5.7	-23±4	-28±4.4	-51±4	<0.01
HM NC	0±5.7	0±4	-11±4	-52±4	<0.01
LM C	0±5.7	-3.6±4	-18±4	-31±4	<0.01
LM NC	0±5.7	-7.6±4	-19±4	-27±4	<0.01
High moisture under cover (HM C), high moisture uncovered (HM NC), low moisture under cover (LM C) and low moisture uncovered (LM NC)					

Table A.4 Change in ash content over time of storage					
	0	30	120	240	360
HM C	5.7±1.18	7.7±1.22	6.2±0.68	12.0±1.95	10.4±1.02
HM NC	6.2±0.87	6.6±0.45	5.5±0.51	7.5±0.71	6.5±0.31
LM C	4.3±0.29	4.6±0.15	4.5±0.21	4.8±0.16	5.1±0.21
LM NC	4.1±0.20	4.7±0.22	4.2±0.09	4.5±0.10	6.4±0.33
High moisture under cover (HM C), high moisture uncovered (HM NC), low moisture under cover (LM C) and low moisture uncovered (LM NC)					

Table A.5 Change in ethanol (gg^{-1}) yield over time of storage					
	0	30	120	240	365
HM C	0.180±0.006	0.179±0.004	0.158±0.005	0.149±0.005	0.116±0.004
HM NC	0.186±0.004	0.180±0.003	0.159±0.003	0.155±0.003	0.118±0.003
OLM C	0.193±0.003	0.183±0.002	0.174±0.002	0.155±0.002	0.161±0.002
LM NC	0.187±0.005	0.177±0.003	0.167±0.003	0.159±0.003	0.155±0.003
High moisture under cover (HM C), high moisture uncovered (HM NC), low moisture under cover (LM C) and low moisture uncovered (LM NC)					

APPENDIX B

Chapter 2 Probability Tables

Table B.1 Probabilities for main effects and interactions for the nutritive value storage study.									
variable	DM	DM wt	OM	CP	CF	ADF	TDN	NE_m	Ash
cover	<0.01	0.87	<0.01	0.06	0.37	0.37	0.34	0.36	0.36
Moist	<0.01	0.38	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cover* moist	<0.01	0.48	0.02	0.61	0.46	0.46	0.45	0.44	0.44
Time	<0.01	0.03	<0.01	<0.01	0.07	0.07	0.07	0.05	0.04
Cover* time	<0.01	0.44	<0.01	0.47	0.58	0.58	0.60	0.60	0.60
Moist* time	<0.01	0.70	<0.01	0.28	<0.01	<0.01	<0.01	<0.01	<0.01
Cover* moist* time	0.01	0.057	<0.01	0.76	0.13	0.13	0.15	0.14	0.14
Dry matter (DM), dry matter weight (DM Wt), organic matter (OM), crude fiber (CF), acid detergent fiber(ADF), total digestible nutrient (TDN), net energy for maintenance (NE _m)									

Table B.2 Definitions of nutrient values used for cover crop and corn stover.	
Nutrient component	Definition
Dry matter:	Part of a feed which is not water
Crude Protein:	Total amount of protein present including true protein and non-protein nitrogen
Crude fiber:	Amount of hard-to-digest carbohydrates
Acid detergent fiber:	Most accurate determinant of forage digestible dry matter and digestible energy.
Total digestible nutrients:	Energy value of the feedstuff with only digestive losses considered
Net energy gain (NE_g):	Evaluate or predict performance of rations fed to non-lactating ruminants
Net energy maintenance (NE_m):	Evaluate or predict performance of rations to non-lactating ruminants
Ash:	Mineral matter of a feed

Table B.3 Probabilities for main effects and interactions for the mineral content storage study.										
variable	Ash	P	Ca	K	Mg	Na	Cu	Fe	Zn	Mn
cover	0.19	0.91	0.40	0.07	0.02	0.23	0.19	0.16	0.39	0.15
Moisture	<0.01	0.90	0.39	<0.01	<0.01	0.99	<0.01	<0.01	<0.01	<0.01
Cover*moist	0.72	0.54	0.40	0.04	0.71	0.98	0.73	0.82	0.52	0.96
Time	0.02	<0.01	0.50	0.02	<0.01	0.70	<0.01	0.03	0.01	0.01
Cover*time	0.54	0.94	0.50	0.30	0.22	0.68	0.41	0.59	0.89	0.81
Moist*time	0.05	0.02	0.50	0.05	0.02	0.39	<0.01	0.03	0.04	0.43
Cover*moist*time	0.09	0.61	0.50	0.31	<0.01	0.37	0.30	0.18	0.53	0.83
Phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn)										

Table B.4 Probabilities for main effects and interactions of the bale study for recovery of nutrients									
Variable	OM	DM	CP	CF	ADF	TDN	NE_m	NE_g	Ash
Cover	0.06	0.59	0.57	0.50	0.49	0.45	0.27	0.13	0.32
Moist	<0.01	<0.01	0.52	<0.01	<0.01	<0.01	<0.01	<0.01	0.05
Cover*moist	0.09	0.52	0.79	0.89	0.89	0.13	0.08	0.05	0.15
Time	<0.01	<0.01	<0.01	0.01	0.01	0.07	0.04	0.10	0.24
Cover*time	<0.01	0.33	0.89	0.19	0.19	0.79	0.87	0.88	0.46
Moist*time	0.13	0.05	0.76	0.99	0.99	0.51	0.37	0.26	0.30
Cover*moist*time	<0.01	0.03	0.11	0.31	0.31	0.03	0.04	0.05	0.17
Organic matter (OM), dry matter (DM), crude protein (CP), acid detergent fiber (ADF), total digestible nutrients (TDN), net energy for maintenance (NE _m) and net energy for gain (NE _g)									

Table B.5.a Nutrient composition of control diet of feedstuffs and the total ration						
	Dry corn	HMC	Corn silage	Distiller's grain/ sol	Supplement	Total ration ¹
Dry matter, %	87±0.69	66±4.5	31±0.05	46±2.8	94±0.61	61±0.78
NE _g ² , Mcal/kg	1.30±0.07	1.50±0.01	0.99±0.04	1.29±0.015	0.0±0	1.27±0.017
	% of DM					
Crude protein	8.2±0.63	8.1±0.47	7.7±1.6	34±2.0	9.1±0.71	15.9±0.95
TDN	88.5±0.34	88±0.62	68.9±1.7	82.3±0.60	0.0±0	79.1±1.4
ADF	2.4±0.22	2.6±0.4	27.2±2.6	15.5±2.6	5.4±0.76	11.1±0.79
Crude fiber	1.9±0.18	2.1±0.32	21.8±2.1	12.4±2.1	4.3±0.61	8.9±0.63
Calcium	0.01±0	0.01±0.01	0.3±0.06	0.12±0.11	12.5±3.5	0.61±0.15
Phosphorus	0.3±0.01	0.3±0.05	0.31±0.05	1.03±0.15	0.52±0.08	0.53±0.06
Potassium	0.39±0.02	0.41±0.06	0.91±0.18	1.3±0.17	1.7±2.7	0.84±0.16
Magnesium	0.11±0.01	0.12±0.02	0.19±0.03	0.39±0.04	1.1±2	0.26±0.1
Sodium	0.01±0	0.01±0	0.01±0	0.36±0.1	3.2±0.2	0.25±0.03
	ppm					
Copper	2.14±1.1	1.86±1.3	7.0±1.7	8.9±1.1	319±25	18.1±2
Iron	17.4±5	35±14	210±143	135±95	542.6±98	114±37
Manganese	3.57±1.3	3.7±1.1	14.1±6.2	18.7±2.7	1043±106	53±5.4
Zinc	16.4±1.4	19.4±2.15	28.7±3.8	69±5.1	1142±110	81.8±4.6
¹ Total ration is the sum of all feedstuff proportion adjusted nutrient concentration						
² Net energy for gain						

Table B.5.b Nutrient composition of 10% stover diet of feedstuffs and the total ration							
	Dry corn	HMC	Corn silage	Distiller's grain/ sol	Supplement	Corn Stover	Total ration ¹
Dry matter, %	87 ±0.69	66±4.5	31±4.8	46±2.8	94±0.61	58±8.5	64±2.9
NE _g ² , Mcal/kg	1.30±0.7	1.50±0.01	0.99±0.04	1.29±0.015	0.0±0	0.41±0.04	1.16±0.19
	% of DM						
Crude protein	8.2±0.63	8.1±0.47	7.7±1.6	34±2	9.1±0.71	5.7±0.71	14.9±2.4
TDN	88±0.34	88±0.62	69±1.7	82.3±0.60	0.0±0	50±1.3	79.9±7.9
ADF	2.4±0.22	2.6±0.4	27.2±2.7	15.5±2.7	5.4±0.76	55.7±2	13.8±1.6
Crude fiber	1.9±0.18	2.1±0.32	21.8±2.1	12.4±2.1	4.3±0.61	44.6±1.6	11±1.3
Calcium	0.01±0	0.01±0.01	0.3±0.06	0.12±0.1	12.5±3.5	0.56±0.14	0.63±0.16
Phosphorus	0.3±0.01	0.30±0.05	0.31±0.05	1.0±0.15	0.52±0.08	0.13±0.05	0.49±0.09
Potassium	0.4±0.02	0.41±0.06	0.91±0.18	1.3±0.17	1.7±2.7	1.1±0.37	0.83±0.22
Magnesium	0.1±0.01	0.12±0.02	0.19±0.03	0.39±0.04	1.1±2.0	0.23±0.07	0.25±0.11
Sodium	0.01±0	0.01±0	0.01±0	0.36±0.1	3.2±0.16	0.01±0.01	0.24±0.04
	ppm						
Copper	2.1±1.1	1.86±1.3	7±1.7	8.8±1	319±25	9.7±3.7	18±2.1
Iron	17±5	35±14	210±143	135±95	542.6±98	1179±660	208±80.6
Manganese	3.6±1.3	3.7±1.1	14.1±6.2	18.7±2.7	1043±106	71.6±17	79.2±3.7
Zinc	16±1.4	19±2.1	28.7±3.8	69±5.1	1142.3±110	23.8±5.6	57.7±6.1
¹ Total ration is the sum of all feedstuff proportion adjusted nutrient concentration							
² Net energy for gain							

Table B.5.c Nutrient composition of 20% stover diet of feedstuffs and the total ration						
	Dry corn	HMC	Corn stover	Distiller's grain/ sol	Supplement	Total ration
Dry matter, %	87±0.7	66±4.4	58±8.5	46±2.8	94±0.6	67±3.5
NE _g , Mcal/kg	1.30±0.07	1.50±0.01	0.42±0.04	1.29±0.015	0.0±0	1.11±0.15
	% of DM					
Crude protein	8.2±0.63	8.1±0.5	5.7±0.7	34±2	9.1±0.7	14±2.8
TDN	88.5±0.34	88±0.6	50±1.3	82.3±0.60	0.0±0	79.5±9.2
ADF	2.4±0.21	2.6±0.4	56±2	15.5±2.7	5.3±0.76	17±1.9
Crude fiber	1.9±0.18	2.1±0.3	44.6±1.6	12.4±2.1	4.3±0.6	13.4±1.5
Calcium	0.01±0.004	0.01±0.009	0.56±0.14	0.12±0.1	12.4±3.5	0.7±0.17
Phosphorus	0.3±0.01	0.3±0.05	0.13±0.05	1±0.1	0.52±0.07	0.46±0.1
Potassium	0.4±0.02	0.4±0.06	1.1±0.4	1.3±0.17	1.7±2.7	0.7±0.17
Magnesium	0.1±0.006	0.1±0.01	0.23±0.07	0.39±0.04	1.1±2	0.25±0.1
Sodium	0.009±0.004	0.01±0.004	0.01±0.007	0.36±0.1	3.2±0.16	0.24±0.04
	ppm					
Copper	2.1±1.1	1.8±1.3	9.7±3.7	8.9±1.1	319±25	18.2±2.1
Iron	17.4±5	35±14	1179±660	135±95	542.6±98	304±131
Manganese	3.6±1.3	3.7±1.1	24±5.6	18.7±2.7	1043±106	63.5±7.3
Zinc	16.4±1.4	19±2	71.6±17	69±5	1142.3±110	78.5±3.3
¹ Total ration is the sum of all feedstuff proportion adjusted nutrient concentration						
² Net energy for gain						

Table B.6 Cattle performance and carcass characteristics probabilities for corn stover feeding trial									
Effect	Marbling	KPH	Dressing ¹	Shrunk ²	Grade	Liver abscess	Over 30 ³	ADG	InWt
Block	0.19	0.14	<0.01	<0.01	0.08	1.0	1.0	0.39	<0.01
trt	0.23	<0.01	0.89	0.89	0.72	0.99	1.0	0.39	0.85
Block*trt	0.03	0.03	0.015	0.015	0.96	1.0	1.0	0.02	0.73
Effect	Dressing wt	Shrunk Wt	Full ADG	Shrunk ADG	REA	BF	QGrade	Muscle	
Block	0.01	0.01	0.98	0.94	<0.01	0.53	0.24	0.77	
trt	0.35	0.35	0.41	0.41	0.55	0.45	0.34	0.43	
Block*trt	0.20	0.20	0.17	0.17	0.51	0.23	0.04	0.40	
Effect	Corn intake	NE _m	NE _g	DMI	G:F	OutWt	Full G:F	Shrunk G:F	
Block	0.045	0.58	0.58	0.12	0.012	<0.01	0.33	0.39	
trt	<0.01	<0.01	<0.01	<0.01	<0.01	0.14	0.01	0.02	
Kidney, pelvic and heart fat (KPH), calculated yield grade (grade), meat manutritry over 30 months (over 30), average daily gain (ADG), initial weight (Inwt), ribeye area (REA), backfat (BF), quality grade (QGrade), muscle (muscle grade)), net energy for maintenance (NE _m), net energy for gain (NE _g), dry matter intake (DMI), feed efficiency (G:F) and final weight (OutWt)									
¹ Carcass adjusted weight, ADG and gain/feed accounts for dressing percentage									
² Shrunk is adjusted weight, ADG and gain/feed to account for shrunk dressing percentage									

Table B.7 Cattle performance probabilities for corn stover feeding trial over time			
Effect	ADG	DMI	G:F
Block	0.38	<0.01	0.16
Trt	0.21	<0.01	<0.01
Period	<0.01	<0.01	<0.01
trt*Period	0.94	<0.01	0.63
Block*trt	0.69	<0.01	0.70
Average daily gain (ADG), dry matter intake (DMI) and feed efficiency (G:F)			

Table B.8 Change in organic matter content with time of storage					
	0	30	120	240	365
C HM	93.1±1.2	94.3±0.83	93.9±0.91	91.7±0.83	91±0.83
C LM	95.5±1.2	95±0.83	95.4±0.83	95.1±0.83	94.9±0.83
NC HM	92.9±1.2	91.4±0.83	95.3±0.83	89.9±0.83	92.6±0.83
NC LM	94.5±1.2	95±0.83	94.5±0.83	94.7±0.83	93.7±0.83
High moisture under cover (HM C), high moisture uncovered (HM NC), low moisture under cover (LM C) and low moisture uncovered (LM NC)					

Appendix C

Chapter 3 Probability Tables and PAR Results

Photosynthetic Active Radiation (PAR)

The PAR measurements were taken at both locations above the corn canopy (PAR_{above}) and on ground level (PAR_{below}). Readings were taken with the LP-80 AccuPAR

PAR/LAI Ceptometer made by Decagon

Devices, INC (Pullman, WA) and then

downloaded to Excel. Measurement

frequency was every other week from the end

of August to mid-October on days with

minimal cloud coverage starting after 11 am.

To determine the percentage of light that was

available to the cover crop PAR_{below} was

divided by PAR_{above} and multiplied by 100.

The percentage of light availability to

be utilized by the cover crop increased as

time went by ($p < 0.01$) with a significant

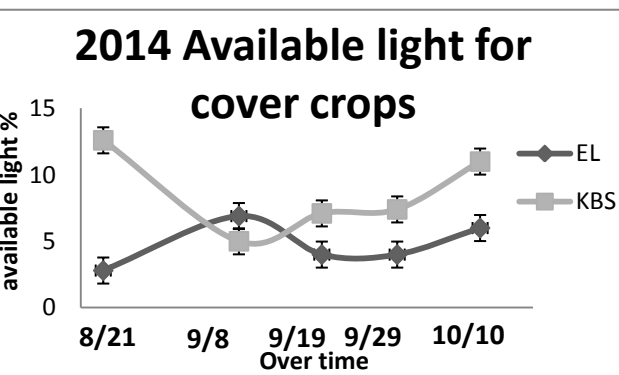


Figure C.1 Available light to cover crops over time for the 2014 growing season

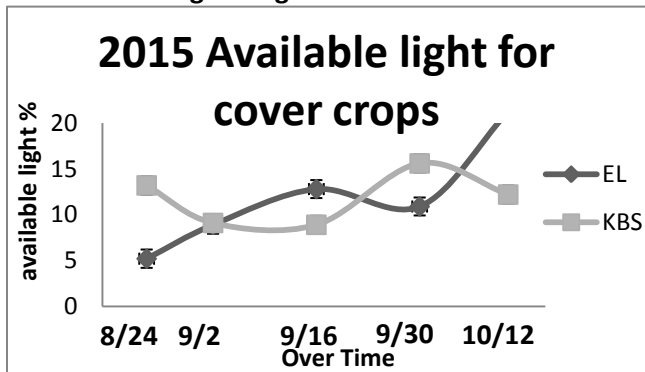


Figure C.2 Available light to cover crops over time for the 2015 growing season.

increase after 9/29/14 and 9/16/15. Light availability was affected by location and year

($p < 0.01$), making a seeding date for optimal germination unclear (Figure C.1, C.2). Treatment

did not have an effect on the light availability ($p = 0.86$). Average percentage of available light for

EL 2014, KBS 2014, EL 2015 and KBS 2015 were found to be 4.7%, 8.6%, 12% and 12%

respectfully. Year, location and period was also found to effect the light availability ($p < 0.01$) and is summarized in figures C.1 and C.2.

Table C.1 Photosynthetic active radiation (PAR) probabilities for the cover crop study	
Effect	Available light
year	<.0001
loc	0.0286
year*loc	0.0103
trt	0.8561
year*trt	0.5439
loc*trt	0.0411
year*loc*trt	0.6177
period	<.0001
year*period	<.0001
loc*period	<.0001
year*loc*period	<.0001
trt*period	0.0421
year*trt*period	0.0132
loc*trt*period	0.3091
year*loc*trt*period	0.3529

Table C.2 Hand harvest probabilities for the cover crop study				
	Pop	Grain Yield	Stover Yield	HI
Treatment	0.45	0.18	0.88	0.24
subplot	0.28	0.95	0.63	0.92
Trt*subplot	0.55	0.99	0.22	0.12
Yr	0.09	<0.01	0.09	<0.01
Trt*yr	0.75	0.29	0.06	0.23
Yr*subplot	1.0	0.24	0.63	0.54
Trt*yr*subplot	0.05	0.99	0.54	0.34
Population (pop) and harvest index (HI)				

Table C.3 Machine harvested yield on a DM basis MT ha⁻¹				
	Af	As	At	B
Stover-only	4.69±0.61	.	4.69±0.61	1.99±1.1
Tritcale + stover	4.54±1.1	1.63±1.1	6.18±2.0	3.18±1.7
Rye+stover	4.65±0.96	2.00±1.1	6.65±1.8	3.42±1.5
Fall harvest of two-harvest system (Af), spring harvest of two-harvest system (As), two-harvest system (At) and spring single-harvest system (B)				

Table C.4 Ethanol yield on land-area basis L ha⁻¹				
	Af	As	At	B
Stover-only	935.6±232	.	935.6±232	499.6±262
Tritcale + stover	930.5±343	309.4±208	1240±528	726.5±398
Rye+stover	937.4±316	372.9±191	1310±463	756.6±329
Fall harvest of two-harvest system (Af), spring harvest of two-harvest system (As), two-harvest system (At) and spring single-harvest system (B)				

Table C.5 Crude protein on a land-area basis MT ha⁻¹				
	Af	As	At	B
Stover-only	0.194±0.06	.	0.194±0.06	0.078±0.05
Tritcale + stover	0.192±0.05	0.131±0.08	0.323±0.12	0.201±0.09
Rye+stover	0.208±0.06	0.144±0.06	0.353±0.11	0.186±0.08
Fall harvest of two-harvest system (Af), spring harvest of two-harvest system (As), two-harvest system (At) and spring single-harvest system (B)				

Table C.6 Total digestible nutrients on a land-area basis MT ha⁻¹				
	Af	As	At	B
Stover-only	2.65±0.38	.	2.65±0.38	0.916±0.60
Tritcale + stover	2.48±0.59	1.10±0.62	3.49±1.41	1.65±0.89
Rye+stover	2.53±0.58	1.18±0.57	3.71±1.36	1.79±0.80
Fall harvest of two-harvest system (Af), spring harvest of two-harvest system (As), two-harvest system (At) and spring single-harvest system (B)				

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