USE OF CORN TREATED WITH VARIOUS APPLICATIONS OF FOLIAR FUNGICIDE TO INCREASE CORN SILAGE QUALITY AND PERFORMANCE OF HOLSTEIN COWS

BY

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THESIS

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ABSTRACT

With increasing feed prices and decreasing profit margins, livestock producers are constantly searching for ways to increase feed nutritive value and consequently improve production per unit of feedstuff. Research on the use of foliar fungicide has shown to increase corn grain yield in recent years. Fungicides can increase forage quality and by changing corn composition and nutritive value. However, little is known on what effects this may have on quality of corn ensiled and production when the silage is fed to cattle. Thus, the objectives of the present study were to explore the associations of corn plant foliar fungicide application on harvested whole plant corn silage quality, aerobic stability, digestibility, and cow performance. Treatments were control **(CON),** corn received no foliar fungicide application; treatment 1 **(1X),** corn received one application of pyraclostrobin foliar fungicide (**PYR**; Headline; BASF Corp. Florham Park, New Jersey) at corn stage V5; treatment 2 **(2X),** in which corn received 2 applications of foliar fungicides, PYR at corn stage V5, and a mixture of pyraclostrobin and metconazole (**PYR+MET**; Headline AMP; BASF Corp. Florham Park, New Jersey) at corn stage R1; and treatment 3 **(3X),** in which corn received 3 applications of foliar fungicides, PYR at corn stage V5, PYR+MET at corn stage R1, and PYR+MET at corn stage R3. Corn was harvested at the ¾ milk line and then ensiled for 7 mo. The first study utilized 64 Holstein cows with parity 2.53 ± 1.5, BW 653 ± 80 kg, and 161 ± 51 days in milk (DIM). Cows were blocked and randomly assigned to one of four treatments to be included in diet (35 % of the DM as corn silage). The trial was conducted in two consecutive periods each consisting of 1 wk for adaptation (covariate) followed by 5 wk of measurements where cows received assigned treatments. Body weight, BCS, and lame scores were assessed weekly. Milk production, and DMI were measured on a daily basis. Milk samples for milk composition analysis were collected during wk 5. Activity was measured using the Hobo

Pendant® G logger. Blood samples were taken on d 1 (covariate) and d 29 to assess blood metabolites. Corn silage was analyzed for nutrients and density weekly, and mold and yeast for each period. All data was analyzed using a MIXED procedure in SAS (v9.4 Institute Inc., Cary, NC). In the second study digestibility of corn silages were then estimated using an in situ procedure. Three rumen-cannulated lactating multiparous Holstein cows (376 ± 28 DIM) were used. Dried unground corn silage was put into 288 (3 per time points/treatment/cow) 10 × 20 cm bags and incubated for 8 different times (0, 2, 4, 8, 12, 48, 72, and 96 h). A sample of unground dried corn silage was also placed into 20 × 40 cm bag and incubated for 48 h. Corn silage treated with fungicide had more sugar concentration (1.2%) than CON (0.75%). Dry matter intake was 23.78, 22.95, 19.54, and 21.33 kg for CON, 1X, 2X and 3X, respectively. There was a linear tendency for DMI. Milk yield (34.5, 34.5, 34.2, 34.4 kg/d) and milk components did not differ among treatments. However, there were trends for increased FCM/DMI (1.65 *vs.* 1.47) and ECM/DMI (1.60 *vs.* 1.43) for cows fed corn silage with fungicide compared with CON. Serum glucose was lower for cows receiving treated corn silage with fungicide when compared with CON (51.1 *vs.* 63.4 mg/dL.). The digestible portion of DM was greater for all corn silage treated with fungicide when compared with CON (0.36, 0.42, 0.40, and 0.47 for CON, 1X, 2X, and 3X, respectively). There was a linear effect of treatment frequency on the proportion of DM digestibility. The two different sizes of Dacron bags used (10 × 20 vs 20 × 40 cm) for the in situ digestibility technique were different for DM, NDF, ADF, CP, and starch for 48 h digestibility. Larger bags had greater digestibility for DM (33 vs 35%), and lower digestibility for NDF (42 vs 35%), and ADF (47 vs 39%) than smaller bags. In conclusion, cows receiving corn silage treated with a foliar fungicide had better feed conversion than CON and corn silage that had fungicide application had higher in situ DM digestibility and a trend for a lower fractional rate of digestion

as well as linear effects for decreasing DM solubility, increasing DM digestibility, and a decreasing fractional rate of DM digestion. The aforementioned changes led to increased predicted and actual milk per ton of silage produced for 2X and 3X, and increases income over feed cost for corn silage treated with fungicide when compared with CON. Treatment 2X had higher income over feed cost than other treatments. Corn treated with foliar fungicide increased corn silage quality, and milk production efficiency and thus seems to be a valuable tool to increase profitability in dairy farms.

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Table of Contents

LIST OF ABBREVIATIONS

ADF	acid detergent fiber
BCS	body condition score
bu	bushel
BUN	blood urea nitrogen
BW	body weight
CP	crude protein
d	day(s)
DM	dry matter
DMI	dry matter intake
DON	15-acteyl deoxynicalenol
ECM	energy corrected milk
FCM	fat corrected milk
FS	fecal score
GA	general appearance
ha	hectare
h	hour(s)
kg	kilogram(s)
LS	lameness score
IVNDFD	*in vitro* neutral detergent fiber digestibility
m	meter
Mg	megagram
min	minute

MUN	milk urea nitrogen
N	nitrogen
NDF	neutral detergent fiber
NDFD	neutral detergent fiber digestibility
NEg	net energy growth
NE_l	net energy lactation
NEm	net energy maintenance
NFC	non-fibrous carbohydrate
OM	organic matter
OTA	ochratoxin
SCC	somatic cell count
TDN	total digestible nutrients
TM	total moisture
TMR	total mixed ration
Wk	week
ZEA	zearalenone

Chapter I:
Literature Review

A BREIF HISTORY OF CORN SILAGE UTILIZATION

Corn silage is a common feedstuff used in many different ruminant feeding systems. It was defined by Woolford (1984) "as a product formed when grass or other material of sufficient high moisture content liable to spoilage by aerobic microorganisms is stored anaerobically." Archeological studies have found that the process of ensiling dates back thousands of years, however, since the 1960's it has become increasingly popular as an animal feedstuff in North America due to the introduction of the forage harvester (Wilkinson et al., 2003). Alonso et al. (2013) estimated that in 2000 the United States produced (32.4 million Mg) 36 million tons of corn silage dry matter, and silage production from 9 other countries surveyed totaled (30 million Mg) 33 million tons of corn silage DM. The National Agricultural Statistics Service (NASS) estimated that in 2014, 6,371,000 acres of corn were harvested for silage, which is more than acres harvested in 2011, which was estimated at 5,567,0000 thousand acres. In 2014, total corn silage production was (116 million Mg) 128 million tons, and average as fed production was estimated at 20.1 tons/acre. Corn silage has been increasing in popularity in recent decades due to its ability to keep the nutritive value of a feedstuff over a long period of time such as a winter or dry season when less or no feed can be grown (Wilkinson et al., 2003), its high yielding nature (Allen et al., 2003), and because corn silage has higher digestibility and crude protein levels when compared with dry hay (Zimmer, 1980). Although some concerns exist regarding corn silage quality and use, one primary concern is the DM loss associated with ensiling of crops, which can be as high as 10 to 30% for good to poor managed silage, respectively (Allen et al., 2003). Other apprehensions pertain to the variation of corn silage, as silage from one bag or bunker may be from many different areas, and proper fermentation as well as feed quality are always major concerns (Allen et al., 2003). Much research has gone into exploring different hybrid qualities, inoculants to ensure proper ensiling, ways to increase digestibility and nutrient content, overall reduced dry matter loss, as well as ways to increase total animal production per ton of silage fed.

USE OF FUNGICIDE TO INCREASE PLANT QUALITY AND YIELD

Recent research regarding the effects of fungicide on plant yield and quality

In recent years the increasing cost of corn and milk production has caused an increase in the demand for higher efficiency and for better profitability in livestock and grain operations. These factors have influenced corn producers to more aggressively pursue methods of getting higher yields per hectare. For the purposes of this paper we will be focusing on the use of fungicide to improve plant quality, as well as improve yield. Bradley and Ames (2009) estimated that in 2007 out of 30.8 million hectares of corn planted, 4.3 to 5.6 million hectares were sprayed with a foliar fungicide, which represents 16% of the total amount planted. The use of foliar fungicides has been researched in order to examine their ability to efficiently increase corn yield for grain production in the presence of disease. From 2008 to 2012, the number of studies evaluating the use of fungicides for increasing corn yield had also increased dramatically from 6 trials in 2008 to 33 trials in 2012 (Wise and Mueller, 2011). From 39 trials which used a Quinone outside inhibitor 18 trials (46%) showed a significant yield increase. The mean response to the Quinone outside inhibitor when disease severity was less than 5% was 1.5 bu/acre; whereas, when disease severity was greater than 5%, mean yield increased 9.6 bu/acre (Wise and Mueller, 2011). The association of fungicide application and increased production may also be significant for corn silage. Laur

(2006) showed that a linear relationship between grain yield and total forage yield can be expected; therefore, increased corn grain yield may lead to increased corn silage yield. One study concluded that when disease favoring environmental conditions occurred, such as high relative humidity, there was a 15% decrease in disease severity for plants that received a pyraclostrobin foliar fungicide. However, there was no overall yield difference shown in this study (Bradley and Ames, 2009). A meta-analysis done by Paul et al. (2011) looked at studies from 2002 to 2009 that were conducted across 14 states in the U.S., and analyzed the use of four different fungicides or combinations of fungicides. This meta-analysis suggested that fungicide on corn is most profitable when disease levels and prices of corn were high. When looking only at the use of pyraclostrobin this study showed that mean grain yield was 255 kg/ha (4.5 bu/acre) higher for treated plots when compared with untreated plots. It was also concluded that for pyraclostrobin, disease severity significantly affected yield response, meaning that yield response was greater when the plants were challenged with disease. One important point to make about this particular study is that, although positive results were seen, the authors concluded that the increase in corn yield did not always make up for the cost of the fungicide application. The two most common reasons for producers to apply foliar fungicides in Illinois were susceptibility of the corn hybrid to diseases, as well as amount of disease pressure. Higher yield, marketing price of crop, and weather were not as important determinants of fungicide use (Bradley, 2012).

Even when disease is not present, fungicide is thought to have yield effects on corn (Köhle et al., 2002). As briefly mentioned before, pyraclostrobin is thought to cause changes in the plant on a physiological level that can help plant growth and grain production. One primary cause for increased grain fill (yield) is decreased leaf senescence of the upper canopy. Adee et al. (2005) showed a linear decrease in yield response to plant defoliation, and an 11% grain yield loss when leaves were dropped prior to silking. Byanukama et al. (2013) showed that area under green leaf incidence curve (incidence of green leaves in each plot, plotted against time of assessment) was significantly higher for treated corn when compared to untreated corn (average over four plots was 1922.4 vs 1339.7 leaves/day for treated vs, untreated corn). This can be due to decreased disease severity or, due to the sole effects of the foliar fungicide. Decreased ethylene production may have decreased the rate of leaf senescence, and leaf dropping (Venancio et al., 2009; and Byanukama et al., 2013). Ethylene is produced by the ACC synthase enzyme in response to ripening, leaf abscission, leaf lesions, oxidative stress, and environmental stressors. The ACC synthase enzyme reduces S-adenosyl-methionine to 1-aminocyclepropane-1-carboxylic acid, which is the first step in synthesis of ethylene. Köhle et al. (2002) showed a decrease in ACC synthase activity in corn plants treated with pyraclostrobin, which led to a decrease in leaf senescence in this study. Decreased ACC synthase activity may have been due to a decrease in oxidative stress associated with reactive oxygen species, as wheat plants treated with a foliar fungicide have been shown to have increased peroxidase activity which favored reactive oxygen species breakdown (Wu and von Tiedemann, 2001). A decrease in leaf dropping causes an increase in leaf area which gives the plant more photosynthetic area; this may allow the grain fill period to last longer for plants treated with pyraclostrobin (Venancio et al., 2009). Köhle et al. (2002) showed that 2 weeks after foliar fungicide application there was a 20% increase in plant biomass. There was also increased abcissic acid levels following fungicide application which can improve water utilization during stress, but may also be linked in an increase of leaf senescence (Grossmann et al., 1999).

It has also been hypothesized that the increase in grain yields must also be accompanied by an increase in nitrogen assimilation. Köhle et al. (2002) concluded that nitrate uptake was increased following a short delay post-pyraclostrobin application. The first step in the assimilation of nitrogen is nitrate to nitrite, and is thought to be the limiting step in the nitrogen assimilation reaction. This reaction is catalyzed by NADH-nitrate reductase, and is thought to be the target of strobilurin-based fungicides (Venancio et al., 2009). Spinach leaf discs that received an application of Kresoxim-methyl (another strobilurin fungicide) and were placed in a nitrogen solution had much higher nitrate concentration when compared with spinach leaves that did not receive an application of fungicide (Glaab and Kaiser, 1999). However, other studies have not seen a direct effect of pyraclostrobin on NADH-nitrate reductase in vitro. Increased nitrogen accumulation in the leaves was hypothesized to be caused by increased NADH-nitrate reductase activity during the overnight period when NADH is not usually active (Venancio et al., 2009). Wu and Von Tiedman (2001) reported a decrease in protein breakdown for plants treated with foliar fungicide, which may have increased the net nitrogen accumulation in the plant.

Mode of action

Fungicides are very broad in their mode of action in the plant, which include interruption of respiration, inhibition of germination (by interfering with growth of germ tubes), interference of the cell membrane, and other indirect modes of action. Two main active ingredients used in the common fungicide Headline AMP® act by modes of respiration inhibitors via the Quinone outside inhibitor (pyraclostrobin), and inhibition of sterol production (metconazole, Vanden Bossche, 1985; Leroux, 1996). Quinone inhibitors act by targeting the cytochrome III (bc) complex of the electron transport chain in mitochondrial respiration in fungi and plants, inhibiting ATP production. This inhibits germination and growth of the fungi. Quinone outside inhibitors are also hypothesized to increase water efficiency and nitrogen use efficiency in plants, as shown by research presented previously. Although pyraclostrobin may have many benefits, it is also a high risk for resistance. However, pyraclostrobin is often combined with metconazole which only carries a medium level risk for resistance (Mueller and Bradley, 2008). The mode of action for the metconazole fungicide, which is from the triazole class, is a demethylation inhibitor (DMI). These work to inhibit the production of sterols in the fungi, which are a necessary component of cell membranes. The demethylation inhibitors are also more systemic than many other fungicides which allow them to move within the stem and leaf (Mueller and Bradley, 2008). The metconazole fungicides are also very effective against *Fusarium spp*. which are a concern in regards to mycotoxin production (Jouany, 2007). Metconazole-based fungicides are used as both a treatment and a preventative.

Interaction with inoculants

Physiological plant changes may be associated with fungicide application, as well as decrease in fungal infestation. Studies have evaluated the effects of antifungal additives on the quality and aerobic stability of corn silage. One particular study evaluated the use of 7 different additives: *Lactobacillus buchneri* at the level of $4x10^5$ cfu/g, *L. buchneri* at the level of $1x10^5$, sodium metabisulfite and amylase, a buffered propionic acid base additive, sodium benzoate, and potassium sorbate. The last 2 treatments are common yeast and mold inhibitors. This study found that dry matter recovery was higher for silages treated with an antifungal additive. It was also found that along with *L. buchneri* at the level of $4x10^5$ cfu/g, silages treated with potassium sorbate and sodium benzoate had higher aerobic stability when measured as the time it took aerated silage temperature to rise 2°C.The antifungal agents had an aerobic stability of 149 h for potassium sorbate, 165 h for and sodium benzoate, and the control was 39 h (Kleinschmit et al., 2005).

A study by Queiroz et al. (2012) analyzed the effect of bacterial and fibrolytic enzymes on corn silage infected with three levels of rust, and analyzed the quality of forages infested with different levels of corn rust. All corn was treated with a fungicide; however, the study hypothesized that the fungicide application may have been uneven due to varying levels of rust. Corn was then categorized into sections of no rust, medium rust, and high rust and harvested in those categories. There was a linear effect for treatment DM, with amount of DM increasing as rust level increased. This study also found that there was lower 30-h neutral detergent fiber digestibility (NDFD) for corn with a high level of rust. There was also a linear effect for amount of rust with a relationship for decreasing NDFD as the amount of rust increased. Overall increased rust in the plants led to a lower quality forage and poorer fermentation qualities (Queiroz et al., 2012).

THE IMPORTANCE OF CORN SILAGE IN THE DAIRY INDUSTRY

Corn silage is used in many dairy rations due to its high fiber, and moderate energy content. It can bring many different components to a dairy herd ration. For example, it can be used as the only source of forage or can be combined with other forages. However, in all cases it must be supplemented with protein, minerals and vitamins, and possibly energy depending on the energy requirements of the cattle for which it is being used (Allen et al., 2003). A combination of grass silage and corn silage allowed cows to produce more milk than with grass silage or corn silage alone (O'Mara et al., 1998). Physically effective fiber is an essential component of all rations in order to maintain proper rumination; corn silage is an appealing feed that provides physically effective fiber, forage NDF, as well as an adequate amount of energy. A survey by Jordan and Fourdraine in 1993 showed that corn silage was the major forage fed to top producing herds across the United States (Wilkinson et al., 2003). Research has been done evaluating alternative fiber sources which are primarily by-product based. One study found that feeding up to 20% of wet corn gluten feed could reduce silage inclusion and potentially increase milk production (VanBaale et al., 2001). However, a different study found that milk production in response to wet corn gluten feed had a quadratic effect and was maximized when inclusion was 18.6% of diet DM (Schroeder, 2003). So, although some inclusion of corn silage may be replaced by a byproduct substitute, the inclusion is usually limited. There are also other limitations due to a large variation in feed quality which is common with byproduct feeds, as well as their lack of physically effective fiber, which limit their ability to replace corn silage or other forages in the diet.

Determining forage quality

The most important factor in determining the nutritive value, which effects total milk production of a specific corn silage, is the energy value (Allen et al., 2003). There are many different ways to measure the energy and nutritive value of a feedstuff, and over past decades much work has been done to evaluate the best way to estimate true feed digestibility. The most common procedures for estimating feed digestibility are rumen *in situ*, animal *in vivo*, laboratory *in vitro* estimates, as well as NRC calculations based on wet chemistry analysis of nutrient value of the feedstuff. In order for an *in vitro* analysis to be considered reliable it must be able to meet certain criteria including but not limited to, repeatability within and between labs, biological relevance, and it also must maintain an economical benefit to comparable methods (Nocek, 1988). *In vitro* methods have been used with varying enzymes for carbohydrates and proteins, as well as utilizing rumen fluid or a combination of both (Nocek, 1988).

Rumen *in situ* techniques have been used since the mid-1900's, and have been determined to be a good estimation of what may be occurring in the rumen; however, a low to moderate level of variance can occur based on a number of factors (Mehrez and Ørskov, 1977). One concern with ruminal *in situ* procedure is that the feed is not subject to all activities of the cow such as mastication, rumination and passage (Nocek, 1988). Nocek (1988) also expressed concern for variability in ruminal *in situ* technique due to bag porosity, particle size, sample size to bag surface area (SS:SA), diet, animal, pre-ruminal incubation, bag insertion, post ruminal washing, incubation times, and expression of results. Vanzant et al. (1998) reported standardization for ruminal *in situ* technique that outlined previous examination of other methods, and put guidelines for the above sources of variation that should be controlled against. One important factor outlined in the document is the SS:SA, which may alter disappearance. Studies have shown a decrease in disappearance as the SS:SA increases; therefore, maintaining a constant, and appropriate SS:SA is essential in order to be able to compare results with previous and future studies. The current recommendation for SS:SA is 10mg/cm^2; however, other studies have used a range of 10-20 mg/cm^2 and have found this SS:SA to be adequate (Vanzant et al., 1998). Recent studies have examined the use of bags larger than the commonly used 10 × 20-cm Dacron® forage bags, due to their ability to hold a larger and possibly more representative sample; however it is unknown what effects this may have on disappearance. One abstract from Ralph Guilherme da Silva Bruno (2000) found that when comparing digestion *in situ* between small (9 × 11 cm) and large (25 × 30 cm) bags that 24h degradability was less for large bags when compared to small bags. However, in this experiment SS:SA was not consistent between the bag size so is a confounding variable; therefore, inferences are limited. Rumen *in situ* technique may have other limitations such as its inability to take into account post ruminal fermentation.

In vivo technique allows for the estimation of post ruminal digestion, but can be costly and may involve post ruminal cannulation and thus is not widely used. One benefit to using energy calculations is that they do not contain other interactions that may be included in the other methods; however, they only take into account nutrient content, not nutrient digestibility (Kitessa et al., 1999). A new method was developed to try to estimate total tract digestibility, which involves collection of the feed offered, and fecal matter output. This method uses indigestible NDF as a marker to measure digestibility of feed ingredients, and concludes that this can be a marker to accurately predict total tract organic matter digestibility, which may provide a commercial method to analyze nutrient content and availability (Schalla et al., 2012).

Plant cell wall

Many factors affect corn silage nutrient content and digestibility. Fiber, or the cell wall portion of the plant cells (made up of hemicellulose, cellulose and lignin) comprises a major portion of corn silage (70%) and thus is a major contributor to corn silage quality. The amount of fiber present in the feed differs depending on the tissue of the plant it comes from. Because corn silage is processed from the whole plant, the amount of fiber can vary greatly and, if proper sampling techniques are not used, poor nutrient composition estimates may result. One possible concern with feeding high amounts of silage to high producing dairy cows is high NDF (hemicellulose, cellulose, and lignin) content, and low NDFD which may lead to decreased dry matter intake. Corn silage has been included in levels of 63% of DM in some dairy cattle diets (Weiss and Wyatt, 2000). Van Soest (1965) proposed that when forage NDF ranged from 55-60% it had little effect on DMI. However, Kendall et al. (2009) found that a four percentage unit decrease in NDF, going from 32% dietary NDF to 28% dietary NDF, increased feed intake (22 kg/d for high NDF compared 23 kg/d for low NDF diets), total milk production increased approximately 3 kg/d, milk fat increased 0.1 kg/d, and milk protein increased by 0.15 kg/d for cows fed the lower NDF diet. The NDFD is a measure of how digestible the NDF present in the forage will be in the rumen. This value is often found using *in vitro* laboratory techniques, NDFD is highly variable, and is not as important for determining energy content. It plays an important role in determining dry matter intake which can limit total energy intake and thus milk production

(Allen, 1993). One study found that when feeding a brown midrib variety of sorghum silage with higher NDFD (44.8% for normal *vs* 46.7% for BMR), the cows ate 5 kg/ day more DMI, and produced 6 kg/d more milk (Grant et al., 1995).

One factor affecting NDFD is lignin content, which is a phenolic compound considered to be indigestible by the animal microbial systems (Jung and Deetz, 1993). It has also been found that when researching varieties of corn bred for low lignin content, there is an increase of 5 kg/ day of milk and 9 kg/ day of DMI when compared to corn with a higher lignin content (Jung et al., 2011). Feeding lower levels of lignin also may increase production of VFA by rumen microbiota thus providing more energy for the cow (Oba and Allen, 1999). Allen et al. (2003) also found that lignification of NDF was closely correlated to *in vitro* NDFD (IVNDFD); IVNDFD decreasing as lignification increased. There are different types of lignin present in the cell wall, and some suggest that all lignins do not have the same impact on digestibility; however, lignin is often used as a direct indicator of NDF quality and digestibility (Jung and Allen, 1995).

Many factors can affect the content of lignin in a plant, such as environmental condition, forage hybrid (as discussed briefly above), and plant maturity. Lignin content can be influenced by plant stress as a response to drought, cold, or other disease such as fungal infestation. Lee et al. (2007) found that in white clover, drought stress does not decrease plant biomass, but can lead to an increase in overall lignification, by causing an increase in the enzymes responsible for lignification (primarily phenylalanine ammonia lyase). It has also been shown that cold and heat stress can cause an increase in phenolic compounds (Rivero et al., 2001). When looking at corn seedlings, it was found that infestation of the root by an endophyte caused increased plant rigidity, and increased the structural components of the plant; this may be due to the plant attempting to protect itself from further fungal infection (Yates et al., 1997).

Another primary component of the fiber portion of corn silage is ADF, which is comprised of lignin and cellulose portions of the cell wall, and is often used to predict energy and digestibility values (Allen et al., 2003). Allen et al. (2003) found that there was a correlation between ADF content and IVNDFD, with IVNDFD decreasing as the content of ADF increased. Acid detergent fiber has been found to be highly correlated with overall plant cell wall and fiber digestibility (Jung and Allen, 1995). Van Soest (1965) found a negative correlation of DMI and ADF content as well, therefore, ADF may not only affect digestibility of the cell wall but intake as well. Acid detergent fiber content will also usually increase as the plant matures (Buxton and O'Kiely, 2003). Using ADF as a measure of total corn silage digestibility is not recommended because it does not relate to the starch digestibility or fat content of the silage (Allen et al., 2003). One study found that corn kernels from corn infected with *Fusarium moniliforme* tended to have more overall fiber content when compared to non-infected corn; reasons for this difference were not discussed (Williams et al., 1992).

Plant cell contents

Non-fibrous carbohydrates (NFC) are another primary component of corn silage. These are predominantly composed of the plant cell contents, but also contain pectins which are usually found in the lamella between plant cells. Besides pectin, the NFC portion of plant carbohydrate contains organic acids, mono- and oligosaccharides, starches, and fructans (Hall et al., 1999). Starch is often the most talked about NFC in relation to corn silage, but all components play an important role in optimal ruminal digestion. The NFC portion of the plant is considered rapidly fermentable and can provide immediate energy for rumen microbiota. It has also been used replace fiber in lower fiber rations but, because of its rapidly degradable nature, this can cause an undesirable drop in pH (Mertens, 1997).

Starch accounts for a major energy source in corn silage, and content as well as digestibility can vary greatly between corn silages. Variability can stem from differences in processing, hybrids, maturity, as well as other environmental factors (Bal et al., 2000; and Allen et al., 2003) When harvested at a more advanced maturity, corn kernels become hardened and this can decrease overall energy content. It was found that when compared to corn harvested at the black-line stage, corn harvest at the ½ milk line stage resulted in more milk production, and better total tract digestibility, even though it had lower starch concentration (Harrison et al., 1996). Bal et al. (1997) reported 1 kg/d more milk when corn was harvested at 2/3 milk-line stage compared to early-dent stage. They also reported that total tract digestibility was higher for corn harvested before the black line stage which included early-dent, ¼ milk-line, and ¾ milk-line (Bal et al., 1997). It has been found that when the same corn hybrid was planted, the DM content differed over the course of 3 years, which shows the impact environmental variability may have on the plant (Johnson et al., 2002). Different hybrids also have differences in corn endosperm (vitreous *vs.* floury), which may affect ruminal digestion; however, this is less of a concern due to post ruminal compensatory digestion (Allen et al., 2003; and Oba and Allen, 2003). However, kernel processing does have a significant effect on starch digestibility and nutritive values (Johnson et al., 2002), and an increase in milk yield of 1 kg/day can result for correctly-processed silage compared to unprocessed silage (Bal et al., 2000). When corn kernels are not processed they may pass through the entire digestive tract without the starch content being digested. This may result in an increase in DMI due to the chemostatic regulators of intake; however, gut fill may limit the cow's ability to meet nutrient requirements (Allen et al., 2003). As mentioned previously, pyraclostrobin may increase the amount of grain present on the corn plant, which is directly related to starch content (Huntington, 1997). This may then increase the amount of gross energy available in the corn silage for use by the animal.

A competition for nutrients between the plant and the fungus can lead to a decrease in the NFC as well as the fat content of plants, which may decrease feed value for use as animal feed. Sugars provide a rapidly degradable energy source for the rumen microbes; however, these can also be readily used by the fungal colonies on infected corn plants. This may decrease the amount present in the corn silage, and decrease its energy content if these nutrients are selectively used by the fungus. These colonies may also use fat from the plant as an energy source as evidenced by a study done by Williams et al. (1992) which found that corn infected with a fungus had less crude fat content when compared to non-infected kernels; however, the infection did not have an effect on gross energy content of the corn. This could be due to higher protein content found in infected plants, which attribute value to the gross energy value. Weiss and Wyatt (2000) found that an increase of 3% of TDN% in a high-oil corn silage led to higher 3.5% FCM (23.9 *vs.* 22.6 kg/d) when fed to dairy cattle. Fat also accounts for 2.25 times more energy when compared to NDF or starch, and can influence digestibility and energy content (Allen et al., 2003). Therefore, a decrease in fat or sugar content due to fungal infection can have negative effects on plant nutritive value.

Protein is also an important component to consider when evaluating the nutritive value of feed. However, because it is not found in high levels in corn silage, protein content of silage is not often discussed in depth (Allen et al., 2003). As mentioned before, there is evidence that pyraclostrobin application may increase nitrogen assimilation, and therefore may increase protein content in the plant (Venancio et al., 2009). However, studies have found an increase in crude protein of corn kernels when infected with a fungus, this was thought to be due to the protein content associated with the fungus (Williams et al., 1992). Fungal pathogens may also use N for its own growth, and decrease the availability of nitrogen use for the plant. Very little research has been done on whether the nutrient composition of the plant changes in absence or presence of infection, but with pyraclostrobin application.

Effects and importance of ensiling corn

Ensiling corn is beneficial because it can start microbial breakdown of NDF which may make it more digestible for the cow. It can also allow a crop like whole corn to be fed year round. However, significant DM losses have been recorded for silage that has been improperly ensiled. Some things that contribute to DM loss in silage are DM at harvest, poor packing which allows air to penetrate into the silage, and air trapped in the process of packing the silo. These factors can lead to aerobic deterioration of the silage (Cherney and Cherney, 2003). There also must be an immediate drop in silage pH in order to prevent the growth of undesirable microorganisms, and help in conserving protein. One main goal of ensiling is to ensure that the nutritional value of the crop which leaves the silo is not negatively impacted by the ensiling process (Muck, 1988). Poorly ensiled forage may also cause growth of bacteria and molds which can decrease feed palatability, and cause an increase the presence of mycotoxins (Pahlow et al., 2003). There has also been work done on enzymes, and their use to start breaking down cell wall contents to increase the digestibility, and therefore the value of the silage while maintaining a stable product (Brown et al., 1993). Much concern has been placed in finding silage additives, and other possible field solutions to help maintain the quality of these forages so they can be used as an effective part of dairy rations. This is particularly important because of the highly dynamic and complex nature of corn silage which is developed around a microbial ecosystem

Negative effects of toxins on a feed and animal

Besides a possible corn yield increase and physiological plant changes associated with using a foliar fungicide, fungicide treated corn silage may have other advantages when being used as a feedstuff fed to cattle. Mycotoxins are produced by the secondary metabolism of the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*, and are low molecular weight substances (Keller et al., 2013). Visual observation is often done to assess the degree of fungal infection and to evaluate whether a pesticide is needed to control the infection; however, visual observation may not be adequate to estimate infection and contamination level. A study done by Eckard et al. (2011) reported that when corn was disease-scored in the field, few disease symptoms were seen. In a study of 1100 ears of corn that were disease scored, only 61 ears were infected, and of those only 43 ears were visibly infected on the surface. When looking at stalks only 1.7% showed signs of disease. These samples were then plated on agar medium formulated for mold growth, and this time 67% of all samples were found to be infected, and 25-75% of these infections were attributed to *Fusarium* species or spores from this genera. This means that even though there may not be visible symptoms of corn infection, the fungi can still be present, and toxins could be present in these feeds. Some common mycotoxins in corn silage are aflatoxin, deoxynivlenol, zearalenone (ZEA), T-2 toxins, fumonisin, and ochratoxin (OTA) (Allen et al., 2003).

Mycotoxin contamination is favored in situations of poor storage which include excessive moisture, dryness, condensation, heating, leaking, and insect infestation (Dos Santos et al., 2003). Alonso et al. (2013) found that fungal spoilage and mycotoxin contamination can lead to loss of nutrients, dry matter, palatability, and dry matter intake which can negatively affect animal performance. Scudamore and Livesy (1998) found that concentrations of fungi greater than 1 x 10^4 CFU/g^{-1} can cause respiratory problems, abnormal rumen fermentation, decreased rumen fermentation, decreased reproductive performance, kidney damage, and skin and eye irritation; although exact fungi species were not indicated in this statement. Mycotoxins are a major concern in today's dairy industry due to their possible impact on animal performance, and employee exposure to mycotoxins while working on the farm and thus mycotoxins pose a threat to the profitability and safety of dairy farms (Richard et al., 2007).

Common field fungi are *Fusarium graminearum, F. verticilloides,* and *Aspergillus flavus* (Alonso 2013). The *Fusarium spp*. are responsible for the production of fumonisin mycotoxins, which include commonly known mycotoxins such as deoxynivalenol, and HT-2, T-2, and zearalenone (Miller et al., 1983). A severe colonization and infestation of the plant can cause the plant to be unfit for human, and even possibly animal consumption, and may reduce nutritional value of the plant (Fandohan et al., 2004). The infection by *F. moniliforme* can be in the form of endophyte, and may cause a symptomless infection which may decrease plant quality for feed purposes (Yates et al., 1997). It was found that *F. moniliforme* may also be responsible for the production of fumonisin B1 (Mesterházy et al., 2012). Other *Fusarium spp*. can cause visible infection such as stalk rots, or ear rots which often result in quality loss as well as direct yield losses due to stock lodging (Mesterházy et al., 2012). However, in post fermented silage *F. verticilliodes* is the most common *Fusarium spp*. pathogen found (Keller et al., 2013). Fumonisin contamination in feed can lead to pulmonary edema in pigs, and esophageal cancer in humans; however, ruminants are more resistant to fumonisin contamination (Keller, 2003). The effects of ZEA and OTA include alteration of immune-mediated activities in bovines (Keller et al., 2003).

Much work has been done to examine if breeding can be used to create resistance in corn against the *Fusarium spp*.; however, because there are such a wide variety of species and toxins produced, it has been difficult to accomplish. It has also been a challenge to accomplish because infection is usually initiated by many different species; however, one species will tend to predominate. So, even if the corn is resistant against the major species, the smaller species may take over as the predominating fungi (Mesterházy et al., 2012). Breeding for resistance may not be possible; therefore, it may be necessary to resort to other alternatives such as a foliar fungicide.

Another common fungi that colonizes corn plants is *A. flavus* which is responsible for the production of aflatoxins. The spores of *A. flavus* can be spread through soil or insects, and damage to the corn plant by insects may increase the risk for infection by *A.flavus* (Diener et al., 1987, Windham et al., 1999). *A. flavus* develops pre-harvest, and it is thought to infect plants during mild temperatures (25.8-27.8 °C) when precipitation is below average, and where nitrogen may be limiting (Diener et al., 1987). *A. flavus* gains access to the kernel via the silks, and it is thought that when *A. flavus* infects the kernel, it remains only on the pericarp and does not infect the inside of the kernel, unless the kernel is physically damaged (Diener et al., 1987, Teller et al., 2012). Aflatoxins have been found to have potential carcinogenic effects, thus pose a threat to human and animal health if consumed. Aflatoxin was found to be carcinogenic and can be transferred to milk; therefore, the aflatoxin concentration in milk is regulated by the FDA. Acute aflatoxicosis is also a possible concern with aflatoxin contamination (Keller et al., 2003). However, there have been recent advances in technology which allow genetic resistance of the fungus, or inhibition of the aflatoxin through plant breeding (Brown et al., 1999).

Overall fungal contamination of feeds can lead to mycotoxin production, decreased palatability, decreased feed intake, may impair the rumen microbiota, and can cause negative health events in dairy cattle. This may also exacerbate the stress at which the animal under due to high milk demand which may decrease the overall efficiency of the animal (Alonso, 2013).

Effect of fungicide on corn silage production and quality

There has been limited published work on the effects of foliar treatment of corn crops with a fungicide when the crop was ensiled and fed to cows; however, some extension work has been done on this topic. A series of experiments were completed by University of Wisconsin extension and found that foliar fungicide application on corn silage significantly increased corn silage output by 0.7 tons DM per acre (1.85 metric tons/ha) compared to untreated corn silage. Fungicide use also led to a numerical increase in nutrients such as CP, and starch, while also significantly decreasing the amount of NDF and increasing its NDFD. There was also an estimated increase of 75lbs (34kg) of milk/ton of silage, and increase of 2,500lbs (1136 kg) of milk per acre of silage, this was calculated using the MILK 2006 system. The MILK 2006 system was developed by the University of Wisconsin in order to aid in determining the relative quality of a forage or feed based on energy value which is predicted from ADF, and potential intake using NDF and NDFD. These plants also showed less premature plant death as well as decreased signs of disease. A 2011 study from the University of Wisconsin extension analyzed the use of foliar fungicide at the R1 and V5 stages of crop growth and found that Headline AMP® when applied at the R1 stage had the highest yield for 1 of 3 counties tested, and had higher moisture in 2 of the 3 counties tested. The corn treated with Headline AMP® also had lower disease severity when compared to untreated corn silage in 1 of the 3 counties (Esker et al., 2012).

Another series of studies were done by University of Wisconsin along with University of Minnesota, and only published as extension articles. A study in 2013 found no significant difference in nutritive value, dry matter yield, milk per ton, or foliar disease scores at harvest for corn treated with various types of fungicide when compared to untreated corn silage. However, before this study another was done by the same parties in 2008 which evaluated fungicide use on two different hybrids. The fungicides evaluated were Headline®, Quilt®, and Stratego®. It was found that using Headline® on the DeKalb DJC57-79 lead to the highest DM yield/ acre at 10.9; however, the Pioneer P34A98 hybrid showed more NDFD overall. No significant difference for milk per ton was found; however, milk per acre was highest for the Dekalb hybrid when Headline® was applied, and for the Pioneer hybrid with no fungicide.

Feed conversion

Feed conversion is a calculation that is commonly used in the industry to help determine the value of a feed, as well as the efficiency of the animal consuming that feed. Corn silages comprise a major portion of many rations, and their ability to provide a fibrous yet energy dense feed stuff makes them an important factor in determining feed conversion (Penn State Extension, 2011). Feed conversion or feed efficiency is the ratio of milk produced to the amount of dry matter a cow is fed. Feed conversion helps determine how efficiently the cow and the rumen use nutrients to make milk and milk components, as well as how digestible that feed is to the cow (Beever and Doyle, 2007). It has been found that changes in digestibility of forages in a ration are directly related to feed efficiency. Beever and Doyle (2007) found that small changes in feed efficiency exhibited as an increase in milk production while maintaining the same level of intake, or by decreasing feed intake while maintaining milk production can greatly increase farm profitability, as a direct decrease in cost per kg of milk produced. This reinforces the need for proper management of corn silage, and the importance of finding ways to increase digestibility and nutritive value of corn silage.

CONCLUSION AND OBJECTIVES

The use of foliar fungicide, more specifically pyraclostrobin and metconazole based foliar fungicides may be beneficial for use in the dairy industry. Although little research has been done evaluating fungicide use for this purpose, findings from previous research highlight the benefits treated corn silage may bring to a feedstuff. These benefits include but are not limited to higher protein accretion, better water efficiency, more tons per acre, higher starch content, overall better nutritive value, and decreased foliar infection which may decrease mycotoxin load and increase cow health. Thus the objectives of the following studies were to examine the use of corn silage treated with foliar fungicide on:

1) Corn silage quality including nutrient content, packing density, and aerobic stability,
2) Cow milk production, efficiency, and health,
3) *In-situ* rumen digestion of the corn silage DM, NDF, ADF, starch, and CP, and
4) The economic benefit fungicide may have to aid in maintaining producer profitability.

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Chapter II: Effects of corn treated with various applications of foliar fungicide on corn silage quality and milk production of Holstein cows

INTRODUCTION

Corn silage is a major component of many dairy rations, and as prices of corn increase, more producers are assessing ways of getting higher corn yield per hectare. The use of foliar fungicides have been studied to examine their ability to efficiently increase corn yield for grain production, and the amount of research has grown in recent years (Wise and Mueller, 2011). A meta-analysis concluded that corn treated with a pyraclostrobin-based fungicide had a mean yield increase of 256 kg/ha when compared with a control crop (Paul et al., 2011). Research has also found that fungicides may cause changes on plant physiology which may be beneficial to plant quality (Venancio et al., 2009).

One of the primary factors affecting corn silage quality is NDF digestibility, as NDF makes up a major portion of corn silage (Allen et al., 2003). Forages with increased NDF digestibility have been shown to increase milk yield in Holstein cows by up to 3 kg/d (Kendall et al., 2009). Going from a low NDF digestibility to a high NDF digestibility can increase milk production by 1 kg/d in Holstein cows fed corn silage genetically modified to have lower lignin content. There has been research indicating that fungal contamination may cause an increase in lignification of the fiber in plants and, therefore, decreased NDF digestibility (Yates et al., 1997). Another primary concern is the availability and total content of starch in the corn silage (Teller et al., 2012). Bal et al. (2000) reported that processing whole-plant corn silage at with a kernel processing method compared with no kernel processing increased total-tract starch digestibility by 4%. This study also reported an increase in milk production of 1 kg/d when corn silage was subjected to kernel processing using rollers and fed to Holstein cows. Damage to ears while in the field can cause a decrease in total starch content and an increase in fumonisin content in corn used for silage which can decrease feed quality (Teller et al., 2012). In addition, damaged crop due to a fungal infection can increase defoliation and decrease the photosynthetic area on the plant. This has the potential to decrease grain yield and starch content (Ward et al., 1997, Adee et al., 2005). Infestation of fungal colonies can also decrease corn silage quality, and can pose a risk for the animals and people exposed to the feed (Dos Santos et al., 2003; and Richard et al., 2007). Some common mycotoxins in corn silage are alfatoxin, deoxynivlenol, zeoralonone (ZEA), T-2 toxins, fumonosin, and ochratoxin (OTA; Allen et al., 2003).

Corn silage is often a large percentage of the lactating cow diet, sometimes upward of 50%. Thus, foliar fungicide application on corn silage could impact milk production and farm overall profitability by increasing feed quality. Therefore, the objective of our study was to evaluate the effects of corn silage produced from corn treated with various fungicide applications and fed to lactating dairy cows on corn silage quality as well as milk production and milk composition.

MATERIALS AND METHODS

Corn

The corn hybrid used for this study was 'LG2636 VT3P RIB' (LG Seeds; Elmhurst, IL) which is a dual purpose hybrid used for either grain or silage. It is advertised as 114 d maturity, with high yield potential, strong stalks, and vigor. This hybrid also is advertised as having a high level of resistance against northern corn leaf blight (caused by the fungus *Exserohilum turcicum*), southern corn leaf blight (caused by the fungus *Bipolaris maydis*), and gray leaf spot (caused by the fungus *Cercospora zeae-maydis*). This hybrid also contains transgenic traits that provide protection against corn earworm (*Helicoverpa zea*). Treatments were randomly assigned to four 0.8-ha plots and all corn was planted on June 5th 2013. The distribution of the treatments was as follows: control (CON), corn received no foliar fungicide application; treatment 1 (1X), in which corn received one application of Pyraclostrobin (PYR) foliar fungicide (Headline; BASF Corp.) at a rate of 0.11 kg active ingredient (a.i)./ha at corn stage V5; treatment 2 (2X), in which corn received 2 applications of foliar fungicides, PYR at 0.11 kg a.i./ha at corn stage V5, and a mixture of PYR + Metconazole (MET; Headline AMP; BASF Corp.) at 0.11 + 0.04 kg a.i./ha at corn stage R1; and treatment 3 (3X), in which corn received 3 applications of foliar fungicide, PYR at 0.11 kg a.i./ha at corn stage V5, PYR + MET at 0.11 + 0.04 kg a.i./ha at corn stage R1, and PYR + MET at 0.11 + 0.04 kg a.i./ha at corn stage R3. The dates for fungicide application for first, second, and third applications were: July 7, July 26, and August 13, 2013.

Corn foliar disease severity was evaluated throughout the growing season. Plots were evaluated at corn silk-emergence (August 2) and kernel-milk stage (August 16). Ten plants were arbitrarily selected within each plot for each evaluation date. For each selected plant, disease severity (percentage of leaf area) was estimated for the ear leaf, the leaf above the ear leaf, and the leaf below the ear leaf. Fungicide applications were made with a 4430 Case IH ground sprayer and applied at 27.27 kg of pressure using 73-60-110 10 VS nozzle tips at a volume of 348.5 L per hectare. The sprayer was driven through all plots for each application timing, including those which did not recive a fungicide application, to account for any physical damage. For each plot, at least 3 samples of chopped corn were used in a composite sample to measure DM. Corn silage was cut at a dry matter of 33%, 30%, 30%, and 32.5% for CON, 1X, 2X, and 3X respectively. The corn silage was harvested and proccessed over a period of 2 d using a New Holland FP240 forage chopper. The processer was set for a ¾ inch theoretical length of chop, and a kernel processor was used in order to ensure mechanical processing of the corn kernel. It was then transported using an

H&P forage wagon. It was bagged with an AG Bagger, using the DM of the silage to adjust the setting on the bagger to ensure adequate and uniform processing of the corn silage. Corn silage was bagged in 2.74 meter diameter bags. It was then allowed to ferment for at least 200 d before opening. The trial finished 300 d post ensiling.

Animal

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee. Sixty-four Holstein cows with parity 2.53 ± 1.5, BW 653 + 80 kg, and 161 ± 51 DIM were randomly selected and assigned to one of four treatments in a randomized complete block-design. Cows were blocked into 16 blocks with regard to lactation number, previous lactation 305-d milk yield, and BCS to ensure that these variables had minimal chance of influencing the outcome variables of the study. The first wk of each period (week – 1) was used to measure baseline values (covariate) for all applicable parameters. The next four weeks (weeks 1 to 4) were considered an adjustment period to the new diet. The 5th wk was used for treatments inferences. The trial consisted of two periods, each containing 32 cows. Cows were housed in 2 barns. When the first period ended and new cows entered the trial, the treatments were moved into opposite barns to decrease the possible effect of barn.

A lactation diet (Table 2.1) was fed to all cows to supply 100% of the NRC (2001) requirements for energy and all nutrients so that only the effects of dietary treatment during the lactation period could be evaluated. All cows received 34.9% of dietary DM as corn silage. The diet was fed once per day at 1400 h. Cows were housed in tie stalls that met or exceeded space requirements as specified in the Ag Guide. Feed and water were provided at all times.

Temperature and humidity were monitored at 5-min intervals for all barns. Temperature humidity index was calculated using the following equation THI = (Dry bulb temperature ^{o}C) + (0.36 x dew point temperature ^{o}C) + 41.2. During the experimental period the average temperature was 17.6 ± 4.3°C.

Sample collection

Feed ingredients and TMR samples were obtained weekly and analyzed for DM content (AOAC, 1995) by drying for 24 h in a forced-air oven at 110°C. Dietary DM was adjusted weekly for changes in feed ingredient DM content. Total mixed ration samples were taken weekly and stored at – 20°C until submitted for analysis. Samples of TMR, refused TMR, and corn silage were sieved with the Penn State particle separator weekly for physical characteristic evaluation (Kononoff et al., 2003). Weekly TMR and corn silage samples were analyzed for contents of DM, CP, soluble protein, ADF, NDF, lignin, starch, fat, ash, NFC, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S using wet chemistry methods (Rock River Lab, Watertown, WI, Schalla et al., 2012). Values for relative feed value, TDN, NE_l, NE_m, NE_g, ME, and DE were provided by the lab, and calculations were based on NRC (2001). Additionally, corn silage was analyzed for mycotoxins (15-acteyl deoxynicalenol, 3-acteyl deoxynicalenol, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, Citrinin, deoxyscirepenol, diacetoxyscirpenol, fumonisin B1, fumonisin B2, fumonisin B3, fusarenon X, HT-2 neosolaniol, nivalenol, ochratoxin A, T-2 toxin, zearalenone), fermentation compounds (lactic acid, acetic acid, propionic acid, butyric acid, succinic acid, formic acid, ethanol, and ammonia N), and 30 h NDF digestibility (Rock River Lab, Watertown, WI). On d 4 of the 5th wk composite fecal and TMR samples were taken for measurement of apparent digestibility of organic matter, crude protein, fat, starch, and NDF (Schalla et al., 2012). Refusals from each cow were measured and DMI was recorded daily. Corn silage was sampled for DM 2X per wk and inclusion was adjusted weekly based on DM content.

Cows were milked 3 times per day at 0600, 1400, and 2100 h. Milk weights were recorded daily and samples were obtained from the 3 consecutive milkings on d 22 and d 24 of the last wk. Samples were composited in proportion to milk yield at each sampling and preserved (800 Broad Spectrum Microtabs II; D&F Control Systems, Inc., San Ramon, CA). Composite milk samples were analyzed for fat, protein, lactose, urea N , total solid and SCC using mid-infrared procedures (AOAC, 1995) at a commercial laboratory (Dairy Lab Services, Dubuque, IA).

Fecal samples were taken once per week, individual and composite samples were taken for each treatment. Fecal score (FS) and general appearance (GA) were recorded daily, fecal score was classified as follows: 1 = runny: liquid consistency, splatters on impact, spreads readily; 2 = loose: may pile slightly and spreads and splatters moderately on impact and setting; 3 = soft: firm but not hard, piles but spreads slightly on impact and settling; 4 = dry: hard, dry appearance, original form not distorted on impact and settling. Cows with FS ≤ 2 were classified as experiencing transient digestive problems (DIGESTIVE); whereas, cows with FS > 2 were classified as healthy (HEALTHY). For GA the scores were: 1: Bright and alert; 2: Depressed; 3: Reluctant to rise. Cows with GA ≥ 2 were classified as sick (SICK); whereas, cows with GA < 2 were classified as healthy (HEALTHY).

Body weight was measured and BCS was assigned in quarter-unit increments (Ferguson et al., 1994) for each cow weekly. More than one individual assigned BCS independently at each time of scoring throughout the experiment. Lameness Score (LS) was also assigned to all cows once per week according to a visual locomotion scoring system with a 5 point scoring system where 1= normal, 2= slightly irregular/asymmetrical gait, 3= favoring of one or more limb, 4=

severely lame, 5=extremely lame (Bicalho et al., 2007). The animal considered lame if the score was ≥ 2.

Blood was sampled from the tail vein or artery on wks 1 and 5 of each period from each cow 2 hours post feeding. Samples were centrifuged and frozen at – 20°C within 2 h of blood collection. Commercially available kits were used to analyze the samples for BUN and glucose. Blood urea nitrogen was measured using the QuantiChrom™ Urea Assay kit (BioAssay Systems, Hayward, CA) and glucose was measured using the glucose autokit (Wako Diagnostics, Richmond, VA).

Activity was monitored using the HOBO pendant® G logger (Hobo Pendant G Acceleration Data Logger, Onset Computer Corp., Pocasset, MA) attached laterally to the distal left hind leg. The activity monitor measured standing and lying behavior as validated by Ledgerwood et al. (2010). The activity logger was attached to the leg using vet wrap. Loggers were set to record at a 60 s interval. Data collected were then used to calculate lying time, bout, and duration of each bout, as well as standing time, bouts, and duration of each bout.

Corn silage aerobic stability was measured using temperature data loggers (Maxim Integrated, San Jose, Ca) set to record the temperature at a 5-min logging interval. Data loggers were placed into buckets with 500 g of a representative sample from each treatment. Corn silage samples were taken immediately prior to feeding and were aerated before they was placed into the buckets.. Three loggers were placed in each bucket, and buckets were kept at ambient daily temperature. One logger was placed outside the bucket to monitor environmental temperature and used as covariate. This procedure was repeated four times (5 replicates per time), and each time the temperature was logged for 38 h.

Corn silage density was also measured 2 times per week throughout the experiment (10 wk). Density was measured using a forage probe attached to a drill (Dairy One, Ithaca, NY). Density was sampled from 5 areas of each treatment silo bag (upper left, upper right, center, lower left, and lower right). Samples were weighed and dried in order to obtain the DM and then density was calculated (kg/m^3).

Feed Conversion

Feed conversion calculations were completed for ECM (ECM/DMI**)**, FCM **(**FCM/DMI), and simple conversion (milk yield/ DMI.)

Statistical Analysis

The 5th wk was used for treatments inferences and daily measurements for DMI, milk yield, and milk components were reduced to weekly means before statistical analysis. Statistical analysis was performed using SAS (v9.4 Institute Inc., Cary, NC). Mixed models were created using the MIXED procedure in SAS to analyze DMI, BCS, BW, milk yield, milk components, conversion, blood metabolites and, TMR digestibility (CP, starch, organic matter, fat, NDF, and NDF fraction of potentially digestible NDF). The model contained the fixed effects of treatment, covariate and block, cow was the experimental unit, and was analyzed as a random variable. Week 1 was treated as a covariate and values for wk 5 were analyzed. Data for density was reduced to average by replicate within week by averaging the 5 core samples taken per treatment and replicate. This used the same model as explained above; however, it included the fixed effect of week, and its interaction with treatment. Treatment within week was treated as a repeated measure using the auto regressive 1 structure for covariance. Corn silage quality, and aerobic stability were also analyzed with the same model. Activity data was first categorized into standing duration, standing time, standing bouts, lying duration, lying time, and lying bouts using SAS. These parameters were then analyzed using the same mixed model as described above. Three predetermined orthogonal contrasts were used, one contrast compares CON with all (1X, 2X, or 3X) corn silage with fungicide application, the second contrast was to determine linear effects, and the third was to determine quadratic effects of the data. Both linear and quadratic contrasts included the control. These contrasts were done for all variables analyzed unless otherwise stated. Lameness scores were analyzed as a binomial distribution (lame and healthy) using the GLIMMIX procedure in SAS. Analyzed content of nutrients were analyzed using the GLM procedure of SAS with treatment being a fixed effect. Only the last week of TMR sample was analyzed in SAS.

The degrees of freedom method was Kenward-Rogers (Littell et al., 2002). Residual distribution was evaluated for normality and homoscedasticity; extreme outliers were deleted for feed conversion (n=2), BCS (n=1), ECM (n=2) and FCM (n=2) conversion, mold (n=1), acetic acid (n=1), lying bouts (n=2), and standing bouts (n=2). The data for SCC, yeast, mold and BUN were log transformed in order to meet criteria for normality and homoscedasticity.

RESULTS

Symptoms of foliar disease were not observed when measured prior to each fungicide application. Corn yield for the whole plant was 61.12 Mg/ha for CON, 59.70 12 Mg/ha for 1X, 63.99 12 Mg/ha for 2X, and 61.22 12 Mg/ha for 3X. Results of disease severity were negative (symptoms not present) for all plants from all treatments at both time points, indicating that no disease was present or extremely low presence of disease (below lower limit of detection).

Nutrient Composition of Basal Diet

Analyzed nutrients from the last week of the experimental period show that the four treatments did not differ ($P > 0.05$). Physical characteristics of the basal diet as sieved by the Penn State particle separator is presented in Table 2.2. The mean particle size of TMR for each treatment was 4595, 5009, 5039, and 5275 µm for CON, 1X, 2X, and 3X, respectively.

Health and fecal scores

Cows had only minor incidence of illness with cows being scored a number other than 3 only 17 times (from a total of 4480 observations, 0.4%) over the duration of the trial with one cow who was treated for fever with unknown cause accounting for 12 counts. The mean GA was 1.03, 1.00, 1.22, and 1.12 for CON, 1X, 2X, and 3X, respectively ($P = 0.40$). Fecal scores were not different between treatments (Mean= 2.91, 2.96, 2.72, and 2.86 for CON, 1X, 2X, and 3X, respectively; $P = 0.58$). Lameness score was not different among treatments (Mean= 1.32, 1.29, 1.19, and 1.15 for CON, 1X, 2X, and 3X, respectively; $P = 0.18$).

Intake, BW, and BCS

No difference in BW ($P = 0.50$) or BCS ($P = 0.87$; Table 2.3) were observed. A tendency for cows consuming corn silage treated with fungicide to consume less than those consuming CON was observed ($P = 0.08$). In addition, a treatment linear effect for DMI was observed, with DMI decreasing as foliar fungicide applications increase ($P = 0.002$; Table 2.3).

Milk Production and Conversion

Milk yield was similar for CON when compared with all cows receiving corn silage treated with fungicide (P = 0.90; Table 2.3). No quadratic or linear effects for milk yield (P >0.86), 3.5% fat corrected milk (FCM; P > 0.69), and energy corrected milk (ECM; P > 0.67) were observed among treatments.

Milk composition did not differ between CON and TRT (P > 0.13). A tendency for a treatment linear effect was observed for SCC (P = 0.09), where, numerically, SCC gets smaller when going from CON to 3X.

All cows fed corn silage treated with fungicide tended (P = *0.09*) to have higher FCM conversion and, ECM conversion than CON. It is seen in Table 2.4 that cows fed corn silage treated with fungicide tended to have higher ECM conversion than CON (P = 0.08). Cows fed treatment 3X tended to have higher simple conversion than those fed CON (P = 0.08) as seen in Table 2.3. A linear effect of treatment for was observed for 3.5% FCM conversion, ECM conversion and simple feed conversion (P < 0.03).

Total Tract Digestibility

Total tract digestibility did not differ between CON and TRT (P > 0.44) between CON and corn silage treated with fungicide for any of the analyzed nutrients that included organic matter, NDF, NDFD% of potentially digestible NDF, starch, crude protein, and fat. There were no linear or quadratic treatment effects as well. These data are presented in Table 2.4.

Blood Metabolites

Blood glucose concentrations for CON, 1X, 2X, and 3X were 63.39, 48.60, 49.17, and

55.53 mg/dL, respectively. All cows fed treated silage had lower glucose than CON ($P = 0.001$). A quadratic treatment effect for blood glucose concentration was observed ($P = 0.0001$; Table 2.3). No differences in blood urea nitrogen were observed ($P > 0.52$).

Activity

No differences were seen between cows in CON and cows fed corn silage treated with fungicide for standing time, standing duration, standing bouts, lying time, and lying bouts. Cows fed the treated silage tended to have a shorter duration of time spent lying when compared to control ($P = 0.10$). No linear or quadratic effects of treatment were detected for any activity variables ($P > 0.12$). These data are in Table 2.5.

Corn Silage Quality

Neutral detergent fiber was lower ($P = 0.05$) in all corn silage treated with fungicide when compared with control ($P = 0.05$). A tendency for a treatment linear effect was observed for NDF% in the corn silage ($P = 0.06$). Sugar content was lower for CON when compared with treated silage ($P < 0.01$). A linear effect of treatment on sugar content was detected, with content increasing as treatments increased ($P = 0.001$). Corn silage treated with fungicide had lower ADF content than CON ($P = 0.008$). A linear effect of treatment for ADF was found ($P = 0.015$). A treatment linear effect for fat content was observed with amount of fat increasing as number of applications increased ($P = 0.013$). A quadratic effect of soluble crude protein was observed with the mid-level treatments having lower soluble CP than the highest and lowest frequency of fungicide applications ($P = 0.0001$). National Research Council energy calculations were numerically higher for treated corn silage; however, there was no significant difference ($P > 0.11$). A linear effect of all the energy calculations was observed, with increasing energy values as number of fungicide applications increased (*P* < 0.04). Overall, ash was lower in corn silage treated with fungicide when compared with CON (*P* = 0.01). A treatment linear effect for percentage of ash in corn silage was observed (*P = 0.01*). Contrasts for all other nutrients and minerals can be seen in Table 2.6.

Fermentation

Content of fermentation compounds in the diet as well as pH were no different among treatments (*P* > 0.39) for pH, and lactic acid. Acetic acid was lower in corn silage treated with fungicide when compared with CON (*P* = 0.02). A quadratic effect of treatment on acetic acid was observed with CON and 3X having the highest content (*P* = 0.0001). There tended to be a linear and quadratic effects of treatment on ethanol (*P* = 0.06). Ammonia N content for CON, 1X, 2X, and 3X was 0.08%, 0.07%, 0.06% and 0.09% respectively (*P* = 0.0001). There was a quadratic effect of treatment on ammonia N content (*P* = 0.0001). Butyric acid, propionic acid, succinic acid, and formic acid were not found in detectable levels in the silage.

The analysis of yeasts and mold showed that there was no difference in the mold and yeast content of the corn silage (*P* > 0.20). A tendency for a treatment linear effect was observed for yeast with levels of yeast increasing as treatments increase (*P* = 0.09*,* Table 2.6)

Aflatoxin

Only 3 toxins were found present over the minimum detectable limits among treatments. In the first period 0.1 ppm of 15-acteyl deoxynicalenol was found in CON, deoxyscirepenol levels were 0.7, 0.6, 0.4, and 0.4 ppm for CON, 1X, 2X, and 3X, and 63.6 ppb of zearalenone was found in CON. In the second period 0.1ppm of 15- acteyl deoxynicalenol was detected in 1X, and 2X, deoxyscirepenol levels were 0.4, 1 , 0.9, and 0.4ppm for CON, 1X, 2X, and 3X, and zearalenone was 57 and 51.8 ppb for CON and 1X respectively.

Density

A week effect for density was observed (*P* = 0.003). No significant week by treatment interaction was detected (*P* = 0.99). The mean corn silage density was 187.2 for CON, 187.2 for 1X, 200.7 for 3X, and kg/m^3 for 3X these values and associated *P* values for main effects and treatments can be observed in Table 2.7. Figure 2.1 shows the density changes over the 10-wk trial.

Aerobic Stability

Figure 2.2 shows the temperature of corn silage after beeing exposed to air. The average temperature for CON, 1X. 2X, and 3X were 32.8, 31.71, 31.62, and 28.84 °C, respectively. Temperature after 38 h for all corn silage treated with fugicide was lower (*P* = 0.001) than CON. A tendency for a treatment linear effect was observed (*P* = 0.06*;* Table 2.7). There was a significant day by treatment interaction (*P* = 0.001*;* Table 2.7)

DISCUSSION

Overall, a decrease in DMI was seen in cows fed treated corn silage; however, cows maintained their milk production in comparison with CON leading to an overall increase in conversion of production in cows fed corn silage treated with fungicide. The blood glucose concentration was lower for cows fed corn silage treated with fungicide when compared with CON perhaps due to decreased DMI. However, a study done by Holcomb et al. (2001) did not reported a difference in blood glucose concentration for cows fed free choice and restricted diets. Standing time, duration and bouts, or lying duration or bouts were not different among treatments; however, there was a trend for longer lying time for cows receiving the corn silage treated with fungicide. Due to a longer amount of time spent lying, while maintaining similar bout number, this may lead to increased time spent lying during each bout, which may be a more efficient use of time for the cow. It has also been reported that amount of time spent feeding, and amount of feeding bouts are correlated with animal health (Huzzey et al., 2007). Exact reasons for this increase in conversion are not known; however, possible hypotheses outlined below may better explain these findings.

One possible reason for why cows fed corn silage treated with fungicide may have had better conversion was the difference in amount of toxins present in the corn silages. The main toxins found were 15-acteyl deoxynicalenol (DON), deoxyscirepenol, and zearalenone (ZEA). These two are known to be often produced by the *Fusarium* species of fungi. Although no visual observation of fungus was noted in the corn in the field, a study done by Eckard et al. (2011) concluded that when corn was diagnosed visually only 1 – 3% of corn showed signs of infection on the surface; however, when the corn particles were plated it was found that the average *Fusarium* incidence was 46%. Corn harvested for this study could have been infected, even though visuals symptoms were not present. Fungal contamination of corn silage can lead to dry matter loss, nutrient loss, and reduced palatability (Alonso et al., 2013). It has also been reported that concentrations of $1x10^4$ CFU/g in forage can cause abnormal rumen fermentation (Scudamore and Livesey, 1998). This effect could be multiplied for cattle raised in intensive operations under any type of stress (Binder, 2006). The presence of these toxins in the silage, although not considered to be at a harmful level, could lead to poorer rumen fermentation leading to poorer nutrient composition and overall lower conversion. Even though the cows fed the treated silage had lower DMI this could be due in part to the cows being able to utilize energy better due to better rumen function and therefore eat less while still maintaining the same level of milk production

Another possible cause could be the higher aerobic stability of the treated corn silage. During the first hours all treatments maintained a temperature below that of the environmental temperature, which is desireble. However, with time, it seemed that corn silage treated with fungicide preserved its quality better than when compared with CON as indicated by the temperature (Figure 2.2). Upon futher analysis of the day by treatment interaction of aerobic stability, it seems that there is much more variablity of CON and 1X treatments, where as 2X and 3X seem to maintain a more consistent and lower temperature over the 38 h (Figure 2.2). It is also known that fungi are the main organisms responsible for heat spoiliage of corn silage, so lower content of fungi in the corn silage treated with fungicide could have contributed to higher aerobic stability (Williams et al., 1995). Other factors that can play a role in aerobic stability are temperature, pH, as well as O_2, and organic acid concentation (Williams et al., 1995). Treatment 3X had higher concentrations of acetic acid present in the silage which are a main factor in the inhibition of yeasts and molds when in aerobic conditions (Weinberg et al., 2011). In general, a greater total amount of volatile fatty acid present usually indicates a more aerobically stable silage (Muck et al., 1991). This inhibition could lead to a higher overall conversion.

Density is also a major contributor to aerobic stability and deterioration. The major purpose for a high packing density is to remove all the O_2 possible to prevent aerobic deterioration. If the packing is not done correctly aerobic deterioration can actually begin in the bag (Muck et al., 2003). In this study no difference was noticed in packing density in any of the silages. However as expected, the density was lower in the beginning and increased throughout the trial.

The final proposed basis for the differences in conversions is the difference in silage composition. There was higher fat and sugar concentration in corn silage treated with fungicide than CON. Fat accounts for nearly two times the net energy of digestible NDF or starch (Allen et al., 2003), so higher fat in the 3X silage may account for higher TDN. A high level of sugar in the forage could lead to *Clostridia* fermentation usually indicated by a high level of butyric acid in the fermentation products (McDonald and Henderson., 1991); however, there was almost no butyric acid present in any of the silage fermentation products for both treated and untreated corn silages. This could be in part due to the fact that *Clostridia* growth is most common in low DM forages which would be classified as less than 30% DM (Cherney and Cherney, 2003) and the corn silages tested in this trial were above 30% DM on average. Higher concentrations of ethanol in the 3X as a fermentation product may in part be due to the higher sugar content in the corn silage treated with fungicide, as ethanol is a main end product of sugar fermentation. Ethanol is not found to have negative effects on health or intake. Overall, the increase in sugar and fat may have contributed to a more nutrient dense, high quality feed.

Corn silage treated with fungicide had less NDF than control silage. It is widely known that there is high variation of NDF and NDFD in different corn silages. This can be due to population density, hybrid difference, or environmental growing conditions or stressors. In the present trial the aforementioned sources of variation were controlled (Allen et al., 2003). Neutral detergent fiber is often associated with the amount of DM that can be consumed this is due to the fact that it represents the total insoluble fiber matrix (Van Soest, 1994). Kendall et al. (2009) found that a four percentage unit decrease in NDF going from 32% dietary NDF to 28% dietary NDF increased feed intake (22 kg/d for high NDF compared 23 kg/d for low NDF diets) and milk production increased approximately 3 kg/d, milk fat increased 0.1 kg/d, and milk protein increased by 0.15 kg/d for cows fed the lower NDF diet. However, DIM for cows in the aforementioned study was 38 ± 15, so the potential to increase intake was much greater. There was no difference in 30-h NDFD between treated silages and CON, so even though the treated silage had less NDF, the NDF had approximately the same digestibility potential for all four corn silages. There was also no difference in total tract digestibility of NDF among treatments. The lignin content of the silage was also not different between corn silages. Lignin can be an indicator of cell wall and NDF digestibility (Van Soest, 1994). This could be a potential reason that 30-h NDFD or total tract NDFD was not significantly different between all tested corn silages. Acid detergent fiber also tended to be lower in silages treated with fungicide; however, this provides no indication of potential digestibility or intake (Van Soest et al., 1991).

All of these factors could be possible contributors to the overall higher TDN found in 3X when compared to CON. Along with the increasing linear effect of TDN there was also an increasing linear effect on NE_L, NE_G, and NE_M. Although net energy for corn silage may be hard to calculate, and values may not be as consistent, a higher TDN found in the corn silage treated with fungicide may lead to higher conversion if fed to cattle. Although affected by other factors, energy intake can often help in determining overall energy performance (Allen et al., 2003). Weiss and Wyatt (2000) found that an increase of 3% TDN in a high oil corn silage led to higher 3.5% FCM (23.9 *vs*. 22.6 kg/d) when fed to dairy cattle at a 63% inclusion rate.

CONCLUSION

Corn treated with foliar fungicide had higher levels of sugar, and lower levels of ADF and NDF when compared to control. Treated silage also had higher aerobic stability. Cows receiving silage treated with foliar fungicide had lower DMI but similar milk production to cows receiving control which resulted in higher conversion in cows receiving corn silage treated with foliar fungicide. These cows also had lower serum blood glucose levels. Foliar fungicide may increase milk production conversion when fed to lactating dairy cows.

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TABLES AND FIGURES

Table 2.1. Ingredient composition of the lactation diet in DM basis fed to cows throughout the experiment.

Ingredient,	**% DM**
Alfalfa hay	6.90
Corn silage[1]	34.9
Alfalfa silage	6.09
Cottonseed	3.25
Wet Brewers grain	8.12
Dry ground corn grain	24.9
Soybean meal, 48%	2.59
Expeller soybean meal[2]	1.26
Soy hulls	4.87
Sodium Bicarbonate	0.81
Limestone	1.20
Dicalcium phosphate	0.29
Calcium Sulfate	0.13
Energy Booster 100®[3]	1.50
Biotin	0.38
Blood meal 85%	1.48
Urea	0.35
Potassium carbonate	0.30
Magnesium oxide	0.13
Salt (plain)	0.30
Mineral and Vitamin mix[4]	0.17

[1] All treatments fed at 34.9% corn silage DM
[2] SoyPlus®
[3] 98% total fatty acids and less than 2% unsaponifiable fat.
[4] Mineral and Vitamin mix was formulated with 5% Mg, 10% S, 7.5% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg/kg of Cu, 250 mg/kg of I, 40 mg/kg of Co, 150 mg/kg of Se, 2,200 kIU/kg of vitamin A, 660 kIU/kg of vitamin D_3, and 7,700 IU/kg of vitamin E.

Table 2.2. Means and standard deviations of the physical characteristics of TMR using the Penn State particle separator for corn silage treated with no foliar fungicide (**CON**), one application of foliar fungicide (**1X**), two applications of foliar fungicide (**2X**) or three applications of foliar fungicide (**3X**).

	Treatment[1]							
	CON		**1X**		**2X**		**3X**	
Pore Size (% in each pan)	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
19 mm	6.2	1.5	6.8	4.0	6.6	2.1	7.0	2.3
7.8 mm	40.1	4.0	41.9	4.1	41.4	2.0	43.5	2.1
1.2 mm	39.8	3.9	39.8	4.8	41.3	3.1	39.5	2.7
Pan	13.9	4.1	11.4	2.2	10.7	3.2	9.9	2.1
Mean particle size, µm	4595		5009		5039		5275	
Log_{10} Standard Deviation	0.48		0.47		0.46		0.46	

[1] Treatment = Treatments were control corn silage (CON, with no application of fungicide), 1X, (with 1 application), 2 (with 2 applications), and 3X (with 3 applications).

Table 2.3. Least squares means and associated standard errors for DMI**,** milk parameters response, and blood metabolites of cows in (**CON**), one application of foliar fungicide (**1X**), two applications of foliar fungicide (**2X**) or three applications of foliar fungicide (**3X**)..

	Treatment[1]					***P*-value**		
						Contrasts[2]		
	CON	**1X**	**2X**	**3X**	**SEM**	**CON vs TRT**	**Linear**	**Quad**
DMI, kg/d	23.78	22.95	19.54	21.33	0.77	0.08	0.003	0.11
BW, kg	661.4	687.5	672.4	694.7	16.9	0.23	0.26	0.91
BCS	3.02	3.03	3.07	3.07	0.50	0.56	0.43	0.93
Milk Yield								
Milk Yield kg/d	34.47	34.50	34.20	34.37	0.78	0.90	0.86	0.94
3.5% FCM	35.43	36.09	33.30	35.27	1.80	0.80	0.69	0.73
ECM	34.49	34.98	32.48	34.48	1.69	0.79	0.73	0.67
Milk Composition								
Fat, %	3.53	3.69	3.58	3.52	0.12	0.64	0.79	0.38
Fat, kg/d	1.24	1.29	1.17	1.24	0.69	0.95	0.69	0.92
Protein, %	2.88	2.89	2.91	2.92	0.06	0.64	0.79	0.38
Protein, kg/d	1.01	0.99	0.95	1.00	0.05	0.65	0.77	0.52
Lactose, %	4.67	4.66	4.70	4.77	0.67	0.63	0.26	0.53
Lactose, kg/d	1.66	1.63	1.58	1.65	0.09	0.69	0.92	0.84
MUN, mg/dL	16.13	14.88	14.99	15.33	0.64	0.15	0.52	0.42
SCC, log transformed	4.45	4.42	4.13	3.90	0.25	0.31	0.09	0.71
3.5% FCM/DMI, kg/kg	1.47	1.51	1.71	1.73	0.09	0.09	0.02	0.94
ECM/DMI, kg/kg	1.43	1.46	1.66	1.69	0.09	0.08	0.01	0.99
Milk/DMI, kg/kg	1.46	1.47	1.70	1.70	0.10	0.14	0.03	0.95
Blood Metabolites								

Table 2.3 (cont).

Urea Nitrogen, mg/dL	53.66	49.84	54.63	46.57	4.73	0.52	0.60	0.70
Glucose, mg/dL	63.39	48.60	49.17	55.53	2.06	<0.001	0.017	0.0002

[1] Treatment = Dietary treatments were control diet (CON, with no application of fungicide), 1X, (with 1 application), 2X (witl applications), and 3X (with 3 applications).

[2] Contrasts were Contrasts were CON vs TRT = no fungicide application (CON) with that of the average of the three treatment with fungicide application; Linear = linear treatment effect; and Quadratic = quadratic treatment effect

Table 2.4. Least squares means and associated standard errors for total tract digestibility of TMR nutrients for cows fed diets with corn silage treated with no foliar fungicide (**CON**), one application of foliar fungicide (**1X**), two applications of foliar fungicide (**2X**) or three applications of foliar fungicide (**3X**).

	Treatment[1]					*P-value*		
						Contrasts2		
	CON	1X	2X	3X	SEM	CON vs Trt	Linear	Quad
OMD,% of OM	54.69	56.02	46.79	45.28	5.56	0.47	0.24	0.81
NDFD,% of NDF	21.18	37.21	26,12	23.34	6.21	0.44	0.90	0.26
NDFD, % of potentially digestible NDF	43.73	67.73	48.35	47.51	11.13	0.52	0.90	0.34
StarchD, % of Starch	92.84	93.93	85.52	93.63	3.00	0.63	0.69	0.36
CPD,% of CP	43.91	45.09	39.94	50.81	5.79	0.85	0.60	0.47
FatD,% of Fat	46.06	41.18	36.46	48.46	6.08	0.62	0.95	0.27

[1]Treatment = Dietary treatments were control diet (CON, with no application of fungicide), 1X, (with 1 application), 2X (w 2 applications), and 3X (with 3 applications).

[2] Contrasts were Contrasts were CON vs TRT = no fungicide application (CON) with that of the average of the three treatme with fungicide application; Linear = linear treatment effect; and Quadratic = quadratic treatment effect

Table 2.5. Least squares means and associated standard error for standing and lying behavior for cows fed corn silage treated with no foliar fungicide (**CON**), one application of foliar fungicide (**1X**), two applications of foliar fungicide (**2X**) or three applications of foliar fungicide (**3X**).

	Treatment[1]					*P*-value		
						Contrasts[2]		
	CON	1X	2X	3X	SEM	CON vs TRT	Linear	Quadratic
Standing time, min	712.48	698.8	731.9	812.3	39.4	0.53	0.17	0.35
Standing duration, min	65.5	61.6	63.2	81.7	7.1	0.73	0.17	0.19
Standing bouts, min	11.0	12.2	13.6	11.0	1.1	0.44	0.81	0.15
Laying time, min	738.0	730.8	699.9	647.8	38.5	0.38	0.12	0.62
Laying duration, min	62.4	51.6	55.4	55.7	3.7	0.10	0.39	0.19
Laying bouts, min	12.9	15.0	14.0	11.8	1.3	0.66	0.50	0.15

[1] Treatment = Treatments were control corn silage (CON, with no application of fungicide), 1X, (with 1 application), 2X (with 2 applications), and 3X (with 3 applications).
[2] Contrasts were CON vs TRT = no fungicide application (CON) with that of the average of the three treatments with fungicide application; Linear = linear effect of treatment frequency; and Quadratic = quadratic effect of treatment frequency

Table 2.6. Least squares means and associated standard errors for nutrient composition, fermentation profile, energy content, and microbial count of corn silage treated with no applications of foliar fungicide (**CON**), one application of foliar fungicide (**1X**), two applications of foliar fungicide (**2X**) or three applications of foliar fungicide (**3X**).

	Treatment[1]					*P*-value		
						Contrasts[2]		
	CON	1X	2X	3X	SEM	CON vs TRT	Linear	Quad
Corn silage composition								
TM[3],% of DM	69.37	69.02	68.59	69.63	0.42	0.56	0.85	0.11
CP,% of DM	8.61	8.72	8.50	8.98	0.12	0.41	0.12	0.13
NDF,% of DM	47.32	45.88	45.41	45.28	0.75	0.05	0.06	0.39
NDFD, 30h	50.09	49.67	49.00	48.82	0.89	0.37	0.27	0.89
ADF,% of DM	29.24	27.72	28.11	27.33	0.46	0.008	0.015	0.43
Fat,% of DM	2.71	2.77	2.74	3.16	0.11	0.16	0.013	0.13
Lignin,% of DM	3.45	3.65	3.36	3.22	0.31	0.62	0.49	0.58
NDICP,% of CP	1.44	1.55	1.45	1.40	0.06	0.64	0.46	0.18
Soluble CP,% of CP	56.18	51.98	54.05	59.04	0.89	0.28	0.01	0.0001
ADICP,% of CP	0.73	0.66	0.70	0.71	0.07	0.64	0.98	0.57
Starch,% of DM	27.61	28.67	29.26	28.70	0.73	0.15	0.25	0.28
Sugar,% of DM	0.72	1.14	1.23	1.25	0.08	0.0001	0.0001	0.02
Ash,% of DM	5.11	4.92	4.79	4.76	0.10	0.01	0.01	0.39
Ca,% of DM	0.22	0.22	0.20	0.22	0.003	0.07	0.06	0.07
P,% of DM	0.19	0.19	0.18	0.19	0.002	0.008	0.15	0.003
Mg,% of DM	0.14	0.13	0.13	0.14	0.003	0.002	0.017	0.008
K,% of DM	1.34	1.27	1.21	1.24	0.02	0.0001	0.0001	0.01
S,% of DM	0.10	0.10	0.09	0.10	0.002	0.03	0.02	0.18

Table 2.6. (Cont.)

NRC 2001 energy calculations								
TDN	65.55	65.92	66.54	67.37	0.62	0.15	0.04	0.71
NE_L	0.65	0.65	0.66	0.67	0.005	0.11	0.02	0.61
NE_G	0.44	0.45	0.46	0.47	0.01	0.15	0.04	0.70
NE_M	0.72	0.72	0.73	0.75	0.01	0.15	0.04	0.72
Fermentation products								
pH	4.88	4.99	4.99	4.84	0.08	0.53	0.69	0.10
Lactic acid, % of DM	4.91	4.25	4.63	4.65	0.45	0.45	0.84	0.46
Acetic acid, % of DM	2.97	2.47	1.31	3.47	0.20	0.02	0.69	0.0001
Ethanol, % of DM	0.20	0.21	0.16	0.32	0.04	0.59	0.09	0.06
Ammonia N, % of DM	0.08	0.07	0.06	0.09	0.005	0.28	0.24	0.0001
Microbial count								
Yeast, CFU/g % of AF	2663	1741	50595	5153	1.96	0.19	0.09	0.18
Mold, CFU/g % of AF	92800	14300	51541	72300	58359	0.66	0.55	0.82

1 Treatment = Dietary treatments were control diet (CON, with no application of fungicide), 1X, (with 1 application), 2X (with 2 applications), and 3X (with 3 applications).

2 Contrasts were CON vs TRT = no fungicide application (CON) with that of the average of the three treatments with fungicide application; Linear = linear effect of treatment frequency; and Quadratic = quadratic effect of treatment frequency

3 Total Moisture.

Table 2.7. Least squares means and associated standard errors for density and aerobic stability for corn silages treated with no applications of foliar fungicide (**CON**), one application of foliar fungicide (**1X**), two applications of foliar fungicide (**2X**) or three applications of foliar fungicide (**3X**).

	Treatment[1]					***P*-value**		
						Contrasts[2]		
	CON	**1X**	**2X**	**3X**	**SEM**	**CON vs TRT**	**Linear**	**Quad**
Density, kg/m^3	187.2	187.2	200.7	197.0	7.8	0.42	0.23	0.86
Aerobic Stability (C°)[3 4]	32.85	31.71	31.62	28.84	0.42	0.001	0.06	0.20

[1] Treatment = Dietary treatments were control diet (CON, with no application of fungicide), 1X, (with 1 application), 2X (with 2 applications), and 3X (with 3 applications)

[2] Contrasts were CON vs TRT = no fungicide application (CON) with that of the average of the three treatments with fungicide application; Linear = linear effect of treatment frequency; and Quadratic = quadratic effect of treatment frequenc

[3] Environmental temperature was used as a covariate in the model

[4] Significant Day*trt effect observed

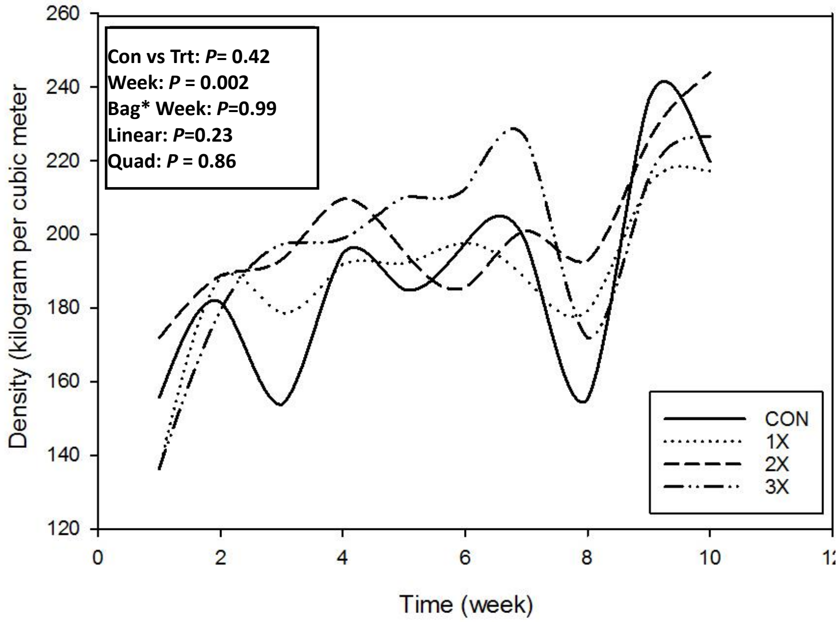

Figure 2.1. Changes in density for control corn silage treated with no applications of foliar fungicide (**CON**), one application of foliar fungicide (**1X**), two applications of foliar fungicide (**2X**) or three applications of foliar fungicide (**3X**).

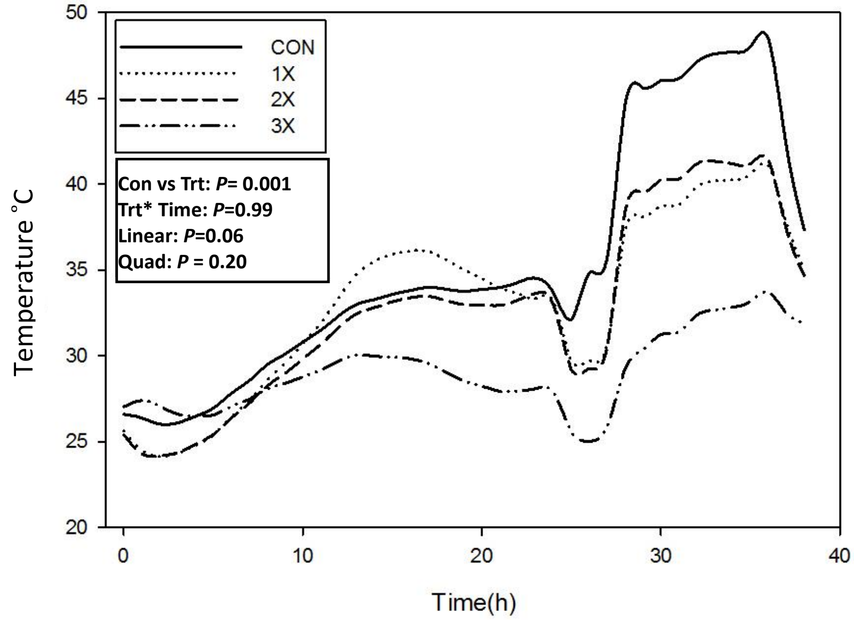

Figure 2.2. Changes in corn silage temperature over a 38 hours period post aeration. Treatments were corn treated with no application of foliar fungicide control (**CON**), one application of foliar fungicide (**1X**), two applications of foliar fungicide (**2X**) or three applications of foliar fungicide (**3X**).

Chapter III: Effects of corn treated with various applications of foliar fungicide on in situ corn silage digestibility in Holstein cows

INTRODUCTION

As prices of feed increase, particularly corn, and nutritional demands of milk production increase, it is important to evaluate potential ways of increasing feed efficiency in the dairy cow leading to increased profitability for dairy farmers. During the course of a growing season corn plants can be exposed to many environmental stressors including heat, cold, drought, and pathogens (Rivero et al., 2001). Plant stress may lead to decreased quality of the plant when used for animal feed, to decreased potential for digestibility, and altered digestibility characteristics (Fahey, 1994).

Fungal colonization on the corn plant causes a competition between the plant and the fungus for nutrients. The plant has many mechanisms (e.g., lignification and leaf shedding) to attempt to hinder the growth of the fungal infestation. These mechanisms may potentially decrease the digestibility of the plant. The fungal infestation itself may also change the chemical composition of the plant in the process of competing for nutrients (Venancio et al., 2009). Research also has examined the different effects of the foliar fungicide on the plant at a chemical level (Köhle et al., 2002). The class of foliar fungicides known as strobulins have been studied for their possible increase in the greening effect of plants as well as their ability to handle stressors (Venancio et al., 2009). The aforementioned physiological changes elicited by the foliar fungicide have been shown to increase yield in plants that are not infected with disease, which may cause a possible increase in digestibility and nutritive content of the plant when used as feed for animals (Wise and Mueller, 2011). This increase in quality can be due to decreased lignification, increased grain fill and starch content, and increased nitrate assimilation and consequently increased protein content (Yates et al., 1997, Venancio et al., 2009).

Digestion techniques are often used in evaluations of the nutritive value of corn silage (Nocek, 1988). The in vitro technique involves drying and fine grinding of whole plant corn silage for analysis. Grinding may minimize quality differences among whole plant corn silage samples related to physical form, such as grain hardness and particle size (Bal et al., 2000). A macro in situ technique using undried, unground whole plant corn silage has been used to evaluate ruminal nutrient disappearance (Doggett et al., 1998). This procedure may provide better estimates of differences in ruminal starch disappearance as it is more influenced by maturity and hybrid than standard in vitro procedures, but because of the occurrence of particle size reduction during eating and rumination it may tend to overestimate the magnitude of mechanical-processing effects on ruminal starch disappearance and likely underestimates ruminal NDF disappearance (Bal et al., 2000).

In situ studies have been used in ruminant animals for many decades to estimate the potential digestibility of feedstuffs, and to attempt to understand the complex interactions of the rumen ecology on feedstuffs. Much research has been conducted to create a standard procedure for rumen in situ techniques, which will not only allow research to be sufficiently compared across laboratories but provides the best estimate of what actually happens in the rumen and is most biologically relevant (Vanzant et al., 1998). It is well known that sample size to bag surface area ratio is very important to obtain correct results. From various studies it has been agreed that an appropriate sample size to surface area ratio is 10 to 30 mg/cm^2 (Varga and Hoover, 1983, Vanzant et al., 1998). The objectives of this study were 1) to evaluate in situ digestibility of corn plant treated at various times with foliar fungicide on corn harvested as whole plant silage for lactating Holstein cows and 2) to determine if there were differences in 48-h *in* situ digestibility between samples ruminally incubated in 10 × 20 cm bags when compared with samples in 20 × 40 cm bags.

MATERIALS AND METHODS

Treatments

Corn was grown for silage on fields owned by the University of Illinois located at 40.08 latitude, and – 88.22 longitude. The four silages evaluated in this experiment were: control **(CON),** corn received no foliar fungicide application; treatment 1 **(1X),** corn received one application of PYR foliar fungicide (Headline; BASF Corp. Florham Park, New Jersey) at a rate of 0.11 kg a.i./ha at corn stage V5; treatment 2 **(2X),** in which corn received 2 applications of foliar fungicides, PYR at 0.11 kg a.i./ha at corn stage V5, and a mixture of PRY and MET (Headline AMP; BASF Corp. Florham Park, New Jersey) at 0.11 + 0.04 kg a.i./ha at corn stage R1; and treatment 3 **(3X),** in which corn received 3 applications of foliar fungicides, PYR at 0.11 kg a.i./ha at corn stage V5, PYR and MET at 0.11 + 0.04 kg a.i./ha at corn stage R1, and PYR and MET at 0.11 + 0.04 kg a.i./ha at corn stage R3. The dates for fungicide application for first, second, and third applications were: July 7, 2013; July 26, 2013; and August 13, 2013. The corn hybrid planted was 'LG2636 VT3P RIB' (LG Seeds; Elmhurst, IL), which is a dual purpose hybrid used for either grain or silage. The hybrid is advertised as 114-d maturity, with high yield potential, strong stalks, and high vigor. This hybrid also is advertised as having a high level of resistance against northern corn leaf blight (caused by the fungus *Exserohilum turcicum*), southern corn leaf blight (caused by the fungus *Bipolaris maydis*), and gray leaf spot (caused by the fungus *Cercospora zeae-maydis*). This hybrid contains transgenic traits that provide protection against corn earworm (*Helicoverpa zea*). All corn was planted on June 5, 2013 and harvested on September 27, 2013 at a dry matter of 33%, 30%, 30%, 32.5% for CON, 1X, 2X, and 3X, respectively. Harvester included kernel processing to have the same theoretical length of chop, set at 1.9 cm. Inoculant (Silo-King WS; 1.5×10^5 cfu/g of *L. plantarum*, *P. pentosaceus*, and *Enterococcus faecium*; Agri-King, Fulton, IL) was applied at a rate of 115 g of inoculant per 1000 kg of corn and then ensiled in silo bags for > 200 d.

Physical characteristics of the corn silage were measured on a weekly basis (3 replicates twice weekly) for 10 wk using the Penn State particle separator (Kononoff et al., 2003). Data from the Penn State box were then fit to a lognormal distribution to estimate mean particle size and the $\log_{10}$ standard deviation.

Animals and housing

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee. Three second-lactation lactating Holstein cows (375 DIM ± 21 d, 19.7 ± 8 kg/d) fitted with a rumen cannula were used for the experiment. Cows were housed in a tie-stall barn with individual feed access and fed diet to meet NRC requirements (NRC, 2001) with ad libitum access to feed. The diet was composed of 50% concentrate and 50% forage, with 72% of the forage consisting of corn silage. Cows were fed once daily at 1400 h, and were milked three times daily at 0700, 1400, and 2200 h. Refusals were removed and weighed before new feed was offered.

Sampling and bag preparation

Corn silage was removed from 6 locations along the face of the bunker silo in order to retrieve a representative sample. Once the sample (4 kg per treatment) was obtained, it was dried and then placed on a sheet. The sample was then divided into 24 equal sections to decrease sample error and increase uniformity of the feedstuff placed into each bag. Two sizes of Dacron forage bags were utilized for this study, one 10 × 20 cm and the other 20 × 40 cm, both had 50-μm pores. The procedure used was similar to the one described by Vanzant et al. (1998). Briefly, bags of both sizes were labeled, dried, and weighed prior to the addition of the feed. Each bag was heat sealed at least twice to ensure no feed particles escaped. Bags were filled to achieve a 20 mg DM/cm^2 of bag surface. Small bags were filled with 8 g of DM and large bags were filled with 32 g of DM. For all bags, the sample was unground and particle length was not altered before it was put into the bags. Three replicates were made for each small bag at each time point for a total of 24 bags per treatment per cow. Bags were placed into large mesh garment bags to prevent the loss of bags in the gastrointestinal tract. All bags were soaked in warm water before they were placed into the ventral rumen at the same time. Bags were then removed at the appropriate time point, noting the identification of each bag at the time of removal. Small bags were removed at 0, 2, 4, 8, 12, 48, 72, and 96 h. The same procedure was followed for the large (20 × 40 cm) bags, except that they were removed from the rumen only at 0 and 48 h. Care was taken to minimize air exposure that could interfere with proper fermentation, and remaining bags were placed back into the ventral rumen. Bags that were removed from the rumen were immediately placed in ice water to stop fermentation. Bags were then rinsed and immediately frozen for at least 24 h. After freezing, the bags were thawed and rinsed on a rinse cycle of a washing machine 2 times to reduce microbial content. Bags were then oven dried for 24 h at 110 °C and disappearance was calculated. In the instance of torn bags or compromised seals post digestion the data were considered missing (n = 14).

Chemical Analysis

The three replicates of each time point from each treatment were combined to make a composite sample that was sent to a commercial laboratory (Rock River Lab, Watertown, WI) for analysis via wet chemistry methods (Schalla et al., 2012). The samples were analyzed for NDF (n = 24/trt), ADF (n = 24/trt), CP (n = 24/trt), and starch contents (n = 24/trt/cow), as well as DM (n = 72/trt). Neutral detergent fiber was analyzed using sulfite and alpha amylase along with a premixed neutral detergent solution (Goering and Van Soest, 1970), and ADF using the Ankom[200] Fiber analyzer (Ankom Technology, Fairport, NY). Crude protein content of the samples was measured using the combustion method to determine N content and then multiplying the N content by 6.25 (AOAC, 1995). Starch was measured using alpha amylase, amyloglucosidase, and Na acetate buffer by the procedure described by Hall and Mertens (2008).

Statistical Analysis

Statistical analysis was performed using SAS (v9.4 Institute Inc., Cary, NC). Statistical analyses were performed using the PROC GLIMMIX, MIXED, and PHREG procedures, considering bag as the experimental unit.

The data were analyzed in two sequential steps. First, a non-linear model of *in situ* digestion was analyzed using the NLIN procedure of SAS, based on the partitioning of feed such that the fractions of soluble feed (A), rumen degradable feed (B), and rumen undegradable feed (C) is summed to 1. The disappearance data from the small bags were first fit to the nonlinear function:

$$\mathrm{Y} = \mathrm{B} + \mathrm{C}\left(e^{-Vk_d(t-t_1)}\right)$$

where B = the portion of potentially digestible feed, C = the portion of rumen undegradable feed, t = time point, Y = the amount of feed reaming at a specified time point (t), k_d = the fractional digestion rate constant, and V = 1 when t ≥ lag, and V = 0 when t < lag (McDonald, 1981, Van

Milgen et al., 1991). The A fraction was then calculated by 100% – (B + C). The parameter of lag was restricted so that lag ≥ 0. Lag was excluded from the parameters when estimated to be zero in order to meet convergence criteria [(Ørskov and McDonald, 1979); (total = 17; 6 for starch, 1 for ADF, 2 for CP, and 4 for DM)]. The equation in which lag was eliminated was:

$$\mathrm{Y} = \mathrm{B} + \mathrm{C}\,(1 - e^{-k_d\,(t)})$$

An anova was then conducted on estimates of soluble feed, rumen degradable feed, rumen undegradable feed, k_d, and lag.

Secondly, a linear mixed model (PROC MIXED) was created to explore associations among parameters calculated and bag size. Treatment and replicate were treated as fixed effects and cow was considered a random effect. Bag type and treatment as well as the interaction between the two were treated as fixed effects. Three predetermined orthogonal contrasts were used for both models. One contrast compared CON with any (1X, 2X, or 3X) corn silage with fungicide application, and the other two contrasts examined linear and quadratic effects of fungicide applications. These contrasts were used for all variables analyzed unless otherwise stated. The degrees of freedom method used was Kenward-Rogers (Littell et al., 1998). Residual distribution was evaluated for normality and homoscedasticity using the UNIVARIATE procedure in SAS Extreme outliers were removed when present (1 value for digestible portion of DM, 1 for k_d of DM, and one for lag of DM). Statistical significance was declared as *P* value lower than 0.05, and tendency declared when *P* value was lower than 0.10.

RESULTS

Physical Characteristics

The mean particle size of corn silage for each treatment was 9134, 9186, 8852, and 9401 µm for CON, 1X, 2X, and 3X, respectively. Results from the analyses of physical characteristics of corn silage are shown in Table 3.1. Corn silage had similar physical characteristics among treatments.

Digestibility Kinetics

Dry Matter. There were no differences among feeds for indigested DM ($P > 0.05$, Table 3.2). There was a linear treatment effect for soluble DM, with the amount decreasing as the number of foliar fungicide applications increased ($P = 0.01$). Digested DM was greater for all silages treated with fungicide when compared with CON ($P = 0.01$). There was also a linear treatment effect for rumen degradable DM in which the portion of rumen degradable feed increased as the number of foliar fungicide applications increased ($P = 0.006$). The fractional rate of digestion of DM tended to be less for all corn silages treated with foliar fungicide when compared with control ($P = 0.08$). There was a linear treatment effect ($P = 0.04$) for the fractional rate of digestion; it decreased as the number of foliar fungicide applications increased. There was a tendency (P = 0.07) for a quadratic treatment effect for lag. A diagram of the DM digestion kinetics for all 4 treatments is shown in Figure 3.1 A.

Neutral Detergent Fiber. There were no differences between CON and all corn silages treated with fungicide for soluble NDF, rumen degradable NDF, rumen undegradable NDF, k_d, or ($P > 0.05$, Table 3.2). However, there was a tendency for a linear treatment effect for soluble NDF with the amount decreasing as the number of applications of foliar fungicide increased ($P = 0.08$).

There was a tendency for a quadratic treatment effect for k_d, with the mid-level treatments having a faster k_d than the CON and 3X treatments ($P = 0.08$). A diagram illustrating the kinetics of NDF digestion for all 4 treatments is shown in Figure 3.1 B.

Acid Detergent Fiber. There were no differences for all contrasts for rumen degradable ADF, indigested ADF, k_d, and lag ($P > 0.05$, Table 3.2). However, there was a tendency ($P = 0.09$) for corn treated with foliar fungicide to have lower soluble ADF portion when compared with control. A diagram of the ADF digestion curve for all 4 treatments is shown in Figure 3.1 C.

Starch. There were no differences between CON when compared to all corn silages treated with fungicide in soluble starch, rumen degradable starch, indigested starch, k_d, and lag ($P > 0.05$, Table 3.2). There were no linear effects for all variables, however there was a tendency for a quadratic treatment effect for k_d. The highest and lowest fungicide treatments tended to have a slightly higher k_d when compared with the mid-level treatments. A diagram of the starch digestion kinetics for all 4 treatments is shown in Figure 3.1 D.

Crude Protein. Three were no differences for soluble CP, rumen degradable CP, indigested CP, k_d, or lag for all contrasts ($P > 0.05$, Table 3.2). A diagram of the crude protein digestion curve for all 4 treatments is shown in Figure 3.1 E.

Comparison of Feed Digestion in 10 × 20 cm vs 20 × 40 cm Dacron forage bags

There was a larger ($P < 0.01$) amount of corn silage DM disappearance for feed in the 20 × 40 cm bags when compared with corn silage in the 10 × 20 cm bags. Feed disappearance at 48 h tended ($P = 0.08$) to be higher for CON than all corn silage treated with fungicide. As the number of fungicide applications increased, DM disappearance decreased ($P < 0.01$). However, there was no interaction between treatment and bag type for total DM disappearance ($P > 0.05$, Table 3.3).

Digestibility of NDF was greater in the 10 × 20 cm bags when compared with 20 × 40 cm bags (*P* = 0.03). There was a treatment effect for NDF (*P* = 0.02) and a linear treatment effect (*P* < 0.01) of NDF with the amount of NDF after 48 h being increased as amount of foliar fungicide application increased. Digestibility of NDF had no interaction between treatment and bag (*P* > 0.05).

Digestibility of ADF was smaller for 20 × 40 cm bags (*P* < 0.01) by 48 h than 10 × 20 cm bags. There was a bag size effect for ADF digestibility, with 20 × 40 cm bags having a smaller (*P* < 0.01) amount of feed digested by 48 h than 10 × 20 cm bags. Larger portion of ADF remained (*P* = 0.01) in corn silage treated with fungicide after 48 hours when compared with CON. Finally, in regards to ADF, there was no interaction between type of bag and treatment (*P* > 0.05, Table 3.3).

Digestibility of starch was higher (*P* < 0.01) in 20 × 40 cm bags when compared with 10 × 20 cm bags. Amount of feed digestibility was the same for all treatments (*P* > 0.05). There was also no interaction between bag type and treatment (*P* > 0.05, Table 3.3). Crude protein digestibility was greater (*P* < 0.01) for feed in the 20 × 40 cm bags when compared with feed in the 10 × 20 cm bags. However, there was no difference (*P* > 0.05) among treatments for digestibility of feed. Interaction of bag type and treatment was not significant for CP (*P* > 0.05, Table 3.3).

DISCUSSION

The major purpose of this study was to evaluate in situ digestibility of corn plant treated at various times of application with foliar fungicide on corn harvested as whole plant corn silage and fed to lactating dairy cattle. A secondary objective was to determine if there were differences in

48-h digestibility between samples ruminally incubated in 10 × 20 cm bags when compared with samples in 20 × 40 cm bags.

Effects of foliar fungicide

Lee et al (2007) found that drought stress on white clover not only led to increased overall lignification of the plant, but caused a significant increase in the enzymes, responsible for lignification phenylalanine ammonia lyase being the primary enzyme. Cold and heat stress also caused an increase in phenolic compounds in tomato and watermelon plants (Rivero, 2001). A study done by Yates et al. (1997) found that an endophyte infection by *Fusarium moniliforme*, recently reclassified as *Fusarium verticillioides*, on seedling corn resulted in more lignin accumulation as a result of the plant defense mechanisms. It is known that lignin is highly indigestible by microbial systems found in the rumen so represents no nutritional value (Jung and Deetz, 1993). Jung et al. (2011) found that corn hybrids with reduced lignin (specifically ferulate-mediated lignin-arabinoxylan cross linking often found in grass plants) content resulted in 5 kg more milk yield and 9 kg higher DMI in Holstein cows when compared with corn with a higher level of lignin content. Oba and Allen (1999) also found higher microbial nitrogen production for low lignin hybrids compared with control. However, since we used the same hybrid for all treatments, corn hybrid was not a confounding factor in the present study. In the present study, digestible feed DM may have been higher in corn treated with fungicide due to a lower amount of lignin, and due to fungal infestation changing the chemical composition of the corn plant in the field or during the ensiling process and therefore competing for nutrients with the plant (Venancio et al., 2009). In future studies, it may be beneficial to analyze at different points of the corn plant growth its portions (e.g., stem and leaves) separately, to better understand the rumen undegradable feed portion, and what is causing differences in digestibility.

The digestible portion of NDF tended to be higher for 3X when compared with CON (Table 3.2). Yates et al. (1997) proposed that when a corn plant had a fungal infestation of the root, the structural components and rigidity increased, which the authors attributed to the plant attempting to decrease further infestation into the upper portion of the plant by increasing the structurally rigid components of the plant such as lignin. This may also have impacted the amount of NDF, or the structural composition of the NDF, and potentially increased the lignification of the NDF making it less digestible in the present study (Buxton and Redfearn, 1997). Williams et al. (1992) found that severely damaged crops exhibited a higher crude fiber content when compared with non-infected plants. The authors also found that gross energy between infected and non-infected plants did not differ, but found that there was more protein and less fat in mold damaged corn kernels. The difference in nutrient composition was due to the fungi using readily accessible carbohydrates as nutrient source first, and the authors hypothesized that this may affect digestibility of the plant. Similar mechanisms may have occurred in the present study because there was a higher proportion of digestible NDF in the silage treated with fungicide.

There were no differences among corn silages in the digestibility of starch or CP, which could be due to the fact that there were no visible signs of infection on the corn ears even from CON, and so corn ears were not damaged. Infection by *Fusarium moniliforme* as a visible ear rot was shown to decrease the fat content, but increased CP content. This is thought to be due to the presence of fungal nitrogen (Williams et al., 1992). When steers were fed increasing amounts of steam-flaked corn infected with fungi, total tract digestibility and ruminal digestibility for OM and N as well as overall DE decreased as inclusion increased (Alvarez et al., 2011).

Even when plants are not compromised with fungal infections, studies have found that application of pyraclostrobin has increased corn yield. A meta-analysis that looked at data from

172 studies from 2002 to 2009 in 14 different states found a mean increase in yield of corn grain of 255 kg/ha (Paul et al., 2011). This is thought to be due to the fact that pyraclostrobins are from the strobilurin class of fungicide, which work as quinone outside inhibitors. Quinone blocks electron transfer in the cytochrome III (bc1) complex, disrupting the electron transport chain and disrupting ATP production (Bartlett et al., 2002). This can also lead to increased N assimilation in the plant, through increased nitrate reductase that can be especially beneficial when the plant has highest N requirements (Venancio et al., 2009). However, in our study we did not see any differences in CP digestibility or availability. There also is some evidence that pyraclostrobin may help to increase water use during drought stress due to increased abcissic acid concentration. Pyraclostrobin works to decrease oxidative stress, due to increased activity of peroxidases that persist weeks after treatment with the fungicide (Venancio et al., 2009).

In the present study the soluble portion of the feed had a linear effect for both NDF as well as total DM, with the portion of soluble feed components decreasing as the number of fungicide applications increased. This could have happened due to the difference in corn silage particle size but our evaluations did not show any difference between particle length and physical characteristics of the feed (Table 3.1). To further investigate this difference it would have been beneficial to complete a more extensive evaluation of particle size of the feed before ensiling and digestion.

It is also important to note that because the samples were not ground before placement in the rumen, digestibility may have been underestimated due to the lack of effects of mastication or rumination. However, grinding the samples may lead to an over estimation of true digestibility (Vanzant et al., 1998). Nonetheless, the relatively small sizes of the standard errors calculated in the present study seem to show that a representative sample was obtained in each bag.

Effects of bag size

The DM disappearance was larger for the 20 × 40 cm bags when compared with the 10 × 20 cm bag, which is similar to the results found by Varga and Hoover (1983) where two different sample sizes (2.5 and 5 g) were put into two different size bags (13 × 21 and 9 × 17 cm bags). The authors analyzed both concentrates and forages (ground at 4 mm) using a sample size to surface area of 9.2 and 14.6 mg/cm^2, respectively. The authors did not analyze NDF in those samples because the amount remaining at 48 h to complete the analyses was not adequate for all sample sizes. There also was no discussion as to why these results were found (Varga and Hoover, 1983). One possible hypothesis would be that because the 10 × 20 cm bags were subjected to some minimal exposure to air when other bags were being removed at the specified time points feed digestion may have been affected, whereas 20 × 40 cm bags were only exposed to air upon removal. However, studies have shown that complete removal of feed, sampling, and reintroduction of the same feed to the rumen did not affect the digestibility, or rate of digestion (Reid, 1965). Bags were treated the same so it is not known why digestibility differed between 10 × 20 and 20 × 40 cm bags (Table 3.3). It was also noted that fiber digestibility was greater in the 10 × 20 cm bags; however, CP and starch digestibility were greater in the 20 × 40 cm bags, so microbial access could differ in the size of the bags. Because there have been limited studies utilizing a bag larger than 10 × 20 cm, we cannot explain why these differed and further research in this area is needed.

The 48-h digestibility of DM and ADF was higher for CON when compared with silage treated with the fungicide (Table 3.2 and 3.3). This could have happened due to a slightly higher k_d in the fungicide-treated corn silages when compared with CON, and a tendency for a linear treatment effect for decreasing k_d as the number of fungicide applications increased was found in the 10 × 20 cm bags alone (Table 3.3). However, this inference to the larger bag should be interpreted cautiously as the digestibility of feedstuff differed between 10 × 20 and 20 × 40 cm bags as stated above. There was also more ADF remaining at 48 h with a linear treatment effect for increasing amount remaining as number of fungicide applications increased. We did not see any differences in digestibility when the 10 × 20 cm bags were analyzed alone, but the difference became apparent when 20 × 40 cm bags were analyzed. It may be beneficial to do more replications of the larger bags and 10 × 20 cm bags at the 48 h time point as well as a longer time point to understand which portions of the feed may be rumen undegradable. As well, it may be beneficial to compare ground and unground samples between the two bag sizes to better understand the differences found in this study.

CONCLUSION

Fungicide application of corn resulted in higher DM digestibility and tended to have a lower k_d as well as linear treatment effects for decreasing DM solubility, increasing rumen degradable DM, and decreasing k_d of corn silage DM. There were no differences in starch, ADF, or CP digestibility among treatments. In situ digestibility results for big bags (20 × 40 cm) and small bags (10 × 20 cm) were different in the present study. Digestion of DM, NDF, and ADF was higher, while starch and CP digestion was smaller in the larger bags. Further investigation is needed to clarify why this occurred. Fungicide application on corn for silage seems promising as changes in digestibility may increase efficiency in ruminant animal production systems.

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TABLES AND FIGURES

Table 3.1. Means and standard deviations of the physical characteristics of corn silage using the Penn State particle separator for corn silages treated with no foliar fungicide (CON), one application of foliar fungicide (1X), two applications of foliar fungicide (2X) or three applications of foliar fungicide (3X).

	Treatment							
	CON		1X		2X		3X	
Pore Size (% in each pan)	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
19 mm	8.7	4.5	10.2	4.7	8.3	5.6	9.6	6.7
7.8 mm	68.9	2.8	67.8	3.5	67.2	5.9	68.0	4.7
1.2 mm	21.5	2.1	21.8	2.1	23.9	6.7	22.0	3.6
Pan	2.1	0.8	2.1	1.1	5.7	0.6	3.6	0.4
Mean particle size, µm	9134		9186		8852		9401	
Log_{10} Standard Deviation	0.34		0.36		0.32		0.32	

Table 3.2. Least squares means and associated standard errors of soluble feed portion, digestible feed portion, rumen undegradable feed portion, fractional rate of digestion (k_d), and lags for corn silages treated with no foliar fungicide (CON), one application of foliar fungicide (1X), two applications of foliar fungicide (2X) or three applications of foliar fungicide (3X), as determined in 10 × 20 cm Dacron bags.

	Treatment					*P*-value for Contrasts[1]		
	CON	1X	2X	3X	SEM	CON vs TRT	Linear	Quadratic
DM								
Soluble	0.35	0.36	0.34	0.23	0.03	0.28	0.01	0.06
Digestible	0.36	0.42	0.40	0.47	0.03	0.01	0.006	0.97
Rumen undegradable	0.25	0.23	0.23	0.25	0.02	0.59	0.96	0.37
K_{d}, h^{-1}	0.05	0.04	0.04	0.03	0.007	0.08	0.04	0.93
Lag, h	2.41	1.92	3.93	0.19	1.20	0.6	0.24	0.07
NDF								
Soluble	0.36	0.38	0.30	0.24	0.04	0.39	0.08	0.37
Digestible	0.38	0.45	0.36	0.46	0.03	0.25	0.26	0.54
Rumen undegradable	0.27	0.18	0.34	0.29	0.05	0.98	0.33	0.68
K_{d}, h^{-1}	0.04	0.18	0.14	0.04	0.06	0.27	0.91	0.08
Lag, h	1.4	1.52	7.83	1.44	1.85	0.34	0.47	0.13
ADF								
Soluble	0.35	0.24	0.33	0.19	0.06	0.20	0.18	0.78
Digestible	0.36	0.54	0.38	0.52	0.10	0.33	0.48	0.86
Rumen undegradable	0.30	0.23	0.28	0.30	0.09	0.74	0.86	0.60
K_{d}, h^{-1}	0.05	0.02	0.06	0.05	0.02	0.55	0.57	0.31
Lag, h	3.68	0.00	5.22	2.60	2.86	0.75	0.88	0.85
Starch								
Soluble	0.29	0.33	0.37	0.41	0.10	0.52	0.41	0.99

Table 3.2. (Cont.)

Digestible	0.57	0.44	0.47	0.46	0.07	0.18	0.35	0.38
Rumen undegradable	0.13	0.23	0.16	0.13	0.04	0.39	0.68	0.15
$K_{d,}$ h^{-1}	0.09	0.07	0.05	0.14	0.02	0.86	0.23	0.09
Lag, h	0.00	0.00	0.00	0.09	0.04	0.58	0.23	0.36
CP								
Soluble	0.33	0.35	0.34	0.27	0.03	0.81	0.17	0.16
Digestible	0.44	0.43	0.46	0.53	0.05	0.59	0.25	0.50
Rumen undegradable	0.23	0.21	0.20	0.20	0.06	0.74	0.74	0.89
$K_{d,}$ h^{-1}	0.02	0.03	0.03	0.04	0.02	0.57	0.52	0.92
Lag, h	0.48	1.33	2.05	2.54	1.78	0.47	0.40	0.92

[1] Contrasts were CON vs TRT = no fungicide application (CON) versus the average of the three treatments with fungicide application; Linear = linear treatment effect; and Quadratic = quadratic treatment effect.

Table 3.3. Least squares means and associated standard errors for proportion of corn silages treated with no foliar fungicide (CON), one application of foliar fungicide (1X), two applications of foliar fungicide (2X), or three applications of foliar fungicide (3X) remaining after 48 h in situ incubation in 10 × 20 or 20 × 40 cm Dacron forage bags.

	Treatment					*P*-value for Contrasts[2]			
	CON	1X	2X	3X	SEM	Bag type[1]	CON vs Fungicide	Linear Trt	Quad Trt
DM	0.29	0.32	0.31	0.40	0.02	< 0.01	0.08	< 0.01	0.14
NDF	0.34	0.36	0.36	0.48	0.03	0.03	0.15	< 0.01	0.09
ADF	0.37	0.40	0.39	0.55	0.03	0.01	0.02	< 0.01	0.03
Starch	0.08	0.14	0.11	0.08	0.02	< 0.01	0.32	0.86	0.11
CP	0.29	0.28	0.25	0.34	0.04	< 0.01	0.98	0.54	0.23

[1] Bag Type: 10 × 20 or 20 × 40 cm Dacron forage bags.
[2] Contrasts were CON vs TRT = no fungicide application (CON) versus the average of the three treatments with fungicide application; Linear = linear treatment effect; and Quadratic = quadratic treatment effect.
All treatment × bag type interactions were not significant (*P > 0.17*).

Figure 3.1. In situ digestion kinetics for proportion of feed fractions remaining after ruminal incubation of corn silages CON (with no application of fungicide), 1X (with 1 application of fungicide), 2X (with 2 applications of fungicide), and 3X (with 3 applications of fungicide). **A**: DM, **B**: NDF, **C**: ADF, **D**: Starch, and **E**: CP.

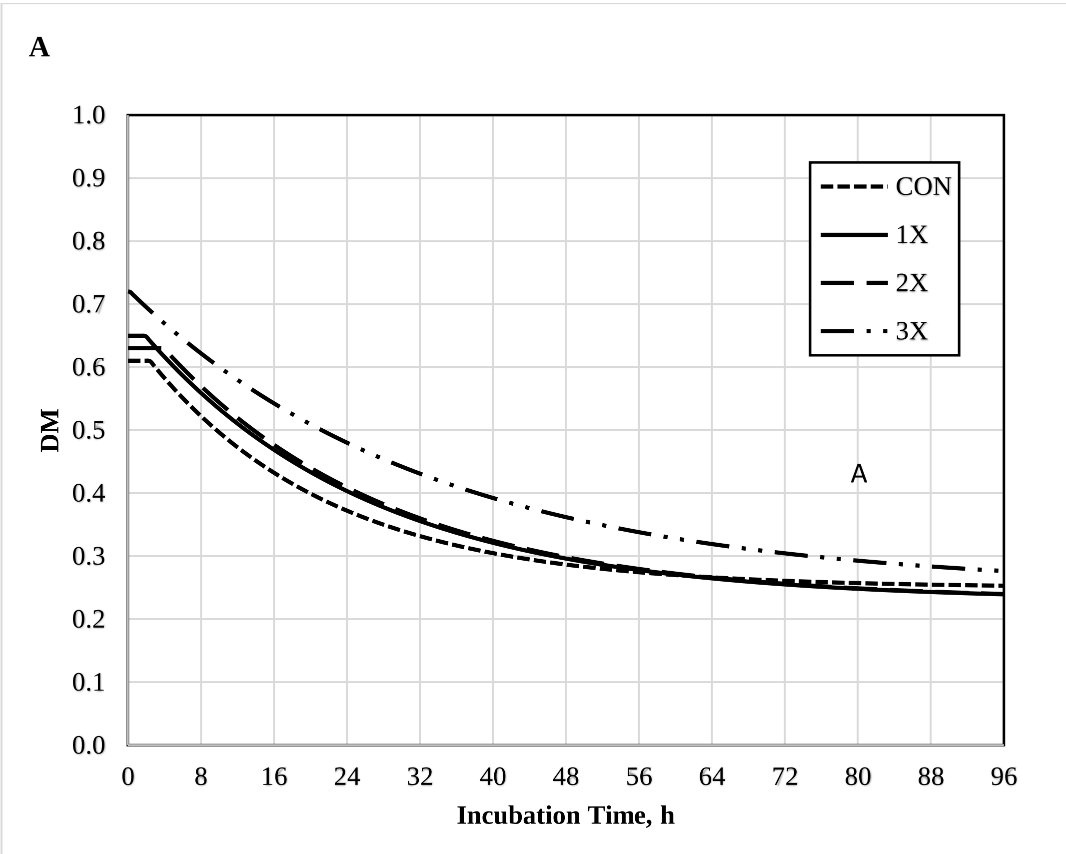

Figure 3.1. (cont.)

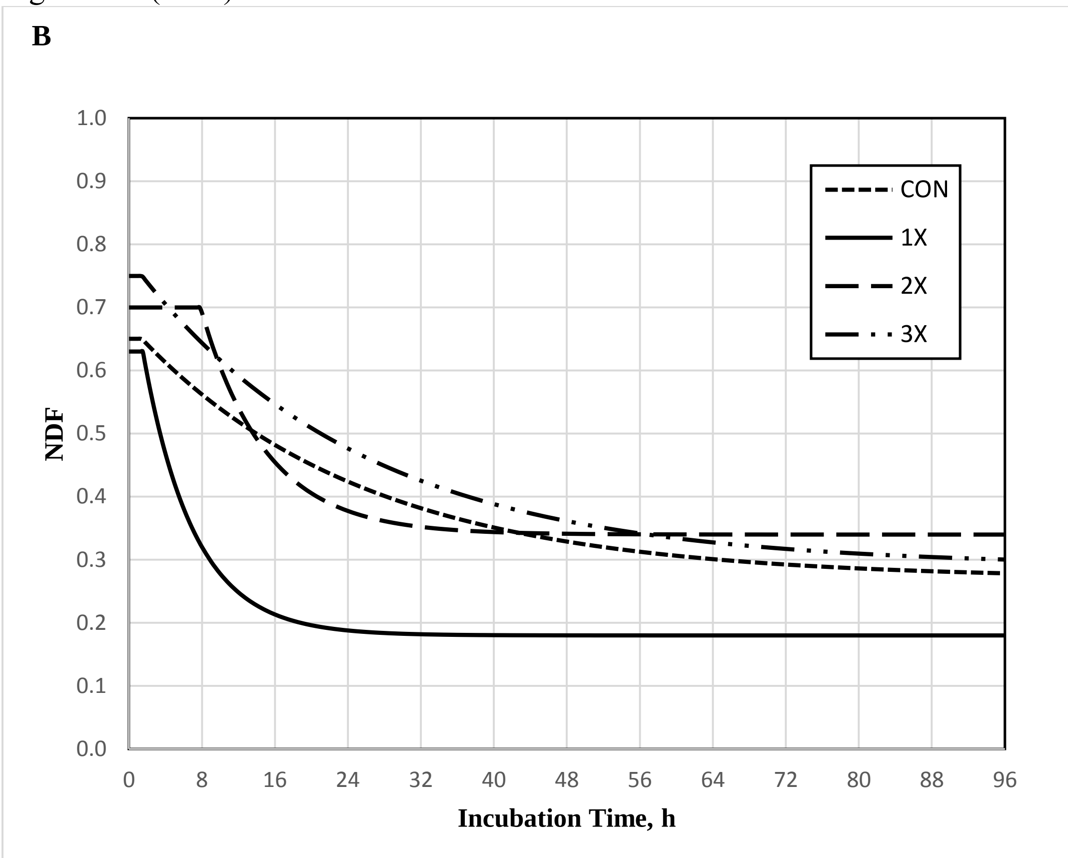

Figure 3.1. (cont.)

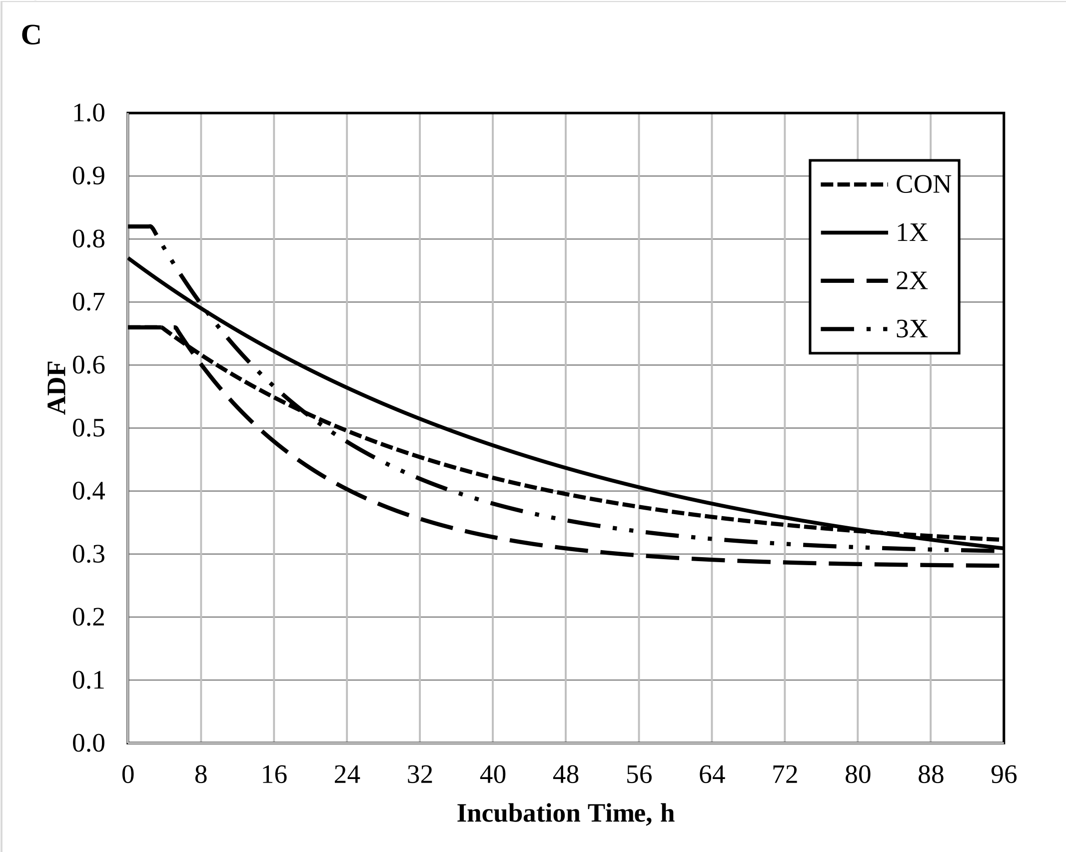

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Figure 3.1. (cont.)

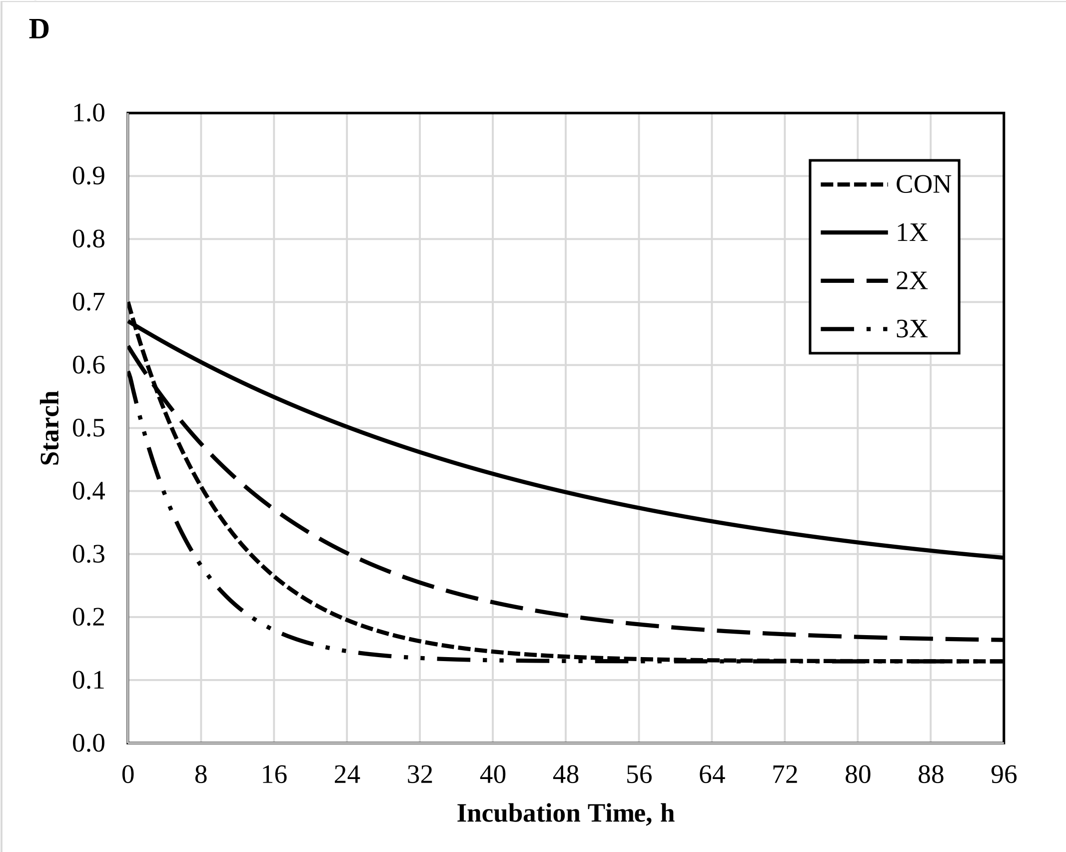
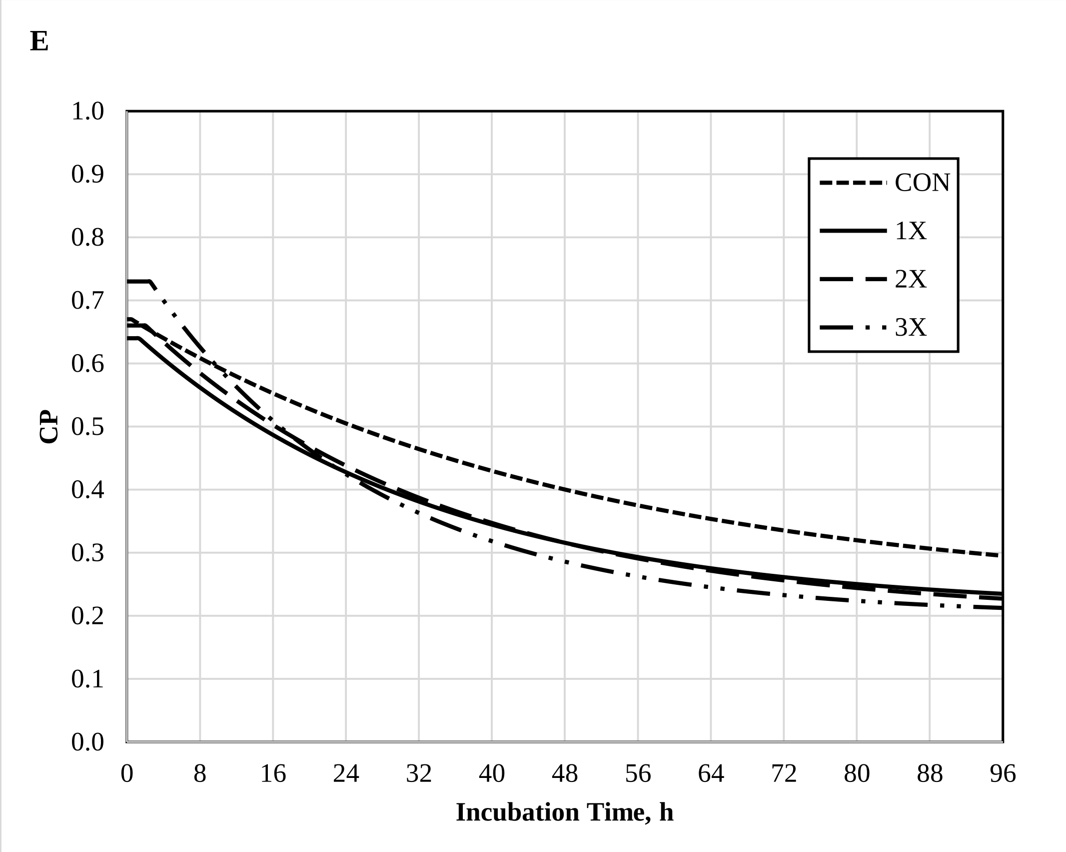


Figure 3.1. (cont.)



CHAPTER IV: CONCLUSIONS

Cows receiving corn silage treated with foliar fungicide had lower DMI, while maintaining milk production, leading to an increase in simple feed conversion, fat corrected feed conversion and energy corrected feed conversion. Exact reasons for this cannot be concluded with the current research done, however we hypothesize that these increases in efficiency were due to increased silage nutrient value, and increase in dry matter digestibility of the corn silage treated with fungicide. These changes may also be due to better aerobic stability of corn treated with fungicide, as well as decreased toxin content of these silages.

Although the cost of fungicide application is considered to be expensive, the advantages of fungicide may have economic benefit to the producer. The average cost for 1 application of fungicide along with labor costs to apply the fungicide is about $30. Thus, the cost for 1X was estimated to be $30, 2X was $60, and 3X was $90 per acre of application. However, these costs may be offset by the increase in efficiency exhibited by cows eating this silage. To estimate the economic value associated with the use of foliar fungicides multiple approaches were taken.

First value of the corn silage alone was estimated by using the MILK 2006 program. This program takes into account the analyzed nutrient values of the silage including NDFD as well as the amount of silage produced as a unit of tons of DM/acre harvested. This program then returns a value of milk per ton of silage, as well as milk per acre of silage produced. These values are shown in Table 4.1. Actual milk per ton produced as well as the milk per acre produced was then calculated using the DMI and milk yield data collected and shown in chapter II. These results are also shown in Table 4.1 along with the differences between the predicted and actual values. It was found that corn silage receiving foliar fungicide application had higher predicted and actual values for milk per ton as well as milk per acre.

As another measure to evaluate potential economic value of corn silage treated with foliar fungicide is the efficiency of corn silage in relation to milk produced. This was done by calculating the cost per pound for each corn silage, multiplying that by the amount consumed by the cows from each treatment, and then finally calculating income over corn silage cost. The price per pound of silage was 0.044, 0.046, 0.047, 0.049 dollars/lb for CON, 1X, 2X and 3X, respectively. Corn silage intake for each treatment was calculated as 18.31, 17.67, 15, 04, and 16.42 lbs/d (8.3, 8.03, 6.8 and 7.46 kg/ d) for CON, 1X, 2X and 3X, respectively. Using the milk yield from chapter II and estimating milk price at 0.18 dollars/ pound the calculated income over corn silage cost was 12.84, 12.85, 12.85, and 12.81 for CON, 1X, 2X, and 3X, respectively. When correcting milk yield for milk fat content results for income over corn silage cost were 13.21, 13.80, 12.45, and 13.13 dollars for CON, 1X, 2X, and 3X, respectively. When correcting milk yield for energy content income over corn silage cost was 12.85, 13.00, 12.15, and 12.84 dollars, respectively. The income over silage cost for milk yield was similar for all treatments. When adjusting milk yield for fat content (FCM) and for energy content (ECM) income over silage cost was higher for 1X when compared to CON, 2X, and 3X. Overall corn silage receiving 1 treatment of foliar fungicide at V5 had the highest income over corn silage cost when analyzed for milk yield, FCM, and ECM.

The final measure to evaluate potential economic benefits of these treatments, efficiency of the rations including corn silage in relation to milk produced was calculated. In order to calculate these values, ration cost was calculated for each treatment and multiplied by the DMI in pounds. Income was then calculated by multiplying pounds of milk, ECM, and FCM for each treatment by price per pound of milk. The total income from milk yield over feed cost was 7.35, 7.54, 8.31, and 7.83 dollars for CON, 1X, 2X, and 3X respectively. When correcting for fat content (FCM) income over feed cost was 7.71, 8.49, 7.95, and 8.14 dollars for CON, 1X, 2X, and 3X respectively. Finally when correcting for energy content produced in milk (ECM) income over feed cost was 7.35, 7.69, 7.69, 7.86 dollars for CON, 1X, 2X, and 3X respectively. Income over feed cost was highest for 2X when looking at milk yield, highest for 1X when correcting for fat (FCM), and highest for 3X in ECM. Income over feed cost was also numerically greater for all treated silages when compared to control.

Overall, there seem to be economical benefits of using corn treated with a foliar fungicide and ensiled. Corn treated with foliar fungicide had higher predicted milk per ton, and measured milk per ton than when compared to CON. Additionally cows receiving treatments 1X and 2X silage produced much more the predicted milk per ton, and milk per acre. The difference in predicted milk per ton for each treatment accounts for the increased silage nutrient content associated with the lower fiber, and higher sugar and fat levels for treated silage observed in chapter II. However, the increase in actual milk production when compared to predicted observed could be due to increase efficiency of the animal, caused by lower toxin content of the feed and creating a better rumen environment, or could be a result of increased total rumen degradable feed content seen in treated silages compared to control. The greatest difference in actual vs predicted milk yield was seen by cows receiving 2X.

There was also an economic advantage for using corn silage treated with fungicide when looking at income over silage cost, and income over total diet (feed) cost. This was due to the decreased DMI seen in 2X, and 3X, while still maintaining milk production levels of CON and 1X, this lead to a higher feed efficiency for cows receiving 2X and 3X. However because of the increased cost of fungicide, and labor of 3X, while observing no differences in efficiency between 2X and 3X there were varying returns on investment. Although fungicides may be expensive to purchase and apply, in this study it was found that use of foliar caused increases in efficiency of production which allowed them to have an economical advantage over the control. Due to the mean separation tests, and the economic analyses it may also be a possibility to discontinue use of the 3X treatment in further studies, because although there were some advantages to the 3X silage when compared to 2X, these are not enough to outweigh the costs associated with the 3rd application of fungicide.

Finally we used these values along with least separation tests to decide what treatments would be best to carry on to a follow up study. In order to evaluate which treatments differed, variables with statistical treatment difference were analyzed and the and differences of least squares means with a Tukey adjustment was used to evaluate treatment differences, this data is shown in Table 4.2. We hypothesized that there may no difference between CON and 1X, and between 2X and 3X, therefore it may be beneficial and cost effective to only use two treatments in following studies. There were differences between CON and 1X for blood glucose, and silage soluble crude protein and sugar content. Although these few differences may not justify the use of a 1X treatment. This data also shows that most of the differences seen in 2X were also seen in 3X. However for most of the silage nutrient contents, differences were seen between 3X and other treatments that were not seen between 2X and other treatments. Also, there were differences between the means of 3X and 2X for acetic acid and aerobic stability. This suggests the use of 2X treatment may be sufficient to see changes caused by silage, and the 3X treatment may not be necessary to carry on to further studies. There were no differences for milk yield, or milk efficiency between any treatments when correcting *P* values for multiple comparisons.

In the future research it would be beneficial to analyze the nutrient composition of the plant before ensiling to establish whether the changes shown in these studies were occurring during corn growth, or during ensiling. In addition if there were differences between the treated and non-treated silages pre-ensiling, it may be advantageous to evaluate the nutrient composition of the different plant structures (for example leaves, stalks, husks, grain). This would allow better understanding of where and how the fungicide is affecting the plant, and how those changes can be beneficial for feed purposes. Another aspect that would be beneficial to understand is the effect of a foliar fungi disease challenge on plant quality. This would help in understanding what negative effects fungal infection has on plant quality, and the ability of foliar fungicide to mitigate these problems. It would also be valuable to assess the use of fungicide with and without the use of inoculants in a factorial design to test if crops receiving fungicide applications still gain benefit from inoculant. Finally, as more research is done, I feel it may be warranted to assess further health status indicators, such as immune response, in cattle which may be attributed to toxin content of the feed. These low level immune responses, if present, may better explain treatment differences for DMI, and efficiency.

In conclusion silage receiving treated silage had better feed quality which lead to an increase in predicted milk per ton, and milk per acre estimates. Cows receiving treated corn silage also had higher efficiency as shown by the difference in actual and predicted milk per ton. This may be due to decrease toxin content, better aerobic stability, or increased rumen degradable DM. This increase in efficiency lead to an economic value of using corn silage treated with foliar fungicide when compared to control. Corn treated with foliar fungicide and ensiled was found to have higher quality, increase efficiency in cows fed treated silage, and may be a valuable tool for producers looking to improve profitability, however more research would be recommended to understand the physiological mechanisms causing these changes.

TABLES AND FIGURES

Table 4.1. Comparison of predicted vs actual milk per ton and milk per acre values using MILK 2006.

	Milk Per Ton			**Milk per Acre**		
Treatment	Estimated	Calculated	Difference	Estimated	Calculated	Difference
CON	2952	2898	-53	26567	26090	-476
1X	3010	3006	-4	24062	24050	-11
2X	3016	3506	490	27563	31907	4344
3X	3057	3222	165	27540	28996	1456

Table 4.2. Mean separation tests to determine treatment differences with Tukey adjustment.

	Trt					***P*-value**
Item	**CON**	**1X**	**2X**	**3X**	**SEM**	**Trt²**
DMI	23.78^{a}	22.95^{a}	19.54^{b}	21.33^{ab}	0.73	0.0015
Milk Yield	34.47	34.50	34.20	34.37	0.78	0.10
FCM Yield/DMI	1.47	1.51	1.71	1.73	0.09	0.08
ECM Yield/DMI	1.43	1.46	1.66	1.69	0.09	0.06
Blood Glucose	63.39^{a}	48.60^{b}	49.17^{b}	55.53^{b}	2.06	0.0003
Silage Nutrients						
CP% of DM	8.61^{ab}	8.72^{ab}	8.50^{ab}	8.98^{a}	0.12	0.06
ADF	29.24^{a}	27.72^{ab}	28.11^{ab}	27.33^{b}	0.46	0.04
Fat	2.71^{b}	2.77^{ab}	2.74^{ab}	3.16^{a}	0.11	0.03
Soluble CP	56.18^{ab}	51.98^{c}	54.05^{bc}	59.04^{a}	0.89	0.001
Sugar	0.72^{b}	1.14^{a}	1.23^{a}	1.25^{a}	0.08	0.0002
Acetic Acid	2.97^{ab}	2.47^{b}	1.31^{c}	3.47^{a}	0.20	0.001
Aerobic Stability	32.85^{b}	31.71^{c}	31.62^{c}	28.84^{a}	0.42	0.0001