Abstract

Bolt, Shelley Marie. The Effects of Feeding Corn Silage that was Exposed to Air for Five Days With or Without Yeast Cell Walls on Production Parameters in Early Lactation Holstein Cows. (Under the direction of Dr. B.A. Hopkins and L.W. Whitlow).

Proper silage management is important in reducing excessive spoilage due to air exposure. The objective of this study was to compare the effects on production of feeding silage that was exposed to air for five days and Yeast Cell Walls (**YCW**) to Holstein cows. Forty-eight early lactation cows were randomly assigned at calving to one of four treatment diets within parity. Diets included: Diet 1 corn silage blended into a TMR (**CON**), 2) CON with added Yeast Cell Walls (**CON+YCW**), 3) silage that was exposed to air for five days blended into a TMR (**EXP**), 4) EXP with added Yeast Cell Walls (**EXP +YCW**). Cows were started on trial at 21 days in milk (**DIM**). Cows received the same silage type for the duration of the experiment, but switched YCW treatment at the experimental midpoint (45 d). CON corn silage was stored in a covered trench silo for the length of the study. EXP silage was taken from the same trench silo and piled under a covered shelter for 5 days before being blended into the TMR based on prior DM change. Blood and rumen fluid samples were collected on days 30, 44, 75, and 89 of the experiment and analyzed for parameters important for health and production. There were no significant differences in %CP, %ADF, and Mcal/kg NE_L for treatment diets. Milk yield (35.87, 36.72, 36.58 and 37.07 kg/d), DMI (23.18, 22.09, 23.44, and 23.88 kg DM), % fat (3.23, 3.22, 3.22, and 3.18%), fat yield (1.16, 1.17, 1.16, 1.19 kg), %CP (2.86, 2.87,

2.80, and 2.79%), and protein yield (1.03, 1.05, 1.02, and 1.03 kg) were not significantly different among CON, CON+YCW, EXP, and EXP+YCW, respectively ($P > 0.10$). Acetate: propionate ratio was not significantly different among treatments CON, CON+YCW, EXP, EXP+YCW, respectively (2.4, 2.3, 2.4, and 2.3; $P > 0.10$). Concentrations of blood urea nitrogen (**BUN**) (19.8, 20.2, 21.0, and 22.8 mg/dl), rumen ammonia (10.7, 10.9, 9.9, and 9.8 mg/dl), as well as rumen pH (6.9, 6.8, 6.9, and 6.8) were not significantly different among treatments CON, CON+YCW, EXP, and EXP +YCW, respectively ($P > 0.10$). DMI and dietary % CP was significantly lower in the EXP silage ($P < 0.10$). Concentrations of BUN (19.9 vs 21.5 mg/dl; $P < 0.01$) were significantly different for CON and EXP silage, respectively. The addition of YCW significantly lowered acetate concentration (59.03 vs 57.56 mol/100 mol; $P < 0.10$). Dual-Flow continuous culture fermentors were used to conduct an *in vitro* experiment. Ruminal fluid was obtained from a non-lactating Holstein cow and transferred into four fermentors which were fed the four treatment diets from the *in vivo* experiment. The *in vitro* experiment was repeated twice and lasted four five days. Control silage suppressed acetate production while increased the production of propionate and butyrate. Addition of YCW to the CON and EXP treatment diets suppressed methane, acetate, butyrate and total VFA production while increased the concentration of propionate.

(**Key words:** silage, mannanoligosaccharide, fermentors)

THE EFFECTS OF FEEDING CORN SILAGE THAT WAS EXPOSED TO AIR FOR FIVE DAYS WITH OR WITHOUT YEAST CELL WALLS ON PRODUCTION PARAMETERS IN EARLY LACTATION HOLSTEIN COWS

By

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Biography

Shelley Bolt was born in Virginia Beach, Virginia on October 14, 1978 to Mr. and Mrs. Kent N. Bolt. She graduated from West Forsyth High School in Winston-Salem, North Carolina. She then began her studies at North Carolina State University in Raleigh, North Carolina in the fall of 1996. Shelley received a B.S. degree in Zoology with a minor in Animal Science in May of 2000. She entered the North Carolina State University animal science program in the fall of 2000 in pursuit of a M.S. degree in nutrition under the direction of Dr. Brinton Hopkins and Dr. Lon Whitlow.

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Table of Contents

List of Tables

Abbreviations

ADF	acid detergent fiber
BAB	butyric acid producing bacteria
BUN	blood urea nitrogen
BW	body weight
CP	crude protein
DHIA	dairy herd improvement association
DIM	days in milk
DMI	dry matter intake
FCM	fat corrected milk
LAB	lactic acid producing bacteria
MUN	milk urea nitrogen
N	nitrogen
NE_L	net energy of lactation
NPN	non-protein nitrogen
TMR	total mixed ration
VFA	volatile fatty acids
WSC	water soluble carbohydrates
YCW	yeast cell walls

Literature Review

Introduction

Proper silage management is important to maximize nutrients, production, and income on commercial dairy farms. Silage is an important part of lactating cow diets especially in the Southeastern United States, and often makes up 60% of the total feed. In North Carolina, 90% of forage fed to ruminants year-round is silage. Silage has advantages over hay making, such as reduced dependence on weather, increased flexibility of conserving the crop at optimum quality, and the ability to mechanize from harvest to feeding.

A major concern with silage is spoilage due to air exposure. Often, the top layers of a horizontal silo will become exposed to air and consequently the silage can suffer a dramatic loss in nutrient density. It is recommended that covering the silo, in addition to adding preservatives, can help reduce the loss of nutrients. If the silage on top or on the face of the silo is exposed to air, it is recommended to discard any spoilage to ensure diets formulated with this silage are accurate in nutrient composition. Feeding spoiled silage to livestock can severely impact the health and productivity of a dairy herd. Producers feeding spoiled silage are more likely to encounter mycotoxins that can result in a decrease in milk production or intake. Therefore, it is recommended to discard the spoilage, and more importantly manage the silo to prevent spoilage.

Yeast cultures have been studied for their beneficial effects on rumen fermentation and the possibility of antibiotic-like growth promotion. Yeast Cell Walls

(**YCW**) include mannanoligosaccharides that constitute the cell wall portion of the yeast *Saccharomyces cerevisiae*, and have been implicated to improve ruminal fermentation (Erasmus et al., 1992; Enjalbert et al., 1999), nitrogen metabolism (Erasmus et al., 1992; Wohlt et al., 1998), fiber digestibility (Dann et al., 2000; Weidmeier et al., 1987), dry matter intake (**DMI**), and milk production (Shaver and Garrett, 1995; Piva et al., 1993). However, these benefits have not been seen in all experiments.

This experiment was designed to use early lactation dairy cows, fed diets based on either a control silage that was well preserved and fed fresh daily or a silage that was exposed to air for 5 days; both with or without the addition of YCW top dressed on the final TMR. The experiment was designed to be a modified split-plot design where cows remained on the same type of silage (control or exposed) while the YCW was either added or removed from the experimental diet. Milk production, intake, digestibility, and rumen parameters were monitored to determine the effects of silage and YCW on early lactation dairy cattle.

This literature review is divided into the following sections:

1. Phases of silage fermentation
2. Effect of air on silage quality
3. Covering the silo to prevent aerobic deterioration
4. Animal and health risks of feeding spoiled silage
5. Supplementation with viable yeast culture
    a. Rumen Ammonia

b. VFA production and rumen fermentation

c. pH

d. Microbial populations

e. Digestibility

f. Intake, growth, and milk production

6. Mannanoligosaccharides

Phases of fermentation

In phase 1 of silage fermentation, the plant material is put into the silo. The plant cells continue to respire and consume oxygen while carbon dioxide and heat are produced. The temperature of the silage increases. Phase 1 lasts about 1 to 2days. In phase 2 of fermentation, acetic acid is produced. The pH drops from 6.0 to about 4.2. This occurs between days 2 to 3. Phase 3 of fermentation is characterized by lactic acid formation and the decline of acetic acid production (day 3). During phase 4 of fermentation, lactic acid formation continues for about two more weeks. Temperatures gradually decline. Bacterial fermentation stops as the pH lowers to about 4.0. Phase 4 lasts from day 4 to about day 20. Finally, in phase 5, the silage remains constant (>20 days) and is described as stable and is acceptable to be fed to livestock.

Effect of air on silage quality

Aerobic stability of silage is especially important in dairy production in the Southeast because large dairies may contract for and take delivery of silage for short term (less than two weeks) feeding. The silage may be stored in a pile unprotected, and the hot weather can encourage rapid aerobic deterioration of the silage (Pitt et al., 1991). In properly fermenting silos, the lactic acid producing bacteria (**LAB**) transform water soluble carbohydrates (**WSC**) into organic acids during the ensiling process. As a result, the pH is lowered and the silage is preserved. When the LAB reach at least 10^5 cfu/g of fresh matter, silage is considered to be well-preserved (Cai et al, 1999). In silage that has been exposed to air, LAB do not produce enough lactic acid to sufficiently reduce the pH to a level that inhibits the butyric acid producing bacteria (**BAB**) (clostridia). The resulting silage is of poor quality. Aerobic deterioration is characterized by an increase in temperature and pH caused by the aerobic metabolism of WSC and organic acids by yeasts, molds, and bacteria. During aerobic exposure of corn silage, silage temperature and pH increase while WSC concentrations decrease. This may occur due to the microbial metabolism of lactate and sugars. The decreased WSC may be a result of metabolism by bacteria and the yeast S*accharomyces,* and the pH is increased most likely due to the yeast *Candida* metabolizing lactate (Sanderson, 1992). Ruxton et al. (1975) noted that silages made from forage rich in WSC or silages with large amounts of WSC because of inhibited fermentation, were more susceptible to aerobic deterioration. Okyama et al. (1975), however, did not find such a correlation. Okyama et al. (1975)

suggested that the mechanism involved in aerobic deterioration was more complex and depended on many chemical, physical, and microbiological interactions.

Respiration of plant material and microorganisms results in considerable generation of heat and loss of digestibility, particularly of the protein fraction. During exposure to air, a secondary fermentation (butyric or clostridial) can occur in silage following the primary fermentation (lactic acid). This secondary fermentation is a deterioration characterized by the production of butyric acid from lactic acid and sugars, and the breakdown of proteins and amino acids to amines, amides, and ammonia. The excess presence of these products is characteristic of poor quality silage. Deterioration is accompanied by a loss of residual sugars and the production of ammonia. The measurement of ammonia production can be an indicator of the extent of deterioration because protein breakdown is a consistent result of aerobic spoilage.

When silage is exposed to air, it is prone to deterioration because air promotes the proliferation of aerobic microorganisms that cause the majority of losses in spoiled silage. Current estimates of their detrimental effects on silage loss are most likely underestimated. It is estimated that DM losses may exceed the storage losses of several months (McGechan, 1990). Therefore, aerobic deterioration is a serious problem, and many researchers have begun to discover means to minimize these losses (Weinberg and Ashbell, 1994). Poor quality silage (high pH, high butyric acid and ammonia, low lactic and acetic acid) is stable in air due to high levels of butyric acid, fatty acids, and ammonia which act as preservatives. Silage that does not have a pH of less than 4.0 is

more stable in air because the BAB will produce enough butyric acid to prevent the growth of yeasts which are inhibited by the acid (Gordon, 1966).

Yeasts, fungi, and bacteria have been implicated in causing spoilage of silage. The main organism believed to be responsible for the vulnerability of silage to air is yeast (Woolford et al., 1990). When exposed to air, silages can spoil rapidly due to the growth of yeasts and molds (Kung et al., 2000). The silage's population of yeast will dictate spoilage rate when air is present. According to Daniel et al. (1970), silage with a yeast population exceeding 10^5 cfu /g DM is particularly prone to be lower in quality. The yeasts implicated in aerobic silage deterioration are of two groups: the acid-utilizers and the sugar-utilizers. High DM silages are particularly vulnerable to yeast growth, early increases in temperature, and other characteristics of deterioration. Fungi have also been implicated in the deterioration of silage, including fungi that are thermophilic and thus thrive during the initial heating phase of silage fermentation. The growth of fungi is much slower than yeasts, and fungi do not grow well at a low pH, therefore, the extent to which they influence spoilage is unknown (Woolford et al., 1990). Bacteria may have a secondary role in promoting the spoilage of silage (Woolford et al., 1990). Weise (1963) indicated that their role is terminal in the process of deterioration due to the presence of bacilli in later microflora when the pH has already risen. However, pH may not be uniform throughout silage. There may be pockets in the silage where a high pH promotes the growth of bacteria and thus enables them to initiate spoilage (Woolford et al., 1990).

Covering the Silo to Prevent Aerobic Deterioration

Air poses detrimental effects on silage including a decrease in conservation efficiency, a loss in nutritive value, and an increased risk of health hazards to animals and handlers. Air deteriorates silage during filling of the silo as well as during storage and feedout. In inadequately sealed silos, where the crop is exposed to air, there is growth of silage microorganisms that utilize a large variety of substrates either in the original crop or produced as fermentation by-products (Woolford et al., 1990). If a silo is not properly sealed, air can also spoil silage during the fermentation process. While silage spoilage organisms can proliferate with exposure to minute quantities of oxygen in the silo, there are recommended management practices that can minimize the effects of air on silage. These include avoiding fungal infected forage, minimizing wilting periods to prevent the buildup of aerobic organisms, rapidly filling and packing the silo, application of additives, and adequately packing and sealing the silo. Also, only enough silage needed for feeding should be removed at one time and the use of specialized equipment such as block cutters to properly manage the feeding face which will minimize the exposure of silage to air (Woolford et al., 1990). Silos that are sized to match the needs of the herd and which results in removal of at least six inches daily from the feeding face is recommended to reduce air exposure and deterioration.

Forage in silos is subject to seasonal weather conditions. In addition, silage in horizontal (trench or bunker) silos is affected by other interactions involving the forage's DM content at ensiling, permeability of the silo walls, the surface area which is exposed

to air during filling, the rate of removal, and length of storage, all of which impact the amount of loss that will occur (Bolsen et al., 1993).

Forage is often ensiled without proper coverage of the exposed surfaces. As a result, a substantial spoilage layer may develop on the top of the silo even before feed is removed. Often, on small farms, the amount of spoilage is not thought to be enough to warrant the time and cost of sealing the silage with polyethylene. Dry matter loss in uncovered silos is inevitable (Muck, 1999). McLaughlin et al. (1978) observed DM losses of up to 60% for the top layer of an unsealed bunker silo. Bolsen et al. (1993) evaluated the effects of sealing on small and pilot-scale bunker silos. There was a rapid and profuse spoilage in the upper 33 cm of the silo and increased spoilage in lower depths of the silo. At 90 d post-filling, there was significant DM loss in the uncovered vs. covered silos.

When the silo is not sealed, or when the seal is not effective, air and moisture seep into the silo affecting both the ensiling process and the quality of the silage. Weighted polyethylene sheeting is the most commonly used seal in horizontal silos. Bolsen et al. (1993) found that alfalfa silage that was uncovered deteriorated completely at 25cm and 0 to 33cm in farm and pilot-scale silos. They also found a large loss in DM and OM in unsealed silos. At a silage depth of 75 cm, the DM (%) was 25.5 vs. 35.9 for unsealed and sealed silage respectively. The pH of the top 25cm was 9.58 vs. 4.88 for unsealed vs. sealed silage respectively (Bolsen et al., 1993).

Bolsen et al. (1993) showed temperature differences between sealed and unsealed silage indicating a secondary fermentation had taken place. Unsealed silage had

persistently higher temperatures (10 to 20 degrees higher) than sealed silage regardless of the depth at which it was measured. Woolford et al. (1990) suggested that during this secondary fermentation, oxygen had entered the silage allowing for more favorable conditions for microbial activity. This is in agreement with McDonald et al. (1991) who suggested that the large temperature increase in the top cm of the silage was a result of increased microbial activity and resulting in nutrient loss.

There have been studies conducted to examine the influence of sealing the silo immediately vs. sealing after a delay. Bolsen et al. (1993) showed that sealing after a delay of 7 d greatly improved silage fermentation characteristics when compared to unsealed silage. However, sealing the silo after a delay resulted in silage that was inferior in quality to silage that was sealed immediately after being filled. Organic Matter (% of ensiled) in the top 25cm was 77.1 vs. 66.9 in silos that were filled immediately and filled after a delay respectively. The pH for silage in silos filled immediately was 4.46 compared to an elevated pH of 4.71 in silage when covering was delayed. Lactic acid (% of the silage DM) was 1.31 vs. 0.83 in silos covered immediately and covered after 7 d, respectively. Finally, temperatures were elevated for silos that were delayed in being covered (32 vs. 29 degrees Celsius). Miller et al. (1962) found that a delay in coverage caused an immediate increase in microbial activity due to oxygen availability and increased buffering capacity that resulted in an increased need for fermentation acids to produce stable silage. However, in an experiment by Bolsen et al. (1993) ensiled forage sorghum showed no differences in silage quality for silage sealed immediately vs. silage sealed after 7 d. This data is in agreement with the data of Henderson and McDonald

(1974) who found no differences in DM content, water-soluble carbohydrates, and ADF between sorghum silages sealed immediately vs. after a delay. Therefore, perhaps forage type will dictate whether or not delayed sealing is fully effective or if silage quality is lost during the unsealed time period. Although the DM and OM was lower for silage when there was a delay in covering, there is a benefit in sealing the silo to prevent further losses in nutrients. After sealing, deterioration slowed significantly in farm and pilot-scale silos (Bolsen et al., 1993).

Oelberg et al. (1983) conducted a study to compare the effects of covering silos with or without black plastic. They found that covered silos had lower silage temperatures than uncovered silos (Oelberg et al., 1983). They also showed that DM recovery was higher for covered silos compared to uncovered silos (96.2 vs. 68.0 %, respectively). The covered silos had higher DM recovery indicating superior fermentation as can be seen by a low pH (4.9). The uncovered silo had significantly less DM recovery indicating less fermentation and represented by a high pH (6.8). As would be expected, covering the silo reduced the silage pH more at the top of the silo than at the bottom where air is more limiting. Lactic acid was higher for the covered than the uncovered silo (3.18 vs. 1.68) (Oelberg et al., 1983). McGuffey and Owens concluded that covering alfalfa silage with plastic lowered ensiling temperatures, pH, lactate, and nonprotein nitrogen (1979). The effects of covering the silage were also observed by Oelberg et al. (1983), to reduced non protein nitrogen (**NPN**), acid detergent insoluble nitrogen (**ADIN**), and ADF. Oelberg et al. (1983) observed that heifers fed covered silage gained weight faster than heifers fed uncovered silage. This may be due to the higher level of

energy in covered silage. Covered silage also may have more protein available for rumen microbial protein synthesis (McDonald et al., 1973). Heifers fed covered silage consumed more DM than heifers consuming uncovered silage (Oelberg et al., 1983). In summary, Oelberg et al. (1983) found that covering a silo with black plastic was as or more effective than propionic acid-treated silage or uncovered silage stored in bunker silos.

In an experiment by Hoffman and Ocker (1997), 18 mid-lactation cows were fed fresh control corn (FC) or unstable control corn (UC). The FC was removed from the silo each day before being fed to the herd. The UC was removed from the silo and piled on a concrete floor at the beginning of each experimental period (14 d). The researchers found milk yield to be significantly different ($P < 0.01$). There was no effect on DMI. The authors concluded that aerobic instability of high moisture corn reduced milk yield (Hoffman and Ocker, 1997).

The amount of DM lost during ensiling is a primary indicator for spoilage losses. The most obvious loss is from seepage. Excess water and sap from the forage is squeezed out due to the weight of the silage mass. Water is also the by-product of fermentation, respiration, and oxidation of silage, but it should not contribute significantly to seepage losses. However, in unsealed bunker silos leaching from precipitation can increase seepage losses dramatically (Gordon et al., 1961). Losses of about 50 % have been observed in unsealed or poorly sealed bunker silos. Before the advent of polyethylene covering, sealing the silo required so much time and labor that it was only used in cases of emergency or very large silos. Plastic sheeting, if properly applied, has changed the amount of spoilage associated with horizontal silos, although poor sealing of the silo can

still produce low quality silage. Brown and Kerr (1965) observed a 12 % loss in covered silos and a 70 % loss in uncovered trenches.

Sealing the silo effectively can be attained by weighting down the plastic sheeting. Plastic without continuous weighting is of little value because air leaking through a small puncture can affect the entire surface of the silo.

In a study by McGuffey and Owens (1979), covering the silo reduced silage temperature at various positions within the silo during a 5 week storage period. They found that total nitrogen (TN) was greater in covered bunker silos while NPN nitrogen and ammonia nitrogen was highest in silage from uncovered bunkers. A live animal experiment found that sheep had greater DM, OM, and nitrogen digestibilities when fed silage from a covered bunker silo. Growth and intake of both heifers and bulls favored silage from covered bunkers (McGuffey and Owens, 1979). An explanation of the increased growth could be attributed to an increase in DM as is suggested by Cowan et al. (1956) who found greater DM recovery of silage removed from covered areas than from uncovered areas of the same trench silo. Also, the covered silage that supported more growth had less ammonia-N, thus provided more true protein and amino acids for absorption (McGuffey and Owens, 1979).

Average weekly temperatures of silage in covered bunkers ranged from 29.9 ºC to 31.7 ºC, but were increased in uncovered bunkers (32.0 ºC to 42.8 ºC). Therefore, it was concluded that covering the silo reduced temperature of silage. Silage pH remained constant in covered bunkers during the storage time. However, silage from uncovered bunkers had an average pH of 7.07 after 38 d of storage. Similar amounts of LA were

produced in covered (2.96) and uncovered (3.11) bunkers (McGuffey and Owens, 1979). Total Nitrogen (%DM) was greater for silage from covered (2.77) than from uncovered bunkers (2.61). Non-Protein Nitrogen and ammonia-N was greater for silage from uncovered bunkers. Acid Detergent Insoluble Nitrogen (%DM) was greater in silage from uncovered bunker silos (McGuffey and Owens, 1979). Silage from this bunker also had a higher temperature which is expected due to the positive relationship between these variables. The data suggest that the large amount of heat damaged protein formed is a problem in uncovered silos.

Animal and health risks

Some microorganisms found in spoiled silage may be harmful to livestock (Cai et al., 1999). Although it is not well documented, producers have experienced drastic drops in milk production when cows are fed spoiled and "hot" silage. In a study by Hoffman and Ocker (1997), feeding spoiled high moisture corn caused a 3.2 kg drop in milk production during a 14-d period. Also, moldy silage poses a health threat to animals and humans who handle the feed especially when stored in a closed space such as a silo. There is an increased risk of respiratory infection associated with the handling and feeding of spoiled silage (May et al., 1986). There is also an increased risk of mycotoxicosis from mycotoxins produced by molds in the silage.

Supplementation with viable yeast culture

For many years, ruminant nutritionists have been interested in manipulating the rumen in order to maximize animal efficiency. Microbial feed additives provide soluble

growth factors (i.e., organic acids, B vitamins, and amino acids) that are required for rumen bacteria (Calloway and Martin, 1997). Live yeast supplements release essential enzymes, vitamins, and amino acids during digestion which may have a beneficial effect on rumen efficiency. These benefits may arise as a result of the metabolites or their interactions with rumen microbes. Yeast cultures are known to be best utilized by animals that are under stress. During times of stress, animals have higher nutrient requirements (Arambel and Kent, 1990). Phillips and von Tungelin (1985) fed yeast culture to post-stressed steers and heifers and found that DM intake and ADG significantly increased compared with controls. Dairy cows in early lactation do not consume enough feed to meet their energy requirements to support high milk production which puts them into negative energy balance. The state of negative energy balance puts a large amount of stress on cows (Arambel and Kent, 1990). Therefore, a yeast supplement has been hypothesized to improve production in early lactation dairy cattle. Several factors affect the response of cows to yeast supplementation including stage of lactation, type of forage that is fed, feeding a TMR or not, and the forage to concentrate ratio (Piva et al., 1993).

Effect of yeast supplementation on rumen ammonia

Yeast supplementation has significantly decreased rumen ammonia (Enjalbert et al., 1999). Yoon et al. (1997) found this same phenomenon in continuous *in vitro* fermentors. The decrease in rumen ammonia is thought to be due to a lower degradation of protein in the rumen (Enjalbert et al., 1999). However, other authors have found that rumen protein degradation did not decrease, and increased microbial N flow to the lower

tract is responsible for the decrease in ammonia. The improved microbial activity of incorporating ammonia into microbial protein has been suggested as the mechanism for lowering rumen ammonia (Erasmus et al., 1992).

Ammonia concentrations were less variable in cows consuming yeast (Harrison et al., 1988). Yeast has been hypothesized to stimulate the uptake of nitrogen sources such as ammonia and protein (Wohlt et al., 1998). There have been few studies on the effect of yeast on the flow of N to the lower tract. Karr et al. (1991) found that there were lower flows of microbial N to the lower tract in sheep that were fed a yeast supplement. However, Erasmus et al. (1992) showed that rate of passage of microbial N tended to increase for lactating dairy cattle, and the amino acid pattern was altered in the lower tract. It was found that the yeast supplement caused an increase flow of lysine and methionine- the main limiting amino acids in lactating dairy cattle rations- to the duodenum. The author suggested that the changes in the amino acid profile were due to changes in the rumen microbe population (Erasmus et al, 1992). The increase in the amino acids lysine and methionine may contribute to the increase in milk production that has been seen in some studies. Putnam et al. (1997) found no increase of individual amino acids to the duodenum conflicting with the findings of Erasmus et al. (1992). The authors also found no evidence that yeast supplementation increased microbial protein synthesis or its flow to the duodenum. They did show that slightly more dietary protein escaped rumen degradation that caused higher non-microbial NAN in the duodenum (Putnam et al., 1997).

Effect of yeast supplementation on VFAs and rumen fermentation

Yeast supplementation has been noted to increase VFA concentration, including increasing propionate ratios, and decrease methane production in the rumen (Enjalbert et al., 1999; Piva et al., 1993). Enjalbert et al. (1999) found that yeast supplementation increased the molar percentage of propionate, and decreased the A:P ratio. The changes in the remaining VFAs (isobutyrate, valerate, and isovalerate) have been conflicting. Carro et al. (1992) found decreased proportions of isobutyrate and isovalerate, whereas Harrison et al. (1988) found decreased iosvalerate and increased valerate. These discrepancies may be due more to the experimental diet than yeast supplementation. Kung et al. (1997) found addition of yeast to continuous culture fermentors showed no effect on VFAs. Piva et al. (1993) found rumen VFAs were not significantly different although acetate and A:P ratio tended to be higher in cows consuming yeast supplements Piva et al. (1993) explained this by suggesting the increase in cellulolytic bacteria as a result of yeast providing growth factors for their proliferation. Other authors have seen a decrease in A:P ratio (Harrison et al., 1988) which they explained by differences in methodology including time of sampling, feeding pattern, ration, or DMI (Piva et al., 1993). Harrison et al. (1988) found that cows supplemented with yeast had lower molar proportions of acetate while higher molar proportions of propionate than cows who did not receive yeast. This resulted in a lower A:P ratio for the cows consuming a yeast supplement. Yeast also caused an increase in branched chain acids (isobutyrate, isovalerate, and valerate); however, this was mainly due to an increase in valerate. The total VFA concentrations were not altered by yeast supplementation (Harrison et al.,

1988). The authors stated that this conflicts with other studies although acknowledges the difficulty in comparing results when diets and intakes are different. The pattern of reduction in isoacids may also be due to the decrease in pH (Harrison et al., 1988). Harrison et al. (1988) suggested that the main effect of yeast on lactating dairy cattle is to stabilize in the rumen. They came to this conclusion because cows receiving a yeast supplement showed less variation samples of rumen fluid. Analysis of *in vitro* gas and VFA production showed lower standard errors in animals fed a yeast supplement. They concluded that feeding a yeast supplement causes more stable fermentation in the rumens of cattle (Harrison et al., 1988). However, the authors noted that some of the control feed also had naturally occurring yeast in it, and therefore experimental results may not be clearly determined. These cells were viable and could have resulted in less difference between treatments than previously thought. However, they still concluded that the differences in rumen fermentation and microbial populations existed between the control and yeast supplemented dairy cattle (Harrison et al., 1988).

Effect of yeast supplementation on rumen pH

Kung et al. (1997) found addition of yeast to continuous culture fermentors showed no effect on pH. These results are consistent with the findings of Carro et al. (1992) who showed no effect on fermenter pH, although it has been suggested that this effect may be due to the high buffering capacity of the fermentors masking a change in pH.

Piva et al. (1993) showed that yeast supplements increase rumen pH. Although, Harrison et al. (1988) observed ruminal pH to be lower in cows consuming a yeast supplement. Yeast is hypothesized to provide growth factors such as malate to rumen bacteria that metabolize lactate, and in turn cause improved ruminal pH (Kung et al., 1997).

Effect of yeast supplementation on rumen microbial populations

Yeast supplementation has been hypothesized to alter the rumen microbial population. Putnam et al. (1997) found an increase in the distribution of rods, cones, and spirochetes with little or no change in bacterial concentrations and a 10 to 20% increase in the numbers of protozoa in the rumen digesta with yeast supplementation. Enjalbert et al. (1999) found increased numbers of cellulolytic bacteria which have been hypothesized to aid in fiber digestion.

Acetate formation is due to the fermentation of structural carbohydrates by cellulolytic bacteria. Propionate formation is mainly due to fermentation of non-structural carbohydrates by amylolytic bacteria. Yeast has been shown to increase or decrease populations of total viable bacteria, cellulolytic bacteria, and amylolytic bacteria (Newbold et al., 1996; Erasmus et al., 1992). Authors have shown that yeast supplements have increased cellulolytic bacteria (Piva et al., 1993). However, Yoon and Stern (1996) found that the addition of a yeast supplement increased numbers of proteolytic bacteria. In the majority of trials, an increase in cellulolytic bacteria and no change in amylolytic was observed which should cause an increase in the A:P ratio (Enjalbert et al., 1999).

However, yeast supplementation could reduce amylolytic bacteria and the effect could be mediated by increased protozoa counts (Carro et al., 1992) since protozoa are known to store starch (Enjalbert et al., 1999).

Effect of yeast supplementation on digestibility

Yeast cultures can increase the nutritive value of poor quality forages and high grain diets by increasing the digestibility of the feed. Wiedmeier et al. (1987) observed that DM, CP, and hemicellulose digestibility increased in non-lactating heifers that consumed a yeast culture supplement. This may be due to a shift in cellulolytic bacteria in the rumen (Arambel and Kent, 1990). There have been some other positive effects of yeast supplementation on digestibility. Wohlt et al. (1998) found digestibilities of CP and ADF were increased by supplementing dairy cows with 10 or 20 g/d of active yeast). Williams et al. (1991) observed steers had a better degradation of hay after the addition of a yeast supplement.

There have been several studies that have shown that yeast has no significant effect on digestibility. In a study performed by Arambel and Kent (1990) there was no difference in CP, NDF, or ADF digestibility. The authors hypothesized that they may have used an amount of yeast that was too small to show treatment effects (Arambel and Kent, 1990). Also, Enjalbert et al. (1999) observed no significant differences in the degradation of DM, NDF or ADF from *in situ* experiments. Carro et al. (1992) found no significant differences in ruminal degradation or total tract digestibility of DM or fiber. Also, *in vitro* studies have shown a decrease in cellulose digestibility when a yeast

supplement was added (Harrison et al., 1988). Addition of yeast to the diets of lactating cows did not affect the apparent digestibilities of DM, NDF, ADF, hemicellulose, or starch. This was surprising because Harrison et al. (1988) observed higher numbers of cellulolytic bacteria in the rumen. The authors stated that one possible explanation was that although yeast stimulated the number of bacteria it somehow decreased their activity (Harrison et al., 1988). Jung and Varel (1987) noted that increases in the number of cellulolytic bacteria did not correspond to increases in digestion of cellulose, cell wall, or hemicellulose. *In vitro* data has also shown a decrease in cellulose disappearance in rumen fluid from cows receiving yeast supplement. Total VFAs produced were also lower (Harrison et al., 1988).

Effect of yeast supplementation on intake, growth, and milk production

Yeast supplementation has been hypothesized to increase DMI, growth, and milk production in heifers and lactating dairy cattle. Feeding strategies that help stimulate DMI in early lactation cows are beneficial to high-producing dairy cattle. Supplemental yeast may be most beneficial to dairy cattle if it is fed before parturition and through peak lactation. Yeast may help counteract the decrease in DMI as parturition approaches (Dann et al., 2000).

Dann et al. (2000) found that DMI increased by 2.1 kg/d during the last week of gestation for cows fed a yeast supplement. Cows fed yeast for the last 21 d of gestation showed a tendency to increase DMI. Cows consuming the yeast also maintained greater DMI as parturition approached (Dann et al., 2000). This increase in DMI was also seen

by Wohlt et al. (1991). Again, the increase in DMI caused by yeast supplementation suggests that yeast supplementation is best supplemented during times of increased stress. Dann et al. (2000) best demonstrated this point when they showed that there was an increase in DMI for cows fed yeast for the first 42 DIM, but no significant increase in DMI for cows fed yeast into 140 DIM. Therefore, yeast is most efficacious during early lactation (Dann et al., 2000). During the stressful time of early lactation, there may not be enough soluble growth factors for rumen microbes. Callaway and Martin (1997) suggested that supplemental yeast provide the needed growth factors. Dann et al. (2000) hypothesized that yeast cells may increase fiber digestion, which could increase the rate of passage through the rumen and therefore improve DMI. Putnam et al. (1997) found that addition of yeast caused an increase in DMI (+0.9 kg/d). They did not, however, find any differences in rumen digestibility of OM, ADF, NDF, CP, or NSC. Therefore, they concluded that DMI was increased due to some other unknown mechanism (Putnam et al., 1997).While, Kung et al. (1997) found there were no significant differences in DMI in research animals, these results conflict with the research of Harris et al. (1992) who found DMI was decreased with the supplementation of yeast, and Erasmus et al. (1992) who found DMI increased.

Milk production and DMI have been seen to improve with the addition of a yeast supplement. It has been suggested that a yeast supplement is beneficial to corn silage based rations fed to cows in early-lactation (Wohlt et al., 1998). Dry Matter intake and milk production improved in commercial dairy herds (Shaver and Garrett, 1995). However, the improvements have not been seen in all trials (Schwartz et al., 1994). The

improvements are thought to be due to ruminal fermentation changes and improved digestion. In a large study, where 46 Virginia dairy herds were fed a yeast supplement, 31 herds reported increased milk yield while 15 reported decreased milk production (McGilliard and Stallings, 1998). The milk production for primiparous cows was higher than for multiparous cows (+0.73 vs. +0.56 kg/d) (McGilliard and Stallings, 1998). This may be due to the yeast reducing the stress on primiparous cows which is thought to be greater than for multiparous cows. In a study conducted by Piva et al. (1993), supplemental dietary yeast increased milk production (26.2 vs. 25.4 kg/d) and FCM (23.6 vs. 21.6 kg/d) in midlactation cows. Putnam et al. (1997) also found an increase in milk production though it was attributed to the slight increase in DMI rather than to an increased flow of amino acids to the duodenum. There needs to be more research in order to understand the interactions between yeast supplementation and diet in order to discover whether yeast influences the amino acid profile and the efficiency of amino acid utilization by lactating cows (Putnam et al., 1997). Dann et al. (2000) found that cows fed yeast reached peak milk yield earlier than cows fed control diets (43 vs. 57 DIM). However, this may be a result of the increased DMI during the first 42 DIM (Dann et al., 2000). Dann et al. (2000) found that cows fed yeast did not have significant differences in prepartum or postpartum BCS. Although cows who did not receive the yeast supplement had significantly lower BW during the first 42 DIM than did control cows. Whether this is a yeast effect or a DMI effect is not clear (Dann et al., 2000). Yeast supplements have improved DMI, milk production, milk composition, and BW gain in dairy cattle (Besong et al., 1993; Dawson et al., 1990; Piva et al., 1993). These improvements have been noted

to be from increased numbers of cellulolytic bacteria, improved fiber degradation in the rumen, and/or changes in VFA patterns (Kung et al., 1997).

Several studies have shown that yeast has no effect or depressed milk production. Arambel and Kent (1990) found that daily milk yields and FCM were not significantly improved with the addition of a yeast supplement. Also, the percentage of milk fat, protein, lactose, and solids not fat (SNF) were not significantly different between treatments. BW was unaffected by yeast supplementation (Arambel and Kent, 1990). The addition of yeast had no effect on milk production, composition or FCM of midlactation cows (Kung et al., 1997). However, when the authors fed the supplement to early lactation cows, yeast tended to increase milk production and FCM more than cows fed the control diet (Kung et al., 1997). This may indicate that yeast supplementation is best when used in early lactation when stress is highest. The authors suggested that milk production was increased due to increased amino acid flow to the lower tract when cows were fed a yeast supplement (Kung et al., 1997; McGilliard and Stallings, 1998).

Mannanoligosaccharides

There may be a benefit to adding an oligosaccharide to the diets of animals. In human nutrition, oligosaccharides have been shown to enhance the non-pathogenic microbes in the intestine. In animal research, they are known to adhere to pathogenic bacteria and therefore reduce disease (Spring, 1998). Oligosaccharides have also been shown to increase the digestibility of fiber fractions (Iji et al., 2001).

There are various oligosaccharide products available on the market that are made from isomerization of disaccharides, enzymatic hydrolysis of starch and other polysaccharides, or direct extraction from the cell wall of microbes such as yeast. Oligosaccharides are classified by the type of backbone to which they adhere: mannanoligosaccharides, which are from yeast cell walls, and fructooligosaccharides, synthesized by transfructosylation of sucrose, or hydrolysis of inulin (Iji et al., 2001). The results seen in animal trials probably vary with products and the process in which they were manufactured (Iji et al., 2001).

Yeast cell wall surrounds the yeast cell and helps give the cell its shape and protects the cell membrane which functions for the diffusion and transport of nutrients into, and products out of, the cell. The cell wall accounts for up to 20% of the cell's weight and is composed of glucan, mannan, and small amounts of chitin, protein and lipids (Fleet, 1991). Structural proteins are bound to the polysaccharides. Unsaturated fatty acids are likely part of the cell membrane, which remains adhered to the cell wall.

Isolated mannanoligosaccharides have been used in swine and poultry diets to aid performance and health, especially in animals that are under large amounts of stress due to high production demands (Spring, 1998). The mode of action for mannans is not fully understood; however, dietary mannan is able to prevent attachment of bacterial pathogens with Type I fimbria to the gut wall and has been shown to reduce cecal colonization of salmonella in poultry (Spring, 1998). The pathogens move through the intestine and are excreted into the feces. However, the host still may be able to mount an immunological defense against the bacteria which is bound to the mannan because the barrier would still

possess its antigenic properties (Parks et al., 2000) This is important because it has the ability to help intensely-reared animals in resisting disease and therefore reduce reliance on antibiotics.

Mannanoligosaccharides serve to prevent attachment of gram-negative pathogens, thereby preventing attachment to enterocytes and enteric infection. They are also used in the grain industry to adsorb mycotoxins (Ingledew, 1999).

Yeast cell walls may be a solution to the growing trend to prevent the feeding of antibiotics to livestock while still maintaining optimum growth results. Antibiotics have come under scrutiny by researchers, consumers, and government agencies because of their potential to aid in the development of antibiotic-resistant bacteria. The agricultural industry uses antibiotics as growth promotants in livestock and poultry operations (Parks et al., 2000). Antibiotics suppress microbial growth in the gut, thereby reducing immunological stress. Also, by eliminating the pathogen, antibiotics likely reduce the microbial by-products and toxins that impart negative effects on the energy needs of the animal. Zimber and Visek (1972) reported that microbial products such as ammonia and lactic acid can increase enterocyte division and alter mucosal barriers. The animal in turn must use energy to sustain this high rate of turnover. This energy is then used for maintenance rather than animal growth and production (Parks et al., 2000).

Although mannanoligosaccharides are the cell walls of the yeast *Sacchromyces cerevisiae,* there still may be a beneficial response in the rumen. It is possible that MOS may yield benefits similar to viable yeast cultures.

Summary

Research has shown that protecting silage from deterioration is an important part of quality farm management. Silage exposed to air exhibits many losses in quality including dry matter recovery and nutrient density. Silage management practices to reduce air exposure can effectively reduce silage deterioration.

Mannanoligosaccharides are a major component of cell walls of viable yeast which may influence rumen fermentation, nitrogen metabolism, dry matter digestibility, and most importantly, milk production in lactating dairy cattle. Although there has been seen some benefit to including MOS or viable yeast culture in livestock diets, results are conflicting and may be influenced by the type of diet that is fed. More research is needed in order to appropriate recommendations of supplementation.

In conclusion, proper silage management is an important component of overall dairy management to maximize milk production, protect the health of animals and employees, and to enhance profits. The inclusion of a yeast derived product, MOS, may aid in increasing production or health of livestock.

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RUNNING HEAD: FORAGE QUALITY IN LACTATING DAIRY CATTLE DIETS

The Effects of Feeding Corn Silage that was Exposed to air for Five Days With or Without Yeast Cell Walls on Production Parameters in Early Lactation Holstein Cows

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Abstract

Bolt, Shelley Marie. The Effects of Feeding Corn Silage that was Exposed to Air for Five Days With or Without Yeast Cell Walls on Production Parameters in Early Lactation Holstein Cows. (Under the direction of Dr. B.A. Hopkins and L.W. Whitlow).

Proper silage management is important in reducing excessive spoilage due to air exposure. The objective of this study was to compare the effects on production of feeding silage that was exposed to air for five days and Yeast Cell Walls (**YCW**) to Holstein cows. Forty-eight early lactation cows were randomly assigned at calving to one of four treatment diets within parity. Diets included: Diet 1 corn silage blended into a TMR (**CON**), 2) CON with added Yeast Cell Walls (**CON+YCW**), 3) silage that was exposed to air for five days blended into a TMR (**EXP**), 4) EXP with added Yeast Cell Walls (**EXP +YCW**). Cows were started on trial at 21 days in milk (**DIM**). Cows received the same silage type for the duration of the experiment, but switched YCW treatment at the experimental midpoint (45 d). CON corn silage was stored in a covered trench silo for the length of the study. EXP silage was taken from the same trench silo and piled under a covered shelter for 5 days before being blended into the TMR based on prior DM change. Blood and rumen fluid samples were collected on days 30, 44, 75, and 89 of the experiment and analyzed for parameters important for health and production. There were no significant differences in %CP, %ADF, and Mcal/kg NE_L for treatment diets. Milk yield (35.87, 36.72, 36.58 and 37.07 kg/d), DMI (23.18, 22.09, 23.44, and 23.88 kg DM),

% fat (3.23, 3.22, 3.22, and 3.18%), fat yield (1.16, 1.17, 1.16, 1.19 kg), %CP (2.86, 2.87, 2.80, and 2.79%), and protein yield (1.03, 1.05, 1.02, and 1.03 kg) were not significantly different among CON, CON+YCW, EXP, and EXP+YCW, respectively ($P > 0.10$). Acetate: propionate ratio was not significantly different among treatments CON, CON+YCW, EXP, EXP+YCW, respectively (2.4, 2.3, 2.4, and 2.3; P > 0.10). Concentrations of blood urea nitrogen (**BUN**) (19.8, 20.2, 21.0, and 22.8 mg/dl), rumen ammonia (10.7, 10.9, 9.9, and 9.8 mg/dl), as well as rumen pH (6.9, 6.8, 6.9, and 6.8) were not significantly different among treatments CON, CON+YCW, EXP, and EXP +YCW, respectively ($P > 0.10$). DMI and dietary % CP was significantly lower in the EXP silage ($P < 0.10$). Concentrations of BUN (19.9 vs 21.5 mg/dl; $P < 0.01$) were significantly different for CON and EXP silage, respectively. The addition of YCW significantly lowered acetate concentration (59.03 vs 57.56 mol/100 mol; $P < 0.10$). Dual-Flow continuous culture fermentors were used to conduct an *in vitro* experiment. Ruminal fluid was obtained from a non-lactating Holstein cow and transferred into four fermentors which were fed the four treatment diets from the *in vivo* experiment. The *in vitro* experiment was repeated twice and lasted four five days. Control silage suppressed acetate production while increased the production of propionate and butyrate. Addition of YCW to the CON and EXP treatment diets suppressed methane, acetate, butyrate and total VFA production while increased the concentration of propionate.

(**Key words:** silage, mannanoligosaccharide, fermentors)

Abbreviation key: YCW = yeast cell walls; **CON** = control silage; **CON+YCW** = control silage with the addition of yeast cell walls; **SP** = spoiled silage; **SP+YCW** = spoiled silage with the addition of yeast cell walls; **BUN** = blood urea nitrogen.

Introduction

Proper silage management is important to insure the feeding of high quality silage which will help optimize production and income on commercial dairy farms. Corn silage is an important part of lactating cow diets in many regions of the United States, and often makes up 60% of the total feed in areas such as North Carolina that depends on corn silage as the major dairy forage. In NC, 90% of forage fed to ruminants year-round is silage. Silage has advantages over hay making, such as reduced dependence on weather, increased flexibility of conserving the crop at optimum quality, and the ability to mechanize from harvest to feeding.

Exposure to air is a major contributor to silage spoilage. Often, the top layers of a horizontal silo will become exposed to air and consequently the silage will suffer a dramatic loss in nutrient density. Covering the silo in addition to adding preservatives can help reduce the loss of nutrients. If the silage on top or on the feeding face of the silo is exposed to air, it is beneficial to discard any spoilage to ensure that diets formulated with this silage are accurate in nutrient composition and free of spoilage by-products. Feeding spoiled silage to livestock can severely impact the health and productivity of a dairy herd as well as endanger farm laborers (May et al., 1986). The

feeding of spoiled silage can expose cows to spoilage organisms, biogenic amines, or other by-products which may result in a decrease in milk production or intake. Therefore, it is recommended to discard the spoilage, and more importantly manage the silo to prevent spoilage. Other ways to preserve silage quality include feeding at least six inches off the feeding face daily. Some producers will bring in silage from other sources other than their own farm. The producer will generally get enough silage to feed the herd for one to two weeks. The result of this is usually a distinct pattern of increasing and decreasing milk production as fresh silage is fed and as the imported silage ages.

Yeast cultures have been studied for their beneficial effects on rumen fermentation and the possibility of antibiotic-like growth promotion. Mannanoligosaccharides are a major component of the cell wall portion of yeast and have been suggested to improve ruminal fermentation (Erasmus et al., 1992; Enjalbert et al., 1999), nitrogen metabolism (Erasmus et al., 1992; Wohlt et al., 1998), fiber digestibility (Dann et al., 2000; Weidmeier et al., 1987), dry matter intake (**DMI**), and milk production (Shaver and Garrett, 1995; Piva et al., 1993). However, these benefits have not been seen in all experiments.

The rationale behind this experiment is that feeding silage that was allowed to spoil for five days may have deleterious organisms in it that may negatively influence production in early-lactation Holstein cows. Therefore, since YCW has been indicated to bind and remove harmful organisms from the gastrointestinal tract, the unwanted

pathogens in the exposed silage should have minimal effects on the production of the cows.

This experiment was designed to use early lactation dairy cows, fed diets consisting of either a fresh control silage or silage that was allowed to spoil for 5 days; with or without the addition of a product containing a mannanoligosaccharide top dressed on a TMR. The experiment was designed to be a modified split-plot design where cows remained on the same type of silage (control or exposed) while the mannanoligosaccharide product was either added or not added to the experimental diet. Milk production, intake, digestibility, and rumen parameters were measured *in vivo* as well as *in vitro* rumen parameters in dual-flow continuous culture fermentors to determine the effects of silage and the mannanoligosaccharide product on early lactation dairy cattle.

Materials and Methods

Diets and cow management

Forty-eight Holstein cows from the Piedmont Research Station in Salisbury, NC were randomly assigned at calving to one of four treatment groups within either primiparous or multiparous groups. At calving, cows were trained to Calan® feeding stations (American Calan Inc., Northwood, NH). At 21 days in milk (**DIM**), cows were fed one of four experimental diets in a total mixed ration (**TMR**) that was fed twice a day through 90 DIM.

The treatment diets were corn silage based and contained 24% ADF and 1.42 Mcal/kg NE_L on a DM basis. Control silage was stored in a covered trench silo. The exposed silage was taken from the same trench silo and piled under a covered shelter for 5 days before being fed and then included in the TMR based on prior change in DM. The mannanoligosaccharide product or Yeast Cell Walls (**YCW**) was top-dressed onto the respective TMRs. Diets included 1) corn silage blended in a TMR (**CON**), 2) Con with added yeast cell walls (**CON+YCW**), 3) silage that was allowed to spoil for five days blended in a TMR (**EXP**), and EXP with added yeast cell walls (**EXP+YCW**). Cows received the same silage type (control or exposed) for the duration of the experiment, but switched to or from YCW treatment at the experimental midpoint (45 days) around 66 DIM.

Cows were housed in a free stall barn and fed to supply approximately 10% orts on an as-fed basis. The daily feed allocation and orts were recorded for each cow to calculate intakes of DM. Feed and orts were analyzed for CP and ADF.

Sample Collection and Analysis

The TMR's fed for each of the four treatment diets were sampled once a week and composited monthly. Whole cottonseed, corn grain, and soybean meal were sampled once for each shipment of ingredient. Ingredient and TMR samples were analyzed for DM, CP, and ADF by the Constable Laboratory (North Carolina Department of Agriculture, Raleigh, NC). The ingredient composition of the treatment diets are reported in Table 1. Temperatures for CON and EXP silage were taken twice

daily at 0900 and 1400 EST, and averaged for a daily silage temperature for each silage type.

Silage, TMRs, cottonseed hulls, and corn grain were analyzed for the mycotoxins Aflatoxin, Deoxynivalenol, Zearalenone, T-2 toxin and Fumonisin at the Mycotoxin Laboratory (North Carolina State University, Raleigh, NC).

Cows were milked twice daily with milk weights recorded at each milking. Milk samples were collected once weekly from consecutive AM and PM samples and composited into one sample. These samples were analyzed for fat, CP and MUN by the United Federation of DHIA Laboratory (Blacksburg, VA). The Bentley ChemSpec® 150 analyzer (Chaska, MN) was used to determine MUN concentration.

Blood samples were collected approximately five hours post-feeding on 30, 44, 75, and 89 days after the treatment diet was begun. Blood was collected via jugular venipuncture into vacutainers containing EDTA and placed on ice for transport to the laboratory. These samples were sent to Antech Diagnostics Laboratory (Atlanta, GA) and analyzed for blood urea nitrogen (**BUN**). Ruminal fluid was also collected approximately five hours post-feeding on 30, 44, 75, and 89 days on trial with a stomach tube connected to a vacuum pump, analyzed for pH, then placed on ice for transport and frozen until analysis. Ruminal fluid was allowed to thaw then centrifuged at 2500 x *g* for 10 minutes at 4° C and the supernatant was removed. Ruminal fluid supernatant was analyzed for ammonia (Beecher and Whitten, 1970). To prepare rumen fluid for VFA analysis, 1 ml of 25% metaphosphoric acid with an internal standard was added to 5 ml of supernatant and centrifuged at room temperature (approximately 22°

C) for 15 minutes at 9300 x *g*. A 1 ml aliquot was removed and analyzed for VFA by gas chromatography (Model CP-3380: Varian, Walnut Creek, CA).

Body weights were recorded weekly through the trial. Cows were body condition scored and the scores were recorded weekly using the guidelines of Ferguson et al. (1994).

In Vitro Fermentation using Dual-Flow Continuous Cultures

Ruminal fluid was obtained from a fistulated non-lactating Holstein cow fed a diet consisting of 100% forage. Ruminal contents were filtered through double-layered cheesecloth and transported to the laboratory in a sealed, preheated thermos. Strained rumen fluid was transferred into fermentors (Teather and Sauer, 1988) with a continuous overflow system at a nominal volume of 700 ml. Prior to the addition of ruminal culture, CO_2 gas was purged through the fermentors to displace O_2 ; the flow of CO_2 was maintained at 20 ml/min throughout the experiment. The pH of the culture was monitored and the temperature was kept constant by a circulating water bath set at 39º C. Ruminal contents were stirred (Fisher Scientific, Yamato Scientific, Japan) continually at 10 rpm. Artificial saliva (pH 6.8) was prepared by mixing two solutions as outlined by Slyter et al. (1966). The concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} in the saliva were 90.5, 4.8, 0.1, and 0.2 m*M*, respectively. Saliva was delivered continuously using a precision pump (Watson Marlo, UK) set at a flow rate of 0.73 ml / min. During a 24-h period, a total of 1.1 L of buffer (saliva) was added to 700 ml of ruminal culture to yield a fractional dilution rate of 6.3% / h.

Feed was frozen and ground through a Wiley Mill fitted with a 6 mm screen. The feed consisted of the four separate treatment diets that were used in the lactation study. Approximately 15 *g* of feed as-fed basis was added daily; 7.5*g* at 0730 and 1630 EST.

Following addition of the ruminal fluid, fermentors were allowed to stabilize for 48 h.

Each fermentor represented a treatment diet (there were four fermentors) and the trial lasted 5d (2d stabilization and 3d sample collection). The trial was repeated twice to provide replication.

Samples of rumen culture were obtained after stabilization (d 3, 4, and 5). Methane was recorded five times daily and averaged to arrive at one daily methane output. Gas samples were withdrawn into a 0.10-ml Gastight® syringe (Hamilton Co., Reno, NV) from one fermentor exit port that was sealed with a butyl rubber stopper (Sauer, 1987). Samples were analyzed with a gas chromatograph (model 3000 CX; Varian, Walnut Creek, CA). Prior to sampling, the contents of the fermentors were thoroughly mixed by manual agitation. Volatile fatty acids were analyzed by gas chromatography (Sauer and Teather, 1987). Ammonia nitrogen was analyzed by a colorimetric assay (Beecher and Whitten, 1970). On d 3, 4, and 5 samples were taken from each fermentor and analyzed for DM and ADF.

Silage Characteristics

Silage was analyzed for temperature, pH, water soluble carbohydrates (Dubois et al., 1956), volatile fatty acids, and ADF.

Statistical Analysis

This experiment used a modified split-plot arrangement of treatments with silage, YCW, parity and order as the main factors. All combinations of interactions were examined. Data recorded as repeated measures were analyzed using the mixed procedure of SAS® as recommended by Littell et al. (1994). Therefore, feed, ruminal fluid, blood, milk, and silage data were analyzed using the mixed procedure . Fermentor data were analyzed using the general linear models procedure of SAS® for a completely randomized design with a factorial arrangement of treatments and two replicates. Model effects included silage, yeast, and silage x yeast. The fermentor data was analyzed using the GLM procedure.

Results and Discussion

Intake

There were no significant differences for the interaction silage by YCW on daily DM, CP, and ADF intakes (Table 2). Exposed silage significantly increased DMI

kg/d and was lower in % CP than CON silage (Table 2). There was no effect of adding YCW to the diets. This is in agreement with Kung et al. (1997) who found there was no difference in DMI with the addition of yeast, but refutes the work of other authors. Harris et al. (1992) found that DMI decreased, while Erasmus et al. (1992) found that DMI increased as a result of feeding a yeast supplement.

As expected, there was a significant parity effect on intake, with multiparous cows consuming more DM ($P < 0.01$) than primiparous cows, resulting in higher intakes of CP and ADF ($P < 0.01$) in multiparous cows than primiparous cows (Table 2). For all intake measures, the interaction between silage and YCW, silage and parity, and YCW and parity was not significant.

Body Weights and Body Condition Scores

There were no significant differences in mean BW, mean BCS, or BW change for the effects of silage, YCW, and order. However, Dann et al. (2000) found that BW did increase for cows supplemented with yeast during the first 45 DIM. There were also no significant differences in the above with regards to the interaction silage and YCW (Table 2). However, there was a significant parity effect ($P < 0.01$) for BW with primiparous cows weighing less than multiparous cows. There was no interaction between silage and YCW, silage and parity, and YCW and parity.

Milk Yield and Composition

As illustrated in Table 2, milk yield, milk CP yield, milk fat yield and milk fat % were not affected by silage or YCW. As a result, 4% FCM yield and feed efficiency, reported as FCM/DMI, were also not significantly different. Therefore, exposed silage and the addition of YCW had no effect on the yield of milk, milk fat, or milk CP compared to control silage without YCW. In a study by Hoffman and Ocker (1997), they reported significant milk production losses when aerobically unstable corn was incorporated into diets. This contradicts the finding in this study that feeding spoiled corn silage to lactating cows did not elicit a response in milk production. It is hypothesized that the corn silage used in this study was not allowed to reheat during the winter months and therefore may skew data. Silage temperatures were significantly higher in EXP silage than CON silage ($P < 0.01$) for all months except December and January ($P > 0.10$). Therefore, during these months, the silage did not show significant spoilage. In a number of studies, milk yield has been increased by yeast supplementation (Shaver and Garrett, 1995), although not in all experiments (Arambel and Kent, 1990). The MUN concentration was not significantly different for silage, YCW, and the interaction silage and YCW (Table 2).

Multiparous cows had higher yields of milk (41.36 kg/d), milk fat (1.29 kg/d) and milk CP (1.15 kg/d), than primiparous cows (31.76, 1.04, 0.91 kg/d, respectively) ($P < 0.01$). Although primiparous cows produced less milk, there was no parity effect on milk fat % and milk CP %. Multiparous cows had significantly higher MUN (20.17,

mg/dl) compared to primiparous cows (18.36, mg/dl) ($P < 0.02$). In addition, there was no silage by YCW by parity interaction for milk yield and composition.

Ruminal Fluid Measures

The interaction silage by YCW did not result in significant differences in acetate to propionate ratios, total VFA production or in molar proportions of rumen acetate, propionate, isobutyrate, butyrate, isovalerate or valerate (Table 3). There was a significant difference in the molar proportion of acetate with respect to YCW. The addition of YCW decreased the molar proportion of acetate compared to the treatments without YCW (57.57 and 59.01, respectively) ($P < 0.07$). Harrison et al. (1988) also observed a decrease in the molar proportion of acetate. It is hypothesized that the YCW may be adhering to the structural carbohydrate utilizing bacteria and transporting them out of the rumen, therefore suppressing acetate production, or YCW may be adhering to the cell wall of the structural carbohydrate preventing the bacteria from also binding.

There was a significant parity effect for propionate and acetate to propionate ratio with multiparous cows producing more propionate than primiparous cow (26.36 vs 25.46, respectively) ($P < 0.07$) and having a lower acetate to propionate ratio (2.30 vs 2.42, respectively) ($P < 0.08$) (Table 3).

Fermentors (*in vitro*)

Silage

There was no significant difference in methane production for CON and EXP silage (12.80 Mmol/d and 13.66 Mmol/d, respectively). There were also no significant differences for ammonia (mg/dl), pH, or ADF (%) for CON and EXP silage (Table 4).

The concentration of acetate was significantly higher for EXP silage than CON silage, although, acetate production (Mmol/d) was not significantly different for silage type (Table 5). Propionate production was significantly increased in the CON silage (7.50 Mmol/d) when compared to EXP silage (6.72 Mmol/d) ($P < 0.03$), although concentration of propionate was similar for silage type (Table 4). Silage type did not affect did not affect ruminal isobutyrate. Butyrate production was significantly lower for EXP silage (9.41 Mmol/d) than CON silage (11.04 Mmol/d) ($P < 0.02$). The same trend was observed for butyrate concentration. This was unexpected, for, it was hypothesized that butyrate would be increased in the EXP silage as it was exposed to air and clostridial organisms were presumed to proliferate increasing butyrate production and concentration (Woolford et al., 1990). Isovalerate concentration was significantly lower in EXP silage (2.29 %) than CON silage (2.71 %) ($P < 0.01$). Isovalerate production was also suppressed with EXP silage (1.05 Mmol/d) being significantly lower than CON silage (1.36 Mmol/d) ($P < 0.01$). Valerate concentration for CON silage (1.87 %) was significantly higher than EXP silage (1.77 %) ($P < 0.10$). Valerate production was also suppressed in EXP silage (0.82 Mmol/d) when compared to CON silage (0.95 Mmol/d) ($P < 0.04$). Finally, EXP silage (45.6 Mmol/d) showed

an over all significant reduction in total VFA production than CON silage (50.92 Mmol/d) ($P < 0.05$). However, the acetate to propionate ratio was not significantly different for silage type (4.04 and 4.07) for CON and EXP, respectively (Table 5). This data suggests that allowing silage to spoil may limit the fermentability of the diet in the rumen of the animal.

Yeast Cell Wall

The inclusion of YCW significantly decreased methane production for +YCW (12.31 Mmol/d) compared to the treatment –YCW (14.15 Mmol/d) ($P < 0.01$) (Table 4). Enjalbert et al. (1999) also saw a reduction in methane when yeast was added to the diet. Acetate production was significantly decreased when YCW was added to the diets (58.98 Mmol/d and 58.71 Mmol/d; $P < 0.01$), although the concentration of acetate remained constant. This is in contrast to research by Piva et al. (1993) who found acetate tended to be higher in cows fed a yeast supplement. Propionate production was significantly decreased when YCW was added to the diet (7.29 Mmol/d and 6.77 Mmol/d; $P < 0.06$); however, propionate concentration was significantly increased with the addition of YCW (14.11 % and 15.31 %; $P < 0.08$). The increase in the molar percentage of propionate is in agreement with Enjalbert et al. (1999) who found that in addition to propionate concentration increasing, the A:P ratio decreased. The data suggest that adding YCW to the diet may shift the population of microorganisms in the rumen in favor of propionate-producing microbes. Isobutyrate production and concentration was not affected by YCW treatment. Butyrate production was

significantly decreased when YCW was added to the diet (11.26 Mmol/d and 9.20 Mmol/d; $P < 0.01$) while butyrate concentration was also significantly lower with the addition of YCW (21.6 % and 20.6 %; $P < 0.01$). The production and concentration of isovalerate was unaffected by YCW (Table 4). Valerate production decreased significantly when YCW was added to the diet (0.95 Mmol/d and 0.82 Mmol/d; $P < 0.04$) while change in valerate concentration was not significantly different (Table 4). The effect of yeast supplementation on the isoacids has been variable. Carro et al. (1992) found that the isoacids decreased, while Harrison et al. (1988) found decreased levels of isovalerate, but increased amounts of valerate. The addition of YCW to the treatment diet suppressed total VFA production (51.96 Mmol/d and 44.56 Mmol/d; $P < 0.01$) for +YCW and –YCW, respectively. Harrison et al. (1988) found that yeast supplementation had no effect on total VFA concentration. Although total fermentability of the diet appeared to decrease with the addition of YCW, YCW did increase propionate concentration as a percentage of total VFAs and also suppressed the production of butyrate. The acetate to propionate ratio for +YCW (3.88) and –YCW (4.23) showed a trend to decrease with the addition of YCW ($P < 0.11$). The tendency for the decrease in the A:P is in agreement with results found by Harrison et al. (1988). Ammonia (mg/dl), pH, and ADF (%) was unaffected by YCW treatment (Table 4). Yeast supplementation has been shown to decrease rumen ammonia concentrations (Enjalbert et al., 1999; Yoon et al., 1997). The consistency of the rumen pH is in agreement with Kung et al. (1997) who found there was no effect of pH when

yeast was added to continuous culture fermentors although, this may be due to the buffer added to the culture.

The interaction of silage by YCW was significant only for propionate production with CON (7.90 Mmol/d) significantly higher than CON+YCW, EXP, and EXP+YCW (6.80 Mmol/d, 6.70 Mmol/d, and 6.74 Mmol/d, respectively; $P < 0.05$) (Table 4).

Silage Characteristics

Silage temperature was significantly higher in EXP silage (30.1 ºC) than CON silage (22.5 ºC) ($P < 0.01$). This same trend was seen for silage pH, WSC (g / 100g DM), and silage ADF (%) (Table 5). The above variables were all significantly higher for EXP silage when compared to CON silage ($P < 0.01$). The increase in temperature was expected because as silage is exposed to air it will reheat as microorganisms rapidly increase in number (Woolford et al., 1990). McGuffey and Owens (1979) also showed an increase in silage temperature for silage left uncovered compared to silage in covered bunker silos. Also, silage which has been exposed to air will often show an increase in pH as aerobic microorganisms decrease the production of acetate and increase the production of butyrate. Silage that has been uncovered also had elevated pH in other studies (McGuffey and Owens, 1997). The increase in WSC in EXP silage was unexpected because microbes use the WSC for growth. However, no microbial counts or analyses were performed; therefore, there may have been organisms in the silage that used substrates other than sugars or produced sugars as a by-product of

fermentation. The decrease in ADF (%) was expected because as silage is exposed to air, it will increase in moisture loss and as a result increase in proportion of ADF.

Acetate concentration was significantly higher in CON (14.27 %) than EXP (9.46 %) silage ($P < 0.01$) while propionate was significantly lower for CON (2.05 %) than EXP (2.57 %) silage ($P < 0.01$). Butyrate was significantly higher in EXP silage (0.27 %) than CON silage (0.13%) ($P < 0.01$). There were no significant differences between CON and EXP silage for isobutyrate, isovalerate, and valerate (Table 2). The increase in acetate in CON silage is possible because of the low pH of the CON silage. A low pH indicates the presence of either acetic or lactic acid. The increase in propionate in the silage may be a coincidence resulting from a lower acetate concentration and a higher concentration of butyrate. Finally, butyrate was expected to increase because spoiled silage has been associated with higher levels of butyrate as butyric acid- producing bacteria increase in numbers which is allowed to proliferate with increasing pH in the silage due to air exposure and reduction in acetate concentration (Woolford et al., 1990).

The silage was analyzed for mycotoxin inclusion and no significant amounts of Aflatoxin, T-2, Zearalenone, or Fumonisin were reported.

Conclusions

In this study, there was no significant effect of silage or YCW treatment on milk production or milk constituents. Cows consuming the EXP ration consumed significantly more DM although the EXP ration was significantly lower in % CP.

Intake of EXP diets may be related to a high pH in the EXP silage as compared to CON (Erdman et al., 1993). In the *in vivo* study, silage type had no effect on VFA production in the rumen although BUN was higher indicating increased proteolysis of protein to free amino acids in the EXP silage. The addition of YCW significantly decreased acetate concentration in the rumen while having no effect on propionate.

In the *in vitro* studies, the continuous culture fermentors showed that CON silage had lower concentrations of acetate while increasing the production of propionate and butyrate. Overall, CON silage had a higher level of total VFAs. This data conflicts with the silage characteristics that were analyzed for in the two silages. The characterization of the CON silage showed it to have more acetate, but less propionate and less total butyrate. The CON silage also had a lower temperature indicating it did not undergo an extensive reheating process. Therefore, the CON silage would be classified as the superior silage, but the EXP silage characteristics did not suggest that it was greatly deteriorated.

The YCW seemed to suppress fermentation of the diet in the continuous culture fermentors. There was significantly less methane, acetate, butyrate, and total VFA production; however, the addition of YCW increased the propionate concentration which may be of benefit to the animal which uses propionate for glucose synthesis. The decrease in methane is beneficial to the animal by decreasing energy losses in the form of gas. The decrease in methane correlates with the increase in the molar percentage of propionate because hydrogen which would be released to the environment may be incorporated into the production of propionate. This results in a more efficient use of

energy for the animal. The depression seen in fermentation may be due to a shift in rumen microbe populations when YCW is added to the diet. The mechanism responsible for this decreased fermentability of the diet is not known.

Based on the *in vitro* data on the characteristics of the CON and EXP silage, silage that was allowed to spoil for 5 days was of lower quality than CON silage although the EXP silage may not have been diluted enough in nutrients to see an impact on milk production or milk constituents. In addition, the EXP silage was already well-preserved which probably reduced the spoilage when it was exposed to air. The EXP silage is also thought to be of lesser quality due to its lower amounts of acetate and its higher amounts of butyrate, a higher pH, and an elevated temperature. Silage that is exposed to air may also contain mycotoxins which may negatively affect the health of the herd. Therefore, it is suggested not to feed silage that has been exposed to air for five days to lactating dairy cattle.

The addition of yeast cell walls has been indicated to aid dairy cattle in the beginning of lactation when stress can suppress milk production and DMI. The addition of YCW to these diets did not seem to benefit early lactation cows when compared to controls. Also, the addition of YCW was shown to decrease fermentability of the diet in *in vitro* studies. However, yeast supplementation decreased methane production which is beneficial to the animal and the environment as well as increased the molar percentage of propionate indicating a more efficient use of energy.

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Table 1. Ingredient and nutrient composition of CON, CON+YCW, EXP, and EXP+YCW.

Item	Diet[1]
Ingredients, % of the DM	
Corn silage[2]	42.9
Whole Cottonseed	15.6
Corn grain	21.7
48% soybean meal	16.7
Sodium bicarbonate	0.62
Salt	0.34
Dicalcium phosphate	0.67
Calcitic limestone	1.30
Vit-TM premix[3]	0.06
Dyna-Mate[4]	0.09

[1]CON = Control silage; CON+YCW= Control silage with Yeast Cell Walls; EXP= Exposed silage; EXP+YCW= Exposed silage with Yeast Cell Walls.

[2] Control silage and Exposed silage were fed in the same amounts and contained the same DM%.

[3]Vitamin-trace mineral premix. Contained 21.5% Ca; 5.5% S; 3.87% Zn; 3.87% Mn; 1.18% Cu; 9650 ppm Fe; 700 ppm I; 590 ppm Co; 250 ppm Se; 1,215,420 IU/kg Vitamin A; 304,545 IU/kg Vitamin D-3; 3,646 IU/kg Vitamin E.

[4]IMC-AGRICO, Bannockburn, IL.

Table 2. ***In vivo.*** Least squares means for nutrient composition, daily intake, milk yield, milk composition, body weight and body condition score as affected by silage by YCW, and parity for cows consuming control or exposed silage with or without the addition of a Yeast Cell Wall product.

	Dietary Treatments[1]					Effect ($P<$)			
Item	CON	CON+YCW	EXP	EXP+YCW	SEM[5]	Silage	YCW	Silage x YCW	Parity
Intake									
DM, kg/d	23.18	22.09	23.44	23.88	0.50	0.10	NS	NS	0.01
DM, %	48.80	49.00	49.20	49.80	0.25	0.05	0.03	NS	NS
CP, kg/d	3.86	3.67	3.77	3.83	0.08	NS	NS	NS	0.01
CP, %	15.64	15.65	15.09	15.06	0.06	0.01	NS	NS	0.09
ADF, kg/d	6.10	5.78	6.08	6.24	0.18	NS	NS	NS	0.01
ADF, %	24.7	24.5	24.2	24.3	0.34	NS	NS	NS	NS
NE_L[2], Mcal/d	39.4	37.6	39.9	40.6	0.9	0.10	NS	NS	0.01
Milk									
Yield, kg/d	35.87	36.72	36.58	37.07	1.22	NS	NS	NS	0.01
CP, %	2.86	2.87	2.80	2.79	0.05	NS	NS	NS	NS
CP, kg/d	1.03	1.05	1.02	1.03	0.03	NS	NS	NS	0.01
Fat, %	3.23	3.22	3.22	3.18	0.10	NS	NS	NS	NS
Fat, kg/d	1.16	1.17	1.16	1.19	0.05	NS	NS	NS	0.01
4% FCM, kg/d	31.89	32.26	32.03	32.82	1.08	NS	NS	NS	0.01
MUN[3], mg/dl	19.23	19.11	19.06	19.66	0.58	NS	NS	NS	0.02
FCM/DMI, kg/kg	1.39	1.42	1.39	1.38	0.05	NS	NS	NS	0.04
BW, kg	563.8	564.7	557.1	558.2	10.20	NS	NS	NS	0.01
BCS[4]	2.22	2.24	2.13	2.12	0.05	NS	NS	NS	NS

[a,b,c]Means in a row with different superscripts differ ($P < 0.10$).

[1]CON = Control silage; CON+YCW= Control silage with Yeast Cell Walls; EXP= Exposed silage; EXP+YCW= Exposed silage with Yeast Cell Walls.

[2]Calculated from *Nutrient Requirements of Dairy Cattle* (2001).

[3]MUN = milk urea nitrogen.

[4]Body condition score (five-point scale where 1 = very thin to 5 = obese (Ferguson 1994).

[5]SEM is only for the interaction silage*YCW.

Table 3. ***In vivo***. Plasma urea nitrogen, ruminal ammonia, and ruminal VFA as affected by silage, YCW, silage by YCW, and parity for cows consuming control or exposed silage with or without addition of a Yeast Cell Wall product.

	Dietary Treatments[1]					Effect (*P*<)			
Item	CON	CON+YCW	EXP	EXP+YCW	SEM[3]	Silage	YCW	Silage x YCW	Parity
BUN[2], mg/dl	19.8	20.2	21.0	22.8	0.75	0.003	NS	NS	NS
Rumen NH_3, mg/dl	10.7	10.9	9.9	9.8	0.82	NS	NS	NS	NS
Rumen pH	6.9	6.8	6.9	6.8	0.07	NS	NS	NS	NS
Total VFA, m*M*	88.5	91.8	91.4	84.8	3.40	NS	NS	NS	NS
Acetate:Propionate	2.4	2.3	2.4	2.3	0.07	NS	NS	NS	0.08
VFA, mol/100 mol									
Acetate	59.3	57.92	58.75	57.2	0.75	NS	0.07	NS	NS
Propionate	25.35	26.02	25.76	26.50	0.54	NS	NS	NS	0.07
Isobutyrate	0.94	0.92	0.93	1.04	0.04	NS	NS	NS	NS
Butyrate	11.2	11.9	11.3	11.9	0.36	NS	NS	NS	NS
Isovalerate	1.79	1.76	1.69	1.85	0.05	NS	NS	NS	NS
Valerate	1.43	1.50	1.55	1.54	0.05	NS	NS	NS	NS

[a,b,c]Means within a row with different superscripts differ ($P < 0.10$).

[1]CON = Control silage; CON+YCW= Control silage with Yeast Cell Walls; EXP= exposed silage; EXP+YCW= exposed silage with Yeast Cell Walls.

[2]BUN = Blood urea nitrogen

[3]SEM is only for the interaction silage*YCW.

Table 4. ***In vitro.*** Least squares means for methane, pH, ADF, ruminal ammonia and ruminal VFA as affected by silage, YCW, and silage by YCW for control or exposed silage with or without the addition of a Yeast Cell Wall product.

	Dietary Treatments[1]					Effect (*P*<)		
Item	CON	CON+YCW	EXP	EXP+YCW	SEM[2]	Silage	YCW	Silage x YCW
Methane, Mm/d	13.5	12.1	14.8	12.5	1.09	NS	0.01	NS
Acetate, Mm/d	32.3	27.0	29.0	25.3	1.77	NS	0.01	NS
Acetate, molar %	58.1	58.1	59.8	59.3	0.65	0.05	NS	NS
Propionate, Mm/d	7.90[a]	6.80[b]	6.70[b]	6.74[b]	0.49	0.03	0.06	0.05
Propionate, molar %	14.4	14.8	13.9	15.8	0.67	NS	0.08	NS
Isobutyrate, Mm/d	0.57	0.50	0.50	0.43	0.06	NS	NS	NS
Isobutyrate, molar %	1.02	1.07	1.03	0.98	0.07	NS	NS	NS
Butyrate, Mm/d	12.22	9.87	10.30	8.53	0.62	0.02	0.01	NS
Butyrate, molar %	22.0	21.2	21.3	19.9	0.59	0.01	0.01	NS
Isovalerate, Mm/d	1.43	1.30	1.10	1.00	0.12	0.01	NS	NS
Isovalerate, molar %	2.59	2.83	2.27	2.31	0.15	0.01	NS	NS
Valerate, Mm/d	1.03	0.88	0.88	0.75	0.09	0.04	0.04	NS
Valerate, molar %	1.85	1.89	1.81	1.74	0.11	0.10	NS	NS
Total VFA, Mm/d	55.5	46.4	48.4	42.8	2.88	0.05	0.01	NS
A:P ratio	4.1	4.0	4.3	3.8	0.21	NS	0.11	NS
Ammonia, mg/dl	8.03	6.72	10.27	8.10	1.61	NS	NS	NS
pH	6.23	6.08	6.25	6.24	0.14	NS	NS	NS
ADF, %	34.8	46.4	43.0	44.0	5.11	NS	NS	NS

[a,b,c]Means within a row with different superscripts differ (P<0.05).
[1]CON= Control silage; CON+YCW = Control silage with Yeast Cell Walls; EXP = Exposed silage; EXP+YCW = Exposed silage with Yeast Cell Walls.
[2]SEM is only for the interaction silage*YCW.

Table 5. Silage Characteristics. Least squares means of pH, water soluble carbohydrates for Control and Exposed silage.

Item	Corn Silage Type[1]		SEM	Effect (*P*<)
	CON	EXP		
pH	3.90	4.16	0.01	0.01
WSC, g/100g DM	2.58	3.38	0.02	0.01
CP, % DM	7.69	7.47	0.03	0.01
ADF, % DM	27.94	29.43	0.01	0.01
VFA, mol/100 mol				
Acetate	14.27	9.46	0.44	0.01
Propionate	2.05	2.57	0.07	0.01
Isobutyrate	0.00	0.02	0.02	NS
Butyrate	0.13	0.27	0.01	0.01
Isovalerate	0.00	0.08	0.04	NS
Valerate	0.03	0.04	0.03	NS
Temperature, ° C	22.5	30.1	0.8	0.01

Significance was reported at the (P<0.01) level of significance.
[1]CON= Control silage; EXP= Exposed silage.
Mycotoxins (Aflatoxin, Deoxynivalenol, Fumonisin, T2, and Zearalenone) were below levels of concern.