EFFECT OF ADDITIVES ON IN VITRO FORAGE DIGESTION AND PERFORMANCE OF CATTLE FED FOUR DIFFERENT SILAGES

by

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(Under Direction of Gary M. Hill and Scott A. Martin)

ABSTRACT

Temperate corn silage is often produced, but increased land utilization may occur if heat and drought resistant alternative forages are ensiled during late summer and autumn for use as primary feeds for growing beef and dairy cattle. Our objectives were to determine the effects of inoculant and additive treatments on chemical composition and fermentation characteristics of temperate corn (CS), pearl millet (PM), tropical corn (TC), and sorghum (S) silages; and, the effects of silage type and inoculant treatment on intake, gain and digestibility of the silages fed to growing beef cattle. All silages were low in DM, and inoculants did not uniformly improve fermentation or digestibility of silages. Extremely low concentrations of lactic and malic acids were observed for PM compared with other silages. Chemical and fermentation data indicated that addition of corn grain at ensiling improved fermentation on TC, PM and S silages. In vivo digestibility was highest for CS and lowest for PM; however, when the four silages with Sun-Cure inoculant were supplemented with energy and protein similar OM, ADF and NDF digestion of total mixed diets was observed. Feedlot results indicated that cattle had lower DM intake ($P < 0.07$) and lower daily gains ($P < 0.01$) on PM compared with

CS when fed silages with energy and protein supplements. Inoculation with Sun-Cure did not improve cattle performance. Results indicate that PM silages are lowest in digestibility, and that substantial amounts of energy supplementation may be required to elevate cattle performance to comparable levels with CS.

INDEX WORDS: Silage, Inoculant, Corn, Pearl Millet, Sorghum, Fermentation, Rumen, *Saccharomyces cerevisiae*

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B.S., Cornell University, 1999

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2002

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August 2002

DEDICATION

I dedicate this thesis to my nephew Kavi, and my niece Kiran. You were both born within the time I began, and finished my thesis. May you reach all your goals.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to Dr. Gary Hill and Dr. Scott Martin for their guidance, advise, and most of all their patience. I would also like to express gratitude to my committee members, Dr. Mark Froetschel, Dr. John Bernard, and Dr. Roger Gates, for answering my endless questions and devoting their valuable time to assisting me. A special thanks to Pat Smith and Trudy Blake for their direction in lab and their friendship.

I would like to thank Brook Hand for all your time and efforts spent in the lab running analyses, shoveling silage and spraying down pens in the individual barn. But most of all I would like to thank you for your friendship and making Tifton/Enigma a great place to live.

I would also like to thank Kristin Adams for her endless support, encouragement and friendship. Most of all for being my best friend and best roommate ever! Finally, a special thanks to my parents for your love and support.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Silage can be defined as the feedstuff resulting from anaerobic digestion by microorganisms, which produce acids that lower the pH of the mass and preserve the forage. Similarly, there is digestion of plant material occurring in the ruminant that involves the symbiotic relationship of the host animal and microorganisms. Through anaerobic fermentation plant material is broken-down within the silo and in the rumen. Addition of microbial colonies (as an inoculant) to forage material at ensiling may enhance fermentation. Furthermore, forage variety may also affect the fermentation in the silo. An understanding of silage fermentation and ruminal fermentation may allow further development of efficient preservation of forages to optimize ruminant digestion and growth.

Rumen

As much as seventy percent of the biomass in the world is not suitable for human consumption. Fortunately, ruminant animals such as bovine species (cows) have the ability to digest fibrous forage components, hemicellulose and cellulose, and convert the resulting nutrients into meat and milk for human consumption. The conversion of organic plant material to edible product involves the symbiotic relationship of the host

ruminant animal and the microorganisms in the rumen. The rumen can be described as a large chambered vat, comprising approximately 54% of the digestive tract, containing 50-70 liters of liquid organic matter, microbes, gases, and end products of metabolism (Yokoyama and Johnson, 1988). The entire rumen is not filled to capacity, with organic matter filling more than half of the rumen and the rest filled with gas.

Within the rumen, there are four compartments that are partitioned by thick muscular walls. Pillars found on the rumen wall, act as baffles and mix the digesta in the rumen, a key function for exposing surfaces of plant material for degradation by the microbes. Populations of microorganisms within the rumen include bacteria, protozoa and fungi. Bacteria produce enzymes that are capable of degrading polymers to monomers (i.e., starch to glucose) which can be fermented to gases (CO_2 and CH_4), ammonia (NH_4), heat, lactate, and volatile fatty acids (VFA). Metabolism of cellulose and hemicellulose to cellodextrins, sucrose, cellobiose and maltose to glucose and fructose monomers provides the microorganisms with a fermentable carbohydrate source that yields VFA, including the major organic acids, acetate, propionate and butyrate. Volatile fatty acids are used by ruminal bacteria and as an energy source by the host animal. Microbes contribute protein to the host animal at the end of the growth cycle and pass to the lower gastrointestinal tract for digestion and absorption. In return, the host animal provides an ideal environment for the microbes in the rumen: anaerobic, constant pH and temperature, supply of masticated feed, addition of buffers, removal of acids and gases, and flushing out the microbial products and indigestible feed particles (Owens and Goetsch, 1988).

Rumen Environment

The rumen is often mislabeled as the stomach of the ruminant animal. In actuality the reticulum, rumen and omasum are forestomach compartments that have no mammalian enzyme secretions, as those found in the stomach. The abomasum, the final compartment before degraded matter reaches the lower gut, secretes mammalian enzymes, similar to the human stomach. Ingested plant matter is held in the first three compartments where particle size is decreased before flushing to the abomasum for enzymatic digestion (Yokoyama and Johnson, 1988).

The rumen is a highly reduced (-0.35 v) environment suitable for the growth of strict anaerobes (Czerkawski, 1986). The temperature remains constant between 38-41°C and pH varies between 5.5 and 7.0 (Yokoyama and Johnson, 1988). Forage-based diets tend to increase the pH to 7.0, while high concentrate diets tend to decrease the pH to 5.0. Copious amounts of saliva produced by the host animal help to buffer the H^+ ions in the rumen and acids produced through fermentation are absorbed across the rumen epithelium, increasing the pH.

Although the rumen is described as an anaerobic environment, the rumen is not completely void of all oxygen. Oxygen enters the rumen when the host animal ingests feed. However, this oxygen is quickly utilized by aerobic or facultative anaerobic microorganisms present in the rumen or is flushed out of the rumen via eructation.

Rumen Microbes

Robert Hungate was the first to isolate strict anaerobes from the rumen using anaerobic culturing techniques and growth stimulating media. Within the rumen there are many morphological forms present including Gram negative, Gram positive, rods, cocci,

and crescent shaped organisms found singly or in clumps (Stewart and Bryant, 1988). Gram negative cells are composed of an outer (phospholipid bilayer) membrane and an inner membrane of structural peptidoglycan. Gram positive cells contain a thick single layer of peptidoglycan and an inner membrane, but are devoid of an outer membrane and are therefore susceptible to changes in the environment (Brock et al., 1994). Besides the archaeic classification of bacterial cells as Gram negative or positive, substrate utilization and production of end products, also categorize and identify bacterial organisms further (Yokoyama and Johnson, 1988). Morphology and substrate utilization plus end product production allows classification of bacterial cells as cellulolytic, hemicellulolytic, amylolytic, methanogenic, lactilytic, and/or proteolytic. Recent research has revealed a more definitive classification system for naming bacterial species because of the discovery and mapping of 16S rRNA for each bacterial species (Krause and Russell, 1996). Within the rumen there are approximately 30 species of bacteria with cell numbers ranging between 10^{7} to 10^{10} cells/ml of ruminal fluid (Bryant, 1959).

Slightly less abundant in ruminal fluid are the protozoal species, with cell numbers of approximately 10^{6} cells/ml fluid. The relatively low concentration of protozoa is compensated by their large size, 40 times the size of a bacterial cell. Protozoa make up nearly half of the ruminal biomass under certain conditions (Williams, 1986). Protozoa are generally predatory to all bacteria, engulfing them through pinocytosis or phagocytosis (Brock et al., 1997). They engulf and store starch granules, retaining carbohydrates in the rumen, which reduces starch digestion by amylolytic bacteria and decreases the incidence of acidosis (Slyter, 1976). Protozoa associate with slower digesting feed particles rather then free living in ruminal fluid to avoid being flushed out of the rumen with digesta. This

association is needed because protozoa reproduce at slower rates than bacteria. In addition to sequestering starch particles and reducing the severity of acidosis, protozoa assist in the digestion of cellulose (Coleman, 1985).

Many studies have looked at the importance of the protozoa for proper rumen function. Defaunation or removal of the protozoa and replacement with bacterial species has not hindered nutritional health of the host ruminant animal (Veira, 1986). This suggests that the protozoa are not essential to the ecology of the rumen.

Fungi constitute a very small fraction (8%) of organisms found in ruminal fluid (Orpin, 1981). Fungi are closely associated with particulate matter and digest lignocellulose through the release of esterase enzymes found in the mycellium. The mycellia are leg-like projections that split apart fibrous cells (Orpin and Letcher, 1979; Orpin and Joblin, 1997).

Bacteria

Bacterial species within the rumen use different substrates for maintenance and growth. Cellulolytic bacteria including, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Butyrivibrio fibrisolvens* (Hungate, 1966) utilize fibrous diets that contain cellulose and hemicellulose, although they are capable of fermenting other carbon compounds. These organisms secrete enzymes which breakdown cellulose and hemicellulose releasing acetate, formate and, depending on the species, some lactate, butyrate, and succinate. These bacteria secrete cellulases from their cell surfaces, which contain a unique protein layer. Outside membranes are sticky and will adhere to the cellulose particles for efficient digestion (Stewart and Flint, 1989; Felix and Ljungdahl,

1993). Fermented products then become the carbon sources for other bacteria and this is called nutrient crossfeeding.

Amylolytic bacteria include: *Prevotella ruminicola*, *Selenomonas ruminantium, Streptococcus bovis*, *Ruminobacter amylophilus* and *Succinomonas amylolytica* (Hungate, 1966; Yokoyama and Johnson, 1988). As the classification implies, these bacteria ferment (or break down) soluble sugars such as glucose, fructose, sucrose, maltose and some pentoses. *P. ruminicola* was identified by Bryant (1959) and is the primary starch digester in the rumen and accounts for 19% of the viable bacterial cells in the rumen. *P. ruminicola* also digests pentose sugars derived from hemicellulose and cellodextrins which allows the organisms to grow on a high-forage diet (Hungate, 1966). In instances where high-starch diets are fed, there is usually a decrease in ruminal pH. Russell and Dombrowski (1980) observed that amylolytic bacteria are more tolerant of lower ruminal pH than other bacteria. Fifty percent of viable cells are *S. ruminantium* (Caldwell and Bryant, 1966), which thrives on high-concentrate diets. Fermentation products produced are acetate, formate, succinate, propionate, butyrate, and lactate (Ricke et al., 1996). *S. bovis* is usually present in low numbers in the rumen, but cell numbers can dramatically increase when rapidly fermentable feedstuffs (i.e., starch) are available. Lactate is a fermentation product that in excess can lead to a significant drop in pH, harming bacteria that are sensitive to abrupt changes in pH. The host animal may experience physiological disorders associated with the accumulation of acid circulating in the blood, a condition called lactic acidosis. *S. ruminantium* subspecies *lactilytica* is able to ferment L-lactate (Stewart and Bryant, 1988) a fermentation product of *S. bovis* via the homofermentative pathway or heterofermentative pathway. *Megasphaera elsdenii* is also

capable of fermenting DL-lactate (Russell and Baldwin, 1978) via the nonrandomizing pathway. Both of these organisms can help control over-production of lactate when the host animal is fed a high grain diet.

No description of the bacteria in the rumen is complete without mentioning the methanogenic species. In order for the rumen to remain reduced there is a need to replace reducing agents (Hino and Russell, 1985). *Methanobrevibacter ruminantium* (Hungate, 1966) an archaebacteria with no peptidoglycan in the cell wall and unusual RNA (Brock et el., 1997) contributes to the difficulty of understanding their structure and function. Carbon dioxide is reduced to CH_4 by a transfer of either H_2 or formate involving the oxidation of pyridine nucleotides (Russell and Hespell, 1981), maintaining a reduced environment in the rumen for enhancing enzymatic reactions. While methanogenesis is instrumental for rumen bacterial function, methane production is an expensive energy loss for the host animal.

Ionophores

Rumen microbiologists and nutritionists constantly work to increase the effectiveness of rumen microbes, ultimately to make more feed energy more available to the host animal. As discussed previously, VFA are products of microbial fermentation that are readily absorbed across the rumen wall into the blood. Propionic acid, a four carbon compound, is converted to glucose (C6) more rapidly than acetic acid, a three carbon compound, via gluconeogenesis. Based on this information, chemical additives such as monensin, a biologically active compound produced by *Streptomyces cinnamonensis* (Haney and Hoehn, 1967), have been identified to inhibit Gram positive microorganisms and their production of acetic acid. According to Schelling (1984), the

mode of action of monensin targets the movement of ions across bacterial cell membranes. Altering the electrochemical gradients across the cell membrane causes instability for the cell. Therefore, inhibition of Gram positive microbes via an ionophore alters VFA proportions. Richardson et al. (1976) proved that monensin increased propionic acid production without changing total VFA production. Adding monensin at 200 mg/head daily to high concentrate or forage diets decreased the amount of acetic acid produced and simultaneously increased the amount of propionic acid produced (Richardson et al., 1976). The outcome of a decreased ratio of acetate to propionate, for cattle on pasture supplemented with monensin, was improved daily gain (Oliver, 1975). When monensin was supplemented at 200 mg/head daily to steers and heifers on pasture (with supplemented concentrate) the daily gain increased 17% (Potter et al., 1976). Raun et al. (1976) reported that steers fed a high concentrate diet, supplemented with 100 mg or 500 mg/head daily of monensin gained at a rate equal to steers not supplemented with monensin. While the ADG was not increased for the cattle on a high concentrate diet, the steers consumed less feed.

Over the years there have been numerous ionophores marketed including monensin, lasalocid, salinomycin, and narasin (Schelling, 1984). Results from 15 trials (greater than 1000 cattle) showed that lasalocid increased the ADG of grazing cattle by 11.1% when supplemented at 200 mg/head daily (Roche, 1985). In addition to the use of ionophores for increasing feed efficiency in feedlot cattle and improving gain in grazing cattle, implants have been used to further maximize gain/growth of cattle.

A normal practice of beef producers today is implanting steers and heifers in all phases of development. The anabolic implants increase ADG while lowering the

production costs of beef production. Implanting feedlot calves can produce gains 18% greater than the unimplanted steers (Duckett et al., 1996). There are various implants available that may contain androgenic and estrogenic hormones such as, estradiol and progesterone.

Specifically, Cain et al. (1984) observed increases in ADG and decreases in feed conversion efficiency for the implants Synovex-S® and Ralgro® compared with control steers. Revalor®-h (trenbolone acetate + estradiol) increased ADG by 15.9% in implanted heifers compared with unimplanted heifers (Brandt et al., 1996). The mode of action of the implants is often disputed; Preston (1975) agreed with Clegg and Cole (1954), who determined increased growth hormone production as the basis for increased growth (Preston, 1975; Clegg and Cole, 1954).

Feeding monensin (Rumensin®) plus implanting has an additive effect on ADG of growing cattle grazing high quality forages (Rouquette, 1976; Hill et al., 1979). Hill and Harpel (1981) reported that steers implanted with zearanol (Ralgro®) and hand-fed monensin had gains greater than control steers grazing Coastal bermudagrass overseeded with ryegrass. The additive gain responses from implanting and feeding ionophores to growing cattle vary, but most reports indicate that ADG of cattle receiving both implants and ionophores often exceed 20% compared with controls receiving neither implants nor ionophores.

Silage

There are various methods for storing crops to be fed to livestock. A hay crop may be dried until the biological activity decreases completely. Another method of preserving is ensiling a slightly dried or direct cut grass or legume crop. The principal concern for

storage is to maintain the quality of the feed from harvest to feeding. Presumably, the storage methods will minimize losses of DM and energy, both affecting the profitability of the crop for feed (Muck, 1988).

As the meat and dairy industries continue to expand and the cost of concentrates increases, there is a demand for quality feed alternatives, including forages with year round availability. Silages provide the answer for efficient harvesting and availability. There have been some reservations about ensiling over the years as mentioned above, including low DM forage, low production for animals fed silage, costs of harvesting equipment and storage facilities, mechanical difficulties with feeding silage, and offensive odors from the silage. Much work has been done to increase the ease of transport, quality through wilting or additives, harvesting and ensiling techniques. Ensiling grass and legumes is an added advantage for the animal industry as it will decrease dependency on the hay crop. Furthermore, the hay harvest depends greatly on the weather, a very unpredictable factor. An increase in mechanized equipment with ensiling lowers the labor costs, increases storing and feeding ease (Chase, 1989).

Forages typically planted for ensiling in the southeastern U.S. are limited to the most heat and drought tolerant, and insect resistant plants. Temperate corn, tropical corn and winter annuals can be planted in a triple cropping sequence, thereby increasing the total forage yield. Tropical corn is well suited to the climate in the southeastern U.S. which is characterized by high temperatures, high humidity and it is tolerant to most diseases and insects (Johnson et al., 1997). Pearl millet commonly grown in Africa, India and Pakistan also contains some of the heat and drought tolerant advantages noted for tropical corn. Pearl millet grain contains more of the essential and limiting amino acids

than wheat or sorghum grains (Burton et al., 1972). Based on these chemical analyses it follows that the CP content of pearl millet grain is higher than corn grain (Hill et al., 1996). Sorghum crops are planted in the midwestern region of the U.S. and have a high yield for the high temperatures and drought in this region (Sanderson et al., 1992). One disadvantage of sorghum silage is the high lignin or fiber content of the plant. Lignin is the indigestible portion of the plant cell wall, which ultimately limits the digestive nutrients available for the animal (Aydin et al., 1999).

In general terms, silage can be defined as the feedstuff resulting from anaerobic digestion by microorganisms, which produce acids that lower the pH of the mass and preserve the forage. Chase (1989) describes five phases for the proper fermentation of forage material. Phase 1 is the respiration phase where oxygen is still available for the aerobic microorganisms and plant cells. Carbon dioxide and heat are produced as well as ethanol, acetic acid, and butyric acids. Once the oxygen supply is depleted Phase 2 or initial anaerobic fermentation commences. Anaerobic organisms begin to ferment and produce acetic acid, which lowers the pH to near 4.2. At this pH Phase 3 begins, lactic acid producing microbes inhibit the production of acetic acid by acetic acid producers. The lactic acid producers ferment water-soluble carbohydrates to lactic acid, lowering the pH to 3.5-4.0. At this point the temperature declines and the microbial activity halts as acidic conditions do not allow further functioning of microbes. Phase 5 is described as the stable phase where the low pH stops fermentation.

The success of ensiling forage material depends on many factors including the exclusion of clostridia. Clostridia are also known as butyric acid bacilli, producing butyric acid. Buchanan and Gibbons (1974) classify clostridia as Gram-positive, spore-

forming, motile, rod-shaped bacteria that can ferment sugars, organic acids, or proteins under anaerobic conditions. The growth of clostridia ensues when insufficient acid is formed during the first phases of fermentation. When there is an insufficient amount of water-soluble carbohydrate, clostridia will ferment lactic acid to butyric acid, via saccharalytic clostridia. Accumulation of butyric acid, a weak acid, allows the proteolytic clostridia to ferment amino acids to ammonia, carbon dioxide and amines (Ohshima and McDonald, 1978). This fermentation results in an increase in pH.

The proliferation of clostridia does not depend on the acidity of the environment, which they can tolerate, but on the amount of moisture available for microbial action. Increased clostridial fermentation of carbohydrates and proteins accounts for the DM and energy losses that adversely affect feed intake of the ruminant animal (Muck, 1988).

Negative Biological Processes

Plant respiration continues following the initial storing of the silage, as explained by Chase (1989). If the silo or bunker is not correctly sealed the aerobic respiration will continue longer than expected. Normal DM and sugar losses during the initial respiration are small fractions of the total losses. When plant respiration continues for longer periods of time, DM and fermentable carbohydrates are lost, diminishing the substrates for fermentation by lactic acid bacteria. The pH remains high, which allows unwanted microbial (clostridial) fermentation and plant activity (Muck, 1988). Respiration produces heat which can produce Mailliard products such as acid-detergent insoluble and unavailable N (Van Soest, 1982). Mailliard products are caramelized proteins and polysaccharides, which can not be digested by the rumen microbes.

Plants produce their own enzymes that continue to hydrolyze starch and hemicellulose to monosaccharides. The hydrolysis provides additional sugars for the lactic acid fermenting microbes and degrades hemicellulose, which lowers the fiber (NDF) content of the forage. Proteolytic enzymes have an adverse effect on the feeding value for ensiled forage. These enzymes convert proteins to nonprotein nitrogen (NPN), in the form of peptides and amino acids. Any further degradation of NPN will produce ammonia and amines, further lowering the protein content of the silage (Muck 1988).

Besides clostridial growth, in the absence of low pH, aerobic microbial activity will reduce the quality of ensiled forage. Exposure to oxygen and degradable carbohydrates, organic acids, or proteins permits the growth of unwanted yeast, fungi, or bacteria. The aerobic exposure from the silo to the feed bunker creates an added concern for the management of quality silage. In one study (Sebastian et al., 1996), the inoculated silage pH increased by 2.18 units when exposed to oxygen. Yeasts and molds proliferate on the organic acid, lactic acid (Lindgren et al., 1985), which is abundant in a properly ensiled forage. Heating during feeding can deteriorate the silage by forming Malliard products, which effects the nitrogen content, making it unavailable. Additionally, molds formed on the surface of aerated silage may contain mycotoxins, which are harmful to cattle (Muck, 1988).

Overcoming Negative Biological Processes

Plant respiration will continue as long as oxygen is available. Effectively packing and sealing the bunker or upright silo reduces energy and DM losses. In terms of plant proteolytic enzyme activity, for legumes, the optimum pH is around 6.0 and declines as the pH falls to 4.0 (McKersie, 1985). Proteolysis is greatest the first day of ensiling and

decreases by day five, though activity never disappears. Drier silages have reduced proteolytic activity and do not require as a rapid decline in pH to hinder proteolysis (Muck, 1987). Ideally, the silage with DM less than 40% will incur better packing and exclusion of oxygen, halting the aerobic microbial activity and plant respiration; then, anaerobic conditions will promote the growth of lactic acid bacteria. Lactic acid bacteria will produce acids and lower the pH quickly, reducing proteolysis (Pitt, 1986).

Fermentation

Lactic acid producing bacteria involved in fermentation include; *Lactobacillus, Pediococcus,* and *Streptococcus.* In quality silage, streptococci initiate the fermentation. Pediococci and leuconostocs compete with, steptococci for viability and finally lactobacilli complete fermentation. Under aerobic conditions, lactic acid bacteria can produce both lactic acid and acetic acid and are classified as heterofermentors. Some lactic acid bacteria are capable of fermenting citric and malic acids found in some forages (McDonald, 1981). In one study (Stirling and Whittenbury, 1963) the source of lactic acid bacteria was from the cut and partially decayed material around the cut surfaces. The cut surfaces were made during harvesting, and contact with the surfaces of equipment helped disperse the lactic acid bacteria through out the forage material. The harvested crop was then ensiled. Muck (1988) found approximately 10^{3} to 10^{4} cfu/g (the population of bacteria normally present) of lactic acid bacteria on chopped alfalfa before loading into the silo. The number of lactic acid bacteria needed to decrease the pH is approximately 10^{8} cfu/g (Pitt et al., 1985). This leads to the next topic, how can the numbers of viable lactic acid bacteria be increased?

Inoculants

Microbial inoculants of various species of bacteria such as, *Lactobacillius acidophilus, Lactobacillius plantarium, Lactobacillius xylosus,* or *Pediococcus acidilactici* are frequently applied to forages prior to ensiling. As already discussed, the bacterial counts are low on the chopped plant material when no inoculant has been added. Research suggests that microbial inoculants contain 10^5 to 10^6 cfu/g, which is lower than the 10^8 cfu/g required to rapidly decrease the pH of the silage. McDonald (1981) suggested using a bacterial strain of lactic acid bacteria that are homofermentors because the lactic acid produced is more acidic (than acetic acid) and will increase the reduction in pH. The inoculant must grow more rapidly than the natural population, which contains the homofermentive and heterofermentive lactic acid bacteria, in order to effectively decrease the pH.

There is much disagreement surrounding the effectiveness of inoculants. Researchers have reported a wide range of responses, from no response to a positive response when forages were inoculated. Ely and Sudweeks (1981) found that addition of *L. plantarium* to alfalfa, corn, sorghum or wheat had little effect on the decline in pH and differences in the nutrient contents between inoculated and control silage. They suggested that the inoculant only produces benefits for the silage in cases where the normal microflora were insufficient to lower the pH of the mass. In another study (Cleale et al., 1990) corn forage was inoculated with *L. xylosus* and *P. acidilactici,* but there were inconsistencies in the chemical composition of the corn silage. Kung et al. (1991) examined the effects of lactic acid bacteria on silage fermentation and composition. In this study, there were higher counts of lactobacilli and the silage pH was lower in the

inoculated treatment silage. Microbial inoculation decreased NH_3-**N** content after 1-3 days of ensiling. It has been suggested that the lower ammonia content in the inoculated silage was due to reduced proteolysis when there is a rapid decline in pH of the forage.

Much work has been done to determine the appropriate strain of *Lactobacilli* for inoculating forage at ensiling. According to Weinberg et al. (1998), the appropriate strain of bacteria depends on the type of water-soluble nutrients that are found in the forage, such as starch, sugars, and proteins. This study also demonstrated how temperature can adversely effect the production of acids by the microorganisms. Different strains of bacteria are temperature sensitive and ensiling experiments are usually conducted at ambient temperature. In an earlier study, Weinberg and Ashbell (1994) determined silage temperatures may reach 40°C when ensiled in a large silo and then decrease to ambient temperatures. Survival and activities of various bacterial strains, used as inoculants, may depend on the temperature fluctuations during ensiling.

Other Bacterial Strains used for Inoculation

Most inoculants do not contain a single strain of bacteria; they are usually composed of a combination of microbial strains. In order to consider possible fermentative strains certain qualifications must be met. These include rapid growth and production of lactic acid from the fermentation of soluble constituents homofermentatively, leading to a decrease in pH, adaptability to temperature and pH in the silo, and absence of proteolytic activity (Whittenbury, 1961). Besides the requirement for non-proteolytic strains, components of the water-soluble plant nutrients degraded must be considered. Some plants contain as much as 50% fructan, which requires a specific lactobacilli strain that possesses the correct enzymes to breakdown the fructans (Winters et al., 1998).

L. plantarum begins fermenting forage water-soluble components only when the pH has dropped to 5. If the forage placed into the silo has a pH of 6, another microbe must initially ferment and produce acid to lower the pH of the forage (McDonald, 1981). Pediococci begin fermenting at pH 6 and produce acids, lowering the pH to promote *Lactobacilli* growth (Langston et al., 1962). In one experiment (Fitzsimons et al., 1992) when *P. acidilactici* and *L. plantarum* were inherent in the grass at a ratio of 10^6:10g of grass then *L. plantarum* was stimulated to grow. At higher concentrations, *P. acidilactici* out-competed lactobacilli for nutrients.

Streptococcus bovis is a starch-digesting ruminal microorganism, which also produces lactic acid. Jones et al. (1991) proposed the use of *S. bovis* for all silage inoculants. As previously discussed, homofermentive microbes will produce one end product and in this case some strains of streptococci will produce lactic acid and thus lower pH rapidly. Typically, *Enterococcus faceium* is used as the starter fermentor of water soluble carbohydrates in the silage, but *S. bovis* is able to ferment the undigested starch fraction of the plant and proliferate fast; thus leading to sharp declines in the initial pH.

Nutritive Problems of Silage Protein and Carbohydrates

Three problems associated with ensiled forage feeds such as tropical corn have been identified. First, inherent low nutrient content of the forage is also evident in the ensiled forage; second, low protein available to the anima;, and third, these factors contribute to low intake by animals (Flores, 1988). Temperate corn silages may have similar problems in addition to the breakdown of protein to ammonia N, amino acid N and amine N, and loss of water soluble carbohydrate source (Thomas et al., 1980). Silages

that are properly ensiled with minimal proteolysis may increase microbial protein synthesis by allowing the transport of the spared oligopeptides into the microbial cells (Stern and Hoover, 1979). Walker et al. (1975) observed lower VFA concentrations in the ruminal fluid of cattle fed a silage diet. This implies that there is less carbohydrate available for synthesizing microbial protein. Although energy can be produced from the fermentation of amino acids, the yield is too low for sufficient protein synthesis. McDonald et al. (1981) suggested that the depletion of water-soluble carbohydrates during ensiling of forage might account for the low carbohydrate availability for protein synthesis.

Tropical corn silage supports the theory that changes in preformed amino acids, degradability of protein in the rumen and energy availability to the rumen microbes are the limiting factors for protein synthesis. Temperate corn silage has a relatively higher starch content from the grain, which provides the energy for protein synthesis. Temperate corn also has a relatively high protein content that is not changed during ensiling and is not degraded by the microbes. There is little change in preformed amino acids (Flores, 1991).

Silages in Feedlot Trials--for Growing Beef Heifers and Steers

Previously, my review has focused on rumen function, the ensiling process, and microbial influence on silage and ruminal fermentation. With this knowledge, there is application to animal production. Silages are valuable feedstuffs for beef and dairy producers because they provide a nutrient rich and inexpensive feed source. Silage can be harvested in the warmer months and fed to growing and producing cattle through the cooler months. Ensiling at first appears to be a simple and efficient procedure to preserve forage, but the difficulties noted earlier in this review are still present. For many years

corn silage has been the main silage produced and fed to ruminants in the United States. According to Moon (1984), 80% of the silage produced in the United States is from corn and sorghum. Many researchers in silage and animal production search for alternate forage species and applications of microbial inoculants that might provide the best silage for nutrient value, animal growth and production with an ultimate goal of decreasing production costs.

Once growth and cost effectiveness are optimized with implantation of steers and heifers, the plane of nutrition becomes an important factor in the growth of the calves. Hill et al. (1999) reported that corn (*Zea mays*) silage provided 7.4% CP, and a TDN of 69.8%. The alternate forage, pearl millet (*Pennisetum glaucum*) provided 12-13% CP and 57-58% TDN. In this example, corn silage may not provide as much CP for the animal, but the TDN available to the animal for metabolism is higher than in the pearl millet silage. Other forages used for silage production include sorghum, tropical corn, and legumes, but the nutrient content and availability vary between plant species.

Besides considering the nutrient value, availability upon ensiling, and ensiling difficulties for a variety of plant species, animal performance must be determined. Feedlot production trials typically utilize growing beef or dairy cattle. In the experiment by Hill et al. (1999), steers and heifers were fed corn silage, millet silage and millet silage plus cracked corn. Average daily gains for the heifers fed the first cut millet silage were 0.15 kg, 0.20 kg for heifers fed second cut millet silage and 0.95 kg for heifers fed corn silage. Following the trend for ADG, intake was least for the first cut millet (2.87 kg), intermediate for second cut millet (3.03 kg) and greatest (6.10 kg) for corn silage. Intake is affected by the nutrient content and fibrous content of a feed. Pearl millet silage was

higher in CP and it had a higher percentage of undigestible stalk and stem. Cattle dry matter intake (DMI) was lower for pearl millet silage because heifers fed this silage reached their requirement for CP and energy more rapidly than when fed a corn silage diet. Additionally, the millet silage has fibrous stalk material that is bulky and fills the rumen, not allowing the steer or heifer to consume more silage. Adding inoculant to the millet silage in a follow-up experiment with growing steers did not improve DMI or ADG for the steers. Adding corn to inoculated millet silage increased DMI, providing more energy for feed conversion.

Intake

In an intake experiment by Campling (1966), larger amounts of digesta remained in the reticulo-rumen of cattle immediately after a hay meal compared with a silage meal. They observed lower voluntary intake for cattle fed a silage diet, and the amount of time spent ruminating was significantly longer on the silage diet. These measurements of retention of digesta and slower rumination indicate that silage organic matter remains in the digestive tract for long periods of time. It was suggested that the retention of organic matter in the digestive tract cause decreased intake of silage.

Cushnahan and Gordon (1995) studied intake and rumen digestibility in cattle, and concluded that differences in intake were more dependent on the chemical composition of the feed, than gut fill. One example of this concept was demonstrated by Oba and Allen (1999). The fresh forages having high NDF digestibility had increased DMI. The authors suggested that increased digestion of NDF was caused by a more rapid hydrolysis of NDF, which allowed faster disappearance of forage material from the rumen.

Fresh forage or hay was ensiled in round bales or a bunker or frozen and fed to cattle and sheep. As mentioned previously, the ensiling of forage or in this case, grass, caused proteolysis and deamination of protein, increasing nitrogen availability, resulting in increased nitrogen apparent digestibility. Contrary to a predicted increase in silage intake, there was a decrease in intake of the ensiled grass. The increases in ammonia nitrogen, butyric acid concentration and buffering capacity in the silage may have lowered the palatability and nutrient value.

Applying an inoculant to silage such as, alfalfa-timothy silage improved the DM consumption according to Kung et al. (1987). In this study, there was only an effect when the inoculated silage contained at least 60% DM; below 40% DM might not allow a rapid fermentation and pH to drop sufficiently to allow proper ensiling. While the inoculation of forage for ensiling did not provide any compositional changes beneficial to the feed product, DM digestion and N retention were improved in a trial with steers (Luther, 1986). Cows fed the inoculated (Ecosyl) grass silage produced 1.3-2.1 kg more milk than cows fed the uninoculated silage (Gordon, 1989). Ruminal acetate production was unaffected by microbial inoculation of the silage in a study by Kung et al. (1987). This might have occurred because of the balance with concentrate, keeping the acetate levels stable for milk production. However, there is some controversy because Petit and Flipot (1990) reported a lower acetate production in the rumen of cows fed inoculated silage. Some argue that there is no beneficial effect of adding inoculant to silage, but the increased intake, possible constancy of acetate production, and increased milk production might improve the animal performance.

Yeast

Yeast are small oval microorganisms that bud and form ascospores, mycelia and pseudomycelia. In the last 15 years there has been an increased interest in using *Saccharomyces cerevisiae* (yeast) as an alternative to antimicrobial feed additives. Nisbet and Martin (1991) reported a *S. cerevisiae* culture increased lactate fermentation by ruminal microorganisms. The addition of yeast to animal feed has the potential to increase rumen pH by assisting *Selenomonas ruminantium* (predominant ruminal bacterium) in uptake of lactate. This research may be beneficial for reducing acidosis (increased lactic acid accumulates from the fermentation of a readily available soluble carbohydrate source and lowers ruminal pH) of cattle fed high grain diets.

Researchers have found that yeast can increase the nutritional value of poor quality forages through alterations of the mixed ruminal microorganism fermentation. Addition of yeast to meadow hay increased the in vitro cellulose digestion by 10% (Wallace and Raleigh, 1960). Arambel and Kent (1990) hypothesized that cattle under stress may benefit from yeast supplementation based on the increase in nutrient requirements for the animal. Although the prospects of yeast supplementation seem promising, many studies have found no effect on either nutrient digestibility or productivity. Wiedmeier et al. (1987) and Carro et al. (1992) reported no effect on rumen pH, or concentrations of ammonia concentration and molar proportions of acetate, propionate, butyrate and valerate. Isovalerate and isobutyrate concentrations were increased. Similarly, yeast culture had no effect on VFA concentrations, but the acetate: propionate ratio was reduced significantly in a study by Williams et al. (1991). Additionally, there was no significant

effect on percent fiber (NDF, ADF, and CEL) digestion with increased amounts of yeast culture (Carro et al., 1992).

In two studies (Harrison et al., 1988; Nisbet and Martin, 1991) a reduction occurred in the acetate: propionate ratio in rumen fluid supplemented with yeast culture. Harrison et al. (1988) studied effects of yeast culture supplementation in fistulated Holstein cows; Nisbet and Martin (1991) studied in vitro effects of yeast culture. Differing from the later findings, ruminal pH and molar acetate concentrations were significantly lower with yeast culture treatment and molar proportions of propionate were significantly greater with addition of yeast culture. Apparent digestibilities were not effected by the addition of yeast culture in the study by Harrison et al. (1988).

Carro et al. (1992) suggested that the inconsistent effects of yeast culture addition might have resulted from the varied diet compositions fed to research animals. High concentrate diets are reported to cause a greater impact on the anaerobic bacteria when yeast cultures are present (Dawson, 1990). In another study (Dawson et al., 1990) cattle fed a roughage diet supplemented with live cell yeast culture had an increase in the numbers of cellulolytic organisms in the rumen. With the increase in cellulolytic bacteria in the rumen with yeast culture addition, it would seem that there should be an increase in the digestion of cellulose and hemicellulose. According to the results of these studies this was not the case. Harrison et al. (1988) suggested that the activity of the cellulolytics was reduced in some way.

Wiedmeier et al., (1987) found that yeast culture supplementation tended to increase the products of cellulolytic bacteria in the rumen, and they suggested that this

must result from nutrients provided by the yeast cultures such as B vitamins and other undetermined growth factors (Bryant, 1973).

Newbold and Wallace (1992) reported that addition of yeast extract to mixed ruminal fermentations did not increase fermentation as suggested by Wiedmeier et al. (1987). This finding was later qualified to include only certain strains of yeast culture were effective for supplying nutrients to the ruminal bacteria (Newbold et al. 1996).

Stewart and Bryant (1988) found rumen microorganisms are very sensitive to oxygen levels in ruminal fluid. Newbold et al. (1996) reported declined dissolved oxygen levels in rumen fluid and the significant uptake of oxygen by specific *S. cerevisiae* strains. This suggests a benefit for yeast culture supplementation, reduction of the oxygen content incorporated into the rumen fluid and support of an anaerobic environment required for the bacteria.

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CHAPTER 2

EFFECT OF SILAGE SOURCE AND ADDITIVES ON COMPOSITION, FERMENTATION, AND DIGESTIBILITY OF CORN, PEARL MILLET, TROPICAL CORN, AND SORGHUM SILAGES

Introduction

There are various methods for storing crops for feeding livestock. One method of preservation is drying grasses and bale as hay. Another method of preservation may be ensiling a wilted or direct cut grass or legume. The principal concern for storage is to maintain the quality of feed from harvest to feeding. Presumably, the storage method will minimize DM and energy losses, which will affect the profitability of the crop as a feed (Muck, 1988).

In general terms, silage is a feedstuff exposed to anaerobic fermentation that yields acids that lower the pH of the plant material and help preserve the forage. Microbial inoculants have been used to improve the preservation of forages although there are contrasting results. Rust et al. (1989) reported an increase in the concentration of one of the major fermentation acids, lactic acid, when lactic acid bacteria were added to corn silage. Even though Phillip and Fellner (1992) observed improved aerobic stability at feedout, there was no evidence of improved performance for cattle fed inoculated high moisture corn silage.

In the southeastern U. S., typically heat- and drought-tolerant plus insect resistant plants are grown for beef and dairy cattle consumption. Tropical corn can be planted as a double or a triple crop in late summer, when the temperatures and humidity are high; temperate corn would be planted earlier in the spring (Johnson et al., 1997). Pearl millet, commonly grown as a grain crop in Africa, India, and Pakistan, tolerates extremes in temperature and humidity, similar to tropical corn. Sorghum crops are grown in the southern high plains, the southeast, and the midwestern U. S. where there are high temperatures and humidity during the growing season (Sanderson et al., 1992). While temperate corn varieties are typically used in silage production, tropical corn, pearl millet, and sorghum forages may serve as alternative silage crops. Increasing the number of crops produced on a field can increase the yield as observed with triple cropping of tropical and temperate corn (Johnson et al, 1997).

In this study, we compared temperate corn, pearl millet, tropical corn, and sorghum silages, and the effects of inoculant and additive treatments at ensiling on silage quality. In addition, in vitro NDF disappearance and in vivo digestibility of the silages were studied.

Materials and Methods

Silages

Four silages were produced during the summer of 1999 using traditional planting and harvesting practices (Table 2-1). Temperate corn (CS) and tropical corn, (TC) were ensiled when the black line was formed across the corn kernels. Sorghum (S) and hybrid grain pearl millet (PM) were harvested when grain was in the dough stage of maturity.

Each wagon load of green chopped forage was weighed, and each silage was stored in an individual plastic silo bag, using a Kelly-Ryan™ silage compactor. The first half of each silo bag was filled with chopped forage with no inoculant (NI) and the second half of the same bag was filled with chopped forage harvested the same day from the same field treated with an inoculant (I). The silage inoculant Sun-Cure™ (Sunbelt Custom Minerals, Sulphur Springs, TX) was added at the manufacturer recommended rate of 0.25 g/kg forage. The Sun-Cure™ inoculant contained calcium carbonate, processed grain by-products, dried *Lactobacillus plantarum* fermentation product, dried *Streptococcus faecium* fermentation, dried *Pediococcus acidilactici* fermentation product, dried *Bacillus subtilis* fermentation extract, dried *Aspergillus niger* fermentation extract, sodium bentonite, cane molasses, dextrose, and calcium silicate.

Experiment 1

On the same day each of the (CS, PM, TC, and S) forages were harvested, approximately 32 kg of untreated fresh green-chopped forage was obtained for use in a mini silo experiment (approximately 3.5 kg green chopped forage/mini silo) as the forages were being compacted into the large silo bags. A total of 144 mini silos (7.5 L, plastic high-density polyethylene and copolymer industrial pail Bennet Industries Inc. #507, Container Resources Inc., Greenville, SC) with one-way gas valves installed in plastic lids sealed with rubber rings were used to make silage from each forage type. Additive treatments (8 mini silos/treatment) for CS included no additive (control), Sun-Cure and Pioneer 1177 inoculants. Corn grain was not added as a treatment because it was thought that there would be no measurable effect on chemical composition, fermentation or

digestion of CS with additional grain. The PM, TC, and S forages were ensiled with five additives (8 mini silos/treatment) including no additive (control), ground shelled corn (5% of green-chopped forage weight), Sun-Cure inoculant, Pioneer 1177 inoculant or a liquid spray-on microbial inoculant, Lact-A-Pro (100 g Lact-A-Pro mixed with 23.7 L water and applied at 0.5 L/ton of silage). Pioneer 1177 contained dried *Lactobacillus plantarum* fermentation product, dried *Enterococcus faecium* fermentation product, and calcium carbonate (Pioneer Hi-Bred Int.,Inc., Johnston, IA) added at manufacturer recommended rate. Lact-A-Pro contained dried *Propionibacterium sp.* fermentation product, dried *Lactobacillus plantarum* fermentation product, dried *Pediococcus acidilactici* fermentation product, sucrose, and sodium silicoaluminate (Lallemand Biochem International, Milwaukee, WI). Each green chopped forage was thoroughly mixed with each additive, and packed into mini silos with a hydraulic press and sealed. Mini silos were stored in an open-air shed for approximately 42 d. Fresh ensiled samples (25 g) were taken from opened mini silos extracted with distilled water (100 mL) and blended in a Waring blender for 2 min. The extract was filtered through four layers of cheese cloth and stored frozen (-20°C).

Experiment 2

An intake and digestion experiment was conducted using beef steers fed each of the four silages (CS, PM, TC, and S) without inoculant (NI) or with (I) the Sun-Cure inoculant applied at ensiling. Steers in this experiment were weaned in September, 1999, at approximately 10 mo of age. The British and Charolais crossbred steers (n = 36; 235 kg ± 16.4 kg initial BW) were ranked by BW and randomly assigned to eight treatments in a

4 x 2 factorial arrangement. Treatment main effects were silage source (SS) and inoculant (I). Steers were implanted with Synovex-S®, and received Totalon™ (anthelmintic; 2.5 ml/242 kg body weight; Schering-Plough Animal Health Corp., USA) on d 1 of the experiment. Collars were fitted for each steer to tether them in individual stalls (1.22 x 2.02 m), equipped with rubber mats in a semi-enclosed barn. Steers were given a four d adaptation period to the stalls and were fed corn silage that they had been eating since weaning. Four steers were randomly assigned to non-inoculated (NI) silage treatments and five steers to inoculated (I) silage treatments. The feeding experiment began in October, 1999 when both ends of each silo bag were opened, with NI silages fed from one end of each bag, and I silage fed from the opposite end of each bag. Silages (Table 2-2) were weighed once daily and supplemental soybean meal was added (soybean meal added daily at: 0.114 kg/steer for CS inoculated (I) and NI; 0 kg/steer for PM, I and NI; 0.171 kg/steer for TC, I, 0.206 kg/steer for TC, NI; and 0.315 kg/steer for S) top dressed on silage to balance CP and energy fed in the 32 d experiment. Silage, water and minerals (Hi-Seven, W. B. Fleming Co., Tifton, GA; minimum (%), respectively, Ca, 16.0; P, 7.0; NaCl, 20; NaCl, 24.0 (maximum); F, 0.07 (maximum); Fe, 1.0; Cu, 0.012; Co,0.003; Mn, 0.12; Mg, 1.0; Zn, 0.12; I, 0.003; S, 1.0; Se, 0.0015) were available ad libitum. Feed refusals were measured daily and subtracted from total feed offered to determine feed intake, and to allow daily adjustments of silages offered. Apparent digestibility of nutrients was determined using Cr as an external marker (Prigge et al., 1981). Chromic oxide (10 g/steer daily) was administered in gelatin capsules (d 22- d 31). Fecal samples (12/steer) were collected at 8 h intervals to account for diurnal variation of digesta from d

28 to d 32. Fecal samples were dried at 60°C for 72 h and ground through a Wiley mill using a 1 mm screen.

Analyses

To determine nutrients in silages from mini silos, bag silos, and fecal samples from the digestion trial, samples were dried at 60°C for 72 h and ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) using a 1mm screen. Dried samples were then analyzed for CP by methods of the AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of Van Soest et al. (1991) and acid detergent lignin (ADL) determined by the method of Van Soest and Wine (1968).

Silage extracts from the mini silos were thawed and analyzed for pH and volatile fatty acids by gas-liquid chromatography (Anonymous, 1975) and lactic acid by gas-liquid chromatography of methylated esters (Holdeman and Moore, 1973). Malate was quantified by HPLC using an organic acid column (Callaway and Martin, 1997; Martin and Park, 1996). Ruminal fluid was collected from a cannulated Hereford steer fed corn silage from the same silo that the weanling calves were fed prior to the beginning of these experiments. In vitro NDF digestibility of the dried and ground ensiled samples was determined using a Daisy Incubator (ANIOM Technology Corp., Fairport, NY). Digestion of NDF was then calculated as original dry sample weight minus dry residue weight, divided by the original sample weight multiplied by 100 to report percent.

Fecal samples collected over time were composited for each steer, and duplicates were analyzed. Fecal samples were analyzed for chromic oxide using the method of Brisson (1956).

In our experiment involving mini silos in which IVDMD-NDF was determined over time, there were 18 treatments which became "pseudoreplications" (Box et al, 1978). For example, among the 18 treatments, there are 10 degrees of freedom for interaction, which is the basis for creating artificial replications. The seven observation times were then fitted with a quadratic trend.

Statistical analyses were conducted for the mini silo experiment and the in vivo digestibility experiment. In Experiment 1 using mini silos, an incomplete factorial was designed using four sources of silage (CS, PM, TC, and S) with five additive treatments (control, corn grain, Pioneer 1177, Sun-Cure and Lact-A-Pro). CS was the first silage harvested, and Lact-A-Pro inoculant was not available at the time of ensiling. Corn grain was not added to the temperate corn forage at ensiling because it was assumed that this additive treatment would have minimal effect on CS, therefore, two treatments were omitted for CS. Least Squares means are not obtainable from any incomplete factorial design (Littell et al., 1996). It was determined that all data (chemical analyses, fermentation data, and in vitro NDF digestibility) could be analyzed using two smaller factorials. First, a 4 x 3 factorial, included the four silage sources and the three additives (Control, Pioneer 1177 and Sun-Cure inoculants) that were used with all silages. Second, a 3 x 5 factorial, included three silage sources (PM, TC and S) with all five additive treatments. Data for each factorial was analyzed using Proc Mixed (SAS, 2000). Factorial arrangement of treatments with the various mini silos treated as sampling units for a particular treatment combination was arranged as a completely randomized design (CRD). Two adjustments were made in order to obtain the correct standard error (SE)

between the two smaller factorials. Ratio of variance from the full model to the variance of the 4 x 3 factorial was computed to obtain the correct SE for interaction. Same procedure was repeated for the 3 x 5 factorial. Interaction means are shown for each factorial, and the SE is lowered to arrive at the correct SE for the main effects using standard adjustments. In the case of the 4 x 3 factorial, the SE for additives = (1/square root of 4) multiplied by the interaction SE, and the SE for silages = (1/square root of 3) multiplied by the interaction SE. Similarly, for the 3 x 5 factorial the SE for additives = (1/square root of 4) multiplied by the interaction SE, and the SE for silages = (1/square root of 3) multiplied by the interaction SE. To discuss main effect means, especially when interaction effect was judged to be significant, the silage main effect and the additive main effect were further adjusted to get differences above the level of interaction (take the square root of the *F* value for interaction multiplied by each of the main effect SE), which equals the adjusted SE that tests differences beyond what is shown in the interaction. This is equal to dividing the mean square for silage main effect by the mean square for silage x additive interaction. Then if the *F* test is significant, one would expect to find at least one difference between main effect means, but if the *F* test is nonsignificant, main effect means are not different). The in vitro NDF disappearance data were obtained from a split plot experiment with no blocking (See suggested analyses, Box et al., 1978). The error terms came from the extra degrees of freedom that are not being used in the interactions between treatments and time, and the fact that measurements were taken at seven times, and only quadratic lines were fitted for each treatment. There were seven observation times, and the mean of sampling times was 24 h. The center of

regression was moved from the normal position at zero to the mean, which was at the 24 h observation point (Draper and Smith,1981). A model was used that fit the slope for each silage, and at the same time a slope was fit for each additive. An earlier analysis indicated no interaction between silage sources and additives. The increase in NDF digestibility was independent of slopes for silages or additives. When linear and quadratic components were fit for effects of silage sources, additives, and their interaction, and no common quadratic effects were found resulting in removal of quadratic effect from the model. In a reduced model, no interaction was determined between slopes of silage and additives, therefore the interaction was removed from the model. The final model used determined slopes for each silage and each additive. The steer digestion trial data were analyzed as a 4 x 2 factorial using a Mixed Model Procedures of SAS (2000), and least squares means for DMI and digestion coefficients were adjusted for initial BW of steers.

Results and Discussion

Mini silo data reported in Experiment 1 were treated as two smaller factorials because it allowed for more efficient discussion of the results. In the case of the 4 x 3 factorial, results were largely determined by one silage, namely PM. In the case of the 3 x 5 factorial, results were influenced greatly by the addition of corn grain at ensiling, which affected the results far greater than any of the inoculant treatments. In this context, the 3 x 5 factorial data reveals a pattern in which the addition of corn grain acted as a positive control, the control treatment acted as a neutral control, which allowed the inoculant treatments latitude to affect treatment responses.

Main effect means for chemical composition of four silages with three additives ensiled in mini silos appear in Table 2-3. Most of the chemical composition data included significant silage source x additive treatment interactions ($P < 0.01$) (Appendix Table 1). In Table 2-3, the overall higher quality of CS compared with other silages was indicated by highest DM for CS and TC ($P < 0.01$), while CS had moderate CP, and lowest ADF, NDF, and ADL ($P < 0.001$). Although PM had the highest CP ($P < 0.001$), it also had the lowest DM, highest ADF, and highest ADL value, that was also similar to ADL in S. Both TC and S had similar CP, but higher ADF, NDF and ADL than CS. The inoculant treatments did not consistently improve chemical composition of the silages.

Interaction means for chemical composition of three silages (PM, TC, and S) and five additives ensiled in mini silos appear in Table 2-4. Comparing silage means for DM content in the 3 x 5 factorial (Table 2-4) resulted in similar observed trends as reported for the 4 x 3 factorial (Table 2-3): TC contained the most DM, S was intermediate and PM silage had the least amount of DM. Opposite trends were observed for CP ($P < 0.001$) content of the three silages with higher CP for PM than TC or S. Pearl millet had consistently higher CP, independent of additive. There was a significant increase in DM and CP when corn grain was added to all three silages, but ADF and NDF were not affected by silage sources or additives. Silage means for ADL were highest for PM, lower for S ($P < 0.001$) and lowest for TC ($P < 0.001$). Addition of corn grain resulted in the largest additive effect. Compared with all other additive treatments, corn grain increased DM and CP ($P < 0.001$) while it decreased ADF and NDF ($P < 0.001$). Inoculants caused varied effects on ADL content in TC. Pioneer 1177 inoculant increased ADL content of

TC while added corn grain decreased ADL in TC, but corn grain addition did not affect ADL in S.

Most of the fermentation components of four silages and three additive treatments had significant silage x additive interactions (Table 2-5). Higher pH ($P < 0.01$) was observed for PM compared with other silages ($P < 0.01$), but other additives applied to CS, TC, and S did not affect pH. The highest ammonia concentrations ($P < 0.001$) were observed for S silages. The addition of Pioneer 1177 and Sun-Cure caused increased ($P < 0.05$) ammonia concentrations in PM silage. Lactic acid concentration was extremely low for PM ($P < 0.001$) compared with CS, TC, or S. Pearl millet had higher pH possibly related to decreased lactic acid concentration. The ammonia levels were higher for S because of low concentrations of WSC to be used by the microorganisms to incorporate available nitrogen, resulting in higher levels of accumulated ammonia in this silage. Pioneer 1177 inoculant caused an increase in lactic acid in TC and S compared with controls and Sun-Cure inoculated silages. As with lactic acid, PM had the least amount ($P < 0.001$) of malic acid, and S had the greatest amount, with no noted additive effects on malic acid concentrations.

Volatile fatty acid concentrations in DM of the silages from the mini silos are shown in Table 2-5. Acetic and butyric acid concentration was low in CS, TC, and S, but PM had greater ($P < 0.001$) concentrations of both VFA. Adding Pioneer 1177 or Sun-Cure inoculant to PM decreased ($P < 0.001$) the amount of acetic acid produced, but they did not affect acetic acid in other silages. Pioneer 1177 inoculant was the only additive that increased butyric acid concentration ($P < 0.005$), and that only occurred for PM. The

observed increase in individual VFA concentrations in PM silage resulted in increased total VFA ($P < 0.001$) compared with the other three silages. Similarly, increased lactic acid concentration and low VFA concentration in CS, TC, and S resulted in a lower ($P < 0.001$) calculated lactic:total VFA ratio for PM. No additive effects were noted for either total VFA or lactic:total VFA ratio.

Table 2-6 shows the fermentation characteristics for the PM, TC and S silages ensiled in mini silos with five additive treatments. Results reported in Table 2-5 for PM, TC, and S with the three additive treatments are similar to those reported in Table 2-6, except that corn grain was added as an ensiling treatment along with the inoculant Lact-A-Pro. As in the chemical composition data (Table 2-4), addition of corn grain greatly affected fermentation of all silages. The pH of PM was drastically reduced ($P < 0.001$) by the addition of corn grain, but pH was actually increased ($P < 0.001$) for S with corn grain compared with controls. The addition of Lact-A-Pro decreased ($P < 0.001$) pH on PM and TC, but not S. The additive main effect indicated no significant differences for pH across silages. Ammonia concentration was highest ($P < 0.001$) for S compared with PM and TC. The three inoculant treatments caused the ammonia levels to be more than twice as high ($P < 0.05$) as control or added corn grain treatments for PM, contrasting sharply with practically minimal additive effects on ammonia measured for TC and S. Additive main effect differences were not significant. Apparently the inoculant treatments caused a rapid release of ammonia from the PM silages, which contained higher N concentrations in the form of CP (Table 2-3).

In Table 2-6, lactic acid concentrations were low ($P < 0.001$) for PM compared with TC and S. The addition of corn grain at ensiling elevated lactic acid concentration for PM ($P < 0.001$), increased lactic acid in TC ($P < 0.001$), but decreased lactic acid in S ($P < 0.001$) compared to controls, contributing to a silage x additive interaction. Pioneer 1177 and Lact-A-Pro increased lactic acid in TC and S. The PM silage contained extremely low levels of malic acid compared with other silages ($P < 0.001$; Table 2-6), as noted previously (Table 2-5). Addition of corn grain, Pioneer 1177 and Sun-Cure inoculants decreased ($P < 0.001$) the malic acid of S silages, but had no effect on PM and TC. Malic acid, or dicarboxylic acid, stimulates lactate uptake as much as 10-fold by the predominant ruminal bacterium *Selenomonas ruminantium* (Nisbet and Martin, 1990; Nisbet and Martin, 1991; Nisbet and Martin, 1993; Nisbet and Martin, 1994; Strobel and Russell, 1991). Intermediates of the citric acid cycle accumulate in plant tissue and malate can comprise up to 1.5% of the DM of mature grasses (Bohman, 1983). Forages that are high in organic acids might provide a vehicle for the inclusion of malate in ruminant diets. Additive treatments in our study may have decreased the organic acids in the silages and therefore the malate was insignificant.

Acetic and butyric acid concentrations were greatest ($P < 0.001$) for PM compared to TC and S (Table 2-6), and interactions of silages with additives were observed. Pearl millet control silage had the greatest ($P < 0.05$) acetic acid concentration with lower acetic acid in Sun-Cure and other inoculant treatments. Added corn grain caused the lowest ($P < 0.001$) acetic acid concentration. Butyric acid concentration appeared to be stimulated by Pioneer 1177 and Lact-A-Pro, but depressed by corn grain addition on PM, with no

additive effect differences for other silages. Pearl millet had the greatest total VFA concentration ($P < 0.001$) and lowest lactic acid ($P < 0.001$), therefore the smallest ($P < 0.001$) ratio of lactic acid:total VFA. Sorghum silage had the greatest ($P < 0.001$) ratio of lactic acid:total VFA and TC had an intermediate value.

The chemical characteristics of PM revealed consistently high CP and low DM content compared with the other silages. El Hag et al. (1982) found that moisture was responsible for the loss of DM of corn silage rather than additive effect. The PM in our study had very low DM when ensiled. Previous chemical evaluations of PM grain reported a superior amino acid profile with greater concentrations of tryptophan, threonine, and valine; greater values than present in sorghum grain and maize (Burton et al., 1972). Although PM silage could potentially provide more CP for rumen degradation or by-pass to promote greater animal performance, the low availability of starch hindered preservation causing the observed results for PM. Davies et al (1998) discussed the importance of availability of water soluble carbohydrates (WSC) for silage microorganisms to ferment and produce lactic acid, reducing silage pH. Although WSC were not measured for the silages in this experiment, but there was evidence of deficient amounts of lactic acid and high pH for PM compared with CS, TC and S silages. Lactic acid, a fermentation end product from lactic acid producing bacteria was not sufficient to lower silage pH and is often associated with less efficient fermentation devoid of a carbohydrate source. In addition, PM had an increased level of ammonia, similar to the ammonia levels in grass silage with low WSC content reported by Davies et al. (1998). They suggested that the high ammonia N levels were produced by proteolytic clostridia.

It is interesting that the three inoculant treatments in our study substantially increased ammonia levels on PM, but not on other silages. In their study, Davies et al. (1998) observed no butyric acid concentration though, which is typically an indicator of spoilage and fermentation by clostridia (specifically, *Clostridium tyrobutyricum*). In our study, PM had increased levels of butyric acid and this may be caused by clostridia fermenting lactic acid. Bender et al. (1941) suggested that when the source of carbon is depleted as with a fermentable carbohydrate, then amino acids are used and yield a volatile base. Silages with added corn grain produced major differences in chemical and fermentative characteristics. Ely et al. (1981) observed no effect on pH decline, fermentation or chemical characteristics for CS or S silages when inoculated with *L. plantarum*. *L. plantarum* was a component of all the inoculants used in our study. Similar results were observed when CS was inoculated with various species of bacteria (*Lactobacillus acidophilus, L. bulgaricus, L. brevis, Streptococcus lactis, and S. cremoris*) in a study by Burghardi et al. (1980) which reported decreased DM content. A possible explanation for the results may be the quantity of normal microflora, of green chopped forages, before ensiling, was sufficient, and additional bacteria did not improve fermentation. Additionally, the silages may not have supplied enough of the proper substrates to allow inoculant response. In the future, a study should estimate or count the numbers of bacteria present on the plant at the time of ensiling

In order to determine the effect of silages and additives on IVDMD, composited samples of the 18 treatments were digested, and the data were separated into the same treatment combinations as outlined for chemical composition, fermentation and in vitro

NDF digestibility data. In the 4 x 3 factorial, IVDMD was affected ($P < 0.01$) by silage source (IVDMD, %, for CS, PM, TC and S were 67.6, 54.3, 60.5, 55.5, respectively; SE 0.50, $P < 0.01$). The three additive treatments did not affect IVDMD (IVDMD, %, control, Pioneer 1177™, Sun-Cure™, 59.3, 58.6, 58.9, respectively; SE 0.40; $P < 0.47$). In the 3 x 5 factorial, silage source affected IVDMD (IVDMD, %, PM, TC, S, were 55.1, 61.1, 55.1, respectively; SE 1.03, $P < 0.01$). In this case where five additive treatments were used, additive treatments affected IVDMD (IVDMD, %, control, corn grain, Pioneer 1177™, Lact-A-Pro, Sun-Cure™, 56.8, 61.4, 55.4, 54.0, 56.1, respectively; SE 1.40, $P < 0.05$). The IVDMD was higher for CS and TC, and similar but lower for PM and S. By far the greatest additive effect resulted from the addition of corn grain at ensiling.

The in vitro NDF disappearance increased over time for all silages and for all additives. The effect of additives on the various silages appears in Appendix Figure 1, with that information only supplied for observation. The ANOVA analyses of the data partitioned into two factorials will be presented in Figures 1 and 2.

When data were separated into the two smaller factorials, the in vitro NDF digestibility over time for the four silages (CS, PM, TC and S) and three additives (control, Pioneer 1177 and Sun-Cure inoculants) are shown in Figure 1, first by silages averaged across additives (1A), and then by additives averaged across silages (1B). In Figure 1A digestibility of CS began at approximately 63% , and continued to increase over time, compared with all other silages beginning at approximately 43% . While trend lines were somewhat steeper over time for the remaining silages, none compared with CS for digestibility over time. Trend lines were similar for TC and S, which were well below

CS, but higher than PM. In Table 2-3, NDF concentration was lowest ($P < 0.001$) for CS, and similar but much higher for PM, TC and S. Similar trend lines were observed for additive effects on NDF digestion averaged across the four silages (Figure 1B), with control and Pioneer 1177 having similar trend lines, both of which were higher than Sun-Cure. Pioneer 1177 and Sun-Cure had similar digestion trends through 24 h, but thereafter Pioneer 1177 had a higher digestion rate than Sun-Cure, but lower than the control treatment.

In the second factorial including three silages (PM, TC and S) with five additive treatments (control, corn grain, Pioneer 1177, Lact-A-Pro and Sun-Cure), in vitro NDF disappearance is shown over a 72 h observation interval in Figure 2. The NDF digestion trends for these three silages averaged across the five additive treatments (Figure 2A) is very similar to those displayed for these particular silages averaged over three additive treatments in Figure 1A. Trend lines were similar for S and TC, and both were higher than PM. The NDF concentrations of these three silages were similar (Tables 2-3 and 2-4), which contributed to the similarity in NDF digestibility over time, but does not explain the apparent higher trend for NDF digestion of S compared with TC. Nicholas et al. (1998) compared in vitro NDF digestibility of TCS and S silages and reported that forage type influenced NDF digestion. The TCS digested at a slower rate than the S silages, but left less residue after 96 h of digestion. When additive effects were averaged over silages (Figure 2B), the average effect of NDF digestibility for the additive corn grain was significantly less than the control or inoculant treatments. In terms of the magnitude of slope, the control converted more NDF per unit time than any other treatment (steepest

slope), while corn grain converted the least amount of NDF per unit time (flattest slope) than the other additives. It was initially theorized that perhaps the inoculant treatments would enhance NDF digestion of PM, TC and S silages since they had a relatively low DM with high NDF concentrations. The inoculant treatments did not keep pace with controls in this experiment, therefore they did not improve NDF digestion over time. These results indicate that corn grain addition to these silages was effective in improving NDF digestibility.

In vitro NDF digestibility does not necessarily predict NDF digestibility in vivo or the energy density of a forage (Oba and Allen, 2000). This was true for this study; all silages had predicted values of in vitro NDF digestibility (at 72 h) greater than actual values of in vivo digestion of NDF (Table 2-8). Corn silage, TC and S had similarly high NDF and ADF digestion and PM had the least NDF and ADF digestion during Experiment 2. Inoculating CS caused a decrease in NDF and ADF digestion, while inoculating PM improved digestion of ADF and NDF. No differences were noted for TC and S silage NDF or ADF digestion when inoculated. The results indicate that in vivo digestion of NDF and ADF for CS decreased when Sun-Cure inoculant was added. The IVDMD data for Sun-Cure similarly showed less DM digestion occurring for all silages treated with that inoculant. Furthermore, the in vitro NDF digestibility for controls compared with Sun-Cure inoculated silages, resulted in less NDF disappearance for the Sun-Cure inoculated treatment. Sun-Cure inoculant decreases digestion of fiber and DM both in vitro and in vivo. Similar results were reported by Waldo and Goering (1976) and Whittenberg et al. (1983). Waldo and Goering (1976) fed lambs corn silage, alfalfa silage,

orchardgrass silage, with and without lactic acid bacteria inoculant. The inoculant improved DM digestibility of alfalfa but not orchardgrass or corn silage. Whittenberg et al. (1983) observed no difference in intake or nutrient digestibility of corn silage inoculated with *L. plantarum* and *S. faecium*. Still, other research (Luther, 1986) reported increases in DM and OM digestibility for inoculated corn silage compared with control corn silage fed to steers in 102 d feedlot trial. Leahy et al. (1981) noted higher digestion coefficients for EE, CF, and TDN for corn silage treated with lactobacillus.

Intake and apparent digestion data for CS, PM, TC and S with and without the inoculant Sun-Cure are shown in Table 2-7. Corn silage and TC had greater DMI than S silage fed steers ($P < 0.01$), and steers fed PM had the lowest DMI during the digestion trial. Added supplements changed the DMI slightly for each silage type, but total DMI was not significantly different from silage intake. Corn silage and TC both had similarly greater DM and OM digestibility than S and PM silages. Crude protein digestibility was greatest for PM ($P < 0.01$) and lowest for CS. The inoculant treatment did not affect DMI or digestibility of DM, OM, or CP. In the case of PM silage (Hill et al.,1999) and corn silage (Kung et al., 1993) Pioneer 1174 inoculant did not effect the preservation of these silages in cattle feeding trials. Addition of Sun-Cure in our study did not improve the quality of the silages that were fed (Table 2-2). The silage sources interacted ($P < 0.05$) with the Sun-Cure inoculant treatments for ADF and NDF digestibility (Table 2-8). The inoculant caused depressed ADF and NDF digestibility in CS, and contrasting increases in ADF and NDF for PM ($P < 0.05$). The inoculant did not affect ADF and NDF digestion for TC and S. In the IVDMD data presented for the 18 silage and additive treatments

including Sun-Cure inoculant, IVDMD of CS, PM, TC, and S were not affected by any inoculant treatment. Hill et al (1999) suggested adding a fermentable carbohydrate source to PM plus the inoculant to improve quality and animal performance. As observed in Experiment 1, added corn grain did improve quality of PM, TC and S.

Conclusions

Our study indicates that PM was an inferior forage when ensiled compared with CS, TC and even S silages. Corn silage was the superior silage, although TC and S may be suggested for silage if a double and triple cropping system is being used. Tropical corn and S are typically more tolerant to many environment extremes. There was evidence of improved silage quality when a fermentable carbohydrate source was added in the form of corn grain. Corn grain provided a carbon source for the microorganisms to ferment which produce high concentrations of lactic acid. Lactic acid caused a rapid decrease in pH stabilizing the silage during storage. The addition of three microbial inoculants at ensiling contributed no effect on the ensiling process, did not improve silage quality, or improve digestibility by the animal. Specifically, Sun-Cure inoculant decreased IVDMD, and in vitro NDF disappearance. Total tract digestibility of OM was greater for CS and TC than PM and S, and ADF and NDF digestibility were improved by the addition of an inoculant, on PM. Microbial populations present on the fresh chopped plants may have been adequate to ensile the forages, resulting in no additional improvement in silage fermentation or silage quality by the addition of commercial silage inoculants.

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Table 2-1. Variety of each silage, dates planted and harvested and total yield.

Item[a]	Date planted	Date harvested	Total ensiled, Mg
CS Dekalb 687	March 29, 1999	July 14, 1999	106
PM HGM-100	May 17, 1999	July 27, 1999	140
TC Pioneer 3098	May 13, 1999	August 16, 1999	100
S Dekalb FS25E	May 25, 1999	August 30, 1999	109

[a]Silage source: CS = corn; PM = hybrid grain pearl millet silage; TC = tropical corn silage; S = sorghum silage.

Table 2-2. Nutrient content of four silages without or with Sun-Cure™ inoculant fed to growing steers in a digestion trial, Experiment 2.

Item[a]	CS	CSI	PM	PMI	TC	TCI	S	SI
Analyses[b]	_____ % DM _____							
DM	27.2	29.5	21.4	20.4	26.2	27.0	22.4	24.3
CP	9.7	8.7	11.7	12.5	8.3	8.1	7.9	7.7
ADF	24.9	22.4	38.5	39.8	35.2	34.7	37.6	37.0
NDF	42.7	42.9	56.0	61.9	55.7	56.7	57.8	59.5
TDN	70.0	67.7	57.1	56.3	66.3	66.3	57.0	56.3

[a]Acronyms: CS = corn silage; CSI = CS with inoculant; PM = pearl millet silage; PMI = PM with inoculant; TC = tropical corn silage; TCI = TC with inoculant; S = sorghum silage; SI = S with inoculant.

[b]Mean of analyses of three samples for each kind of silage obtained before mixing in TMR during the intake and digestion experiment.

Table 2-3. Chemical composition of four silages treated with Pioneer 1177 or Sun-Cure inoculants at ensiling in mini silos.

Item	DM	CP	ADF	NDF	ADL
Component			% DM		
Silage[a]					
CS	29.70[b]	7.59[c]	21.67[d]	40.02[c]	2.84[d]
PM	19.24[d]	10.28[b]	36.94[b]	59.62[b]	5.81[b]
TC	28.67[b]	7.46[c]	33.91[c]	60.61[b]	4.12[c]
S	24.29[c]	6.66[c]	34.29[c]	58.84[b]	5.00[bc]
SE[e]	0.32	0.21	0.33	0.39	0.16
Additive					
Control	25.72[b]	7.94[b]	31.83[b]	54.84[b]	4.37[b]
Pioneer 1177	25.36[b]	8.10[b]	31.66[b]	54.86[b]	4.63[b]
Sun-Cure	25.34[b]	7.96[b]	31.62[b]	54.61[b]	4.32[b]
SE[e]	0.27	0.18	0.29	0.34	0.14

[a]Interaction of silage × additive ($P < 0.01$); interaction means shown in Appendix Table 1. Overwhelming effect of silage source shown as main effect means, no differences for additives. The interaction df = 6; integrity of main effect mean separation was protected by the size of the interaction.

[bcd]Within silage and within additive, means in same column with different superscript letters differ ($P < 0.001$).

[e]Two adjustments were made to SE for main effects: First, correction of interaction SE was obtained by computing the ratio of variance from the full model to variance of the 4 x 3 factorial, by taking the square root, and multiplying by the interaction SE. Second, SE was adjusted to guard against differences not justified based on interaction means.

Table 2-4. Interaction means for chemical composition of three silages with five additives ensiled in mini silos.

Item	Additive (A)	Silage source (SS)			A mean	Statistics				
		PM	TC	S		Effect	SE[e]	*df*	LSD[f]	P ≤[f]
Component[a]		% DM								
DM	Control	19.04[c]	29.74[c]	24.18[c]	**24.32[n]**	SS × A	0.46	105	1.70	0.01
	Corn grain	23.47[b]	32.59[b]	26.88[b]	**27.65[m]**	SS	0.34	8	2.40	0.001
	Pioneer	19.45[c]	28.07[c]	24.53[c]	**24.02[n]**	A	0.44	8	3.10	0.001
	Lact-A-Pro	18.94[c]	29.68[c]	24.75[c]	**24.46[n]**					
	Sun-Cure	19.22[c]	28.20[c]	24.15[c]	**23.86[n]**					
	Mean	**20.03[z]**	**29.66[x]**	**24.90[y]**						
CP	Control	9.80[d]	7.48[c]	6.86[c]	**8.05[n]**	SS × A	0.11	105	0.48	0.001
	Corn grain	10.98[b]	8.48[b]	7.48[b]	**8.98[m]**	SS	0.10	8	0.91	0.001
	Pioneer	10.53[b][c]	7.56[c]	6.53[c]	**8.20[n]**	A	0.13	8	0.47	0.01
	Lact-A-Pro	10.35[c]	7.53[c]	6.58[c]	**8.24[n]**					
	Sun-Cure	10.51[b][c]	7.35[c]	6.58[c]	**8.14[n]**					
	Mean	**10.43[x]**	**7.68[y]**	**6.86[y]**						
ADF	Control	37.48[b]	33.44[b]	34.91[b]	**35.28[m]**	SS × A	0.34	105	1.59	0.001
	Corn grain	29.59[c]	29.38[c]	30.34[d]	**29.77[n]**	SS	0.52	8	3.14	0.01
	Pioneer	36.46[b]	33.88[b]	34.48[b]	**34.94[m]**	A	0.67	8	3.65	0.001

	Lact-A-Pro	37.66^{b}	33.04^{b}	32.62^{c}	**34.44^{m}**					
	Sun-Cure	36.88^{b}	34.41^{b}	33.48^{b}	**34.92^{m}**					
	Mean	**35.61^{x}**	**32.83^{x}**	**33.17^{x}**						
NDF	Control	60.41^{b}	60.47^{b}	59.14^{b}	**60.01^{m}**	SS × A	0.61	105	2.92	0.001
	Corn grain	48.69^{c}	52.92^{c}	52.13^{c}	**51.25^{n}**	SS	0.59	8	2.46	0.05
	Pioneer	59.51^{b}	60.34^{b}	59.00^{b}	**59.62^{m}**	A	0.76	8	4.16	0.001
	Lact-A-Pro	60.23^{b}	62.40^{b}	57.53^{b}	**60.05^{m}**					
	Sun-Cure	58.95^{b}	61.00^{b}	58.38^{b}	**59.44^{m}**					
	Mean	**57.56^{x}**	**59.43^{x}**	**57.24^{x}**						
ADL	Control	5.68^{b}	3.70^{cd}	5.31^{b}	**4.90^{m}**	SS × A	0.15	105	0.74	0.001
	Corn grain	4.28^{c}	3.27^{d}	4.73^{b}	**4.09^{m}**	SS	0.18	8	1.64	0.001
	Pioneer	6.01^{b}	4.49^{b}	5.02^{b}	**5.17^{m}**	A	0.23	8	1.27	0.001
	Lact-A-Pro	6.11^{b}	3.74^{cd}	4.81^{b}	**5.17^{m}**					
	Sun-Cure	5.73^{b}	4.16^{bc}	4.67^{b}	**4.86^{m}**					
	Mean	**5.56^{x}**	**3.87^{z}**	**4.91^{y}**						

[a]Abbreviations and terms: Additives, Control = no additive; Corn grain = 5% ground corn added at ensiling; Inoculants = Pioneer 1177 Lact-A-Pro, Sun-Cure added at ensiling . SS = Silage Source; A = Additive.
[bcd]Interior means within components and silage source and within columns with different letters differ (P < 0.01; DM); (P < 0.001; CP, ADF, NDF, ADL).
[e]Two adjustments were made to SE for main effects: First, correction of interaction SE was obtained by computing the ratio of variance from the full model to variance of the 3 x 5 factorial, by taking the square root, and multiplying by the interaction SE. Second, SE adjusted to guard against differences not justified based on interaction means.
[f]The LSD = [SE multiplied by the square root of 2, product multiplied by *t* value, at the error degrees of freedom (*df*) and probability level]. The probability level was determined from analysis of variance from the reduced factorial such that differences reflect at a level compatible with *F* value from the analysis**.** The *t* value used was then at the probability level (Box et al. 1978).
[mn]Main effect means for additives with different superscript letters differ (P < 0.001; CP P < 0.01).
[xyz]Main effect means for silage sources with different superscript letters differ (P < 0.001; DM, CP, ADL; P< 0.01, ADF; P < 0.05, NDF).

Table 2-5. Fermentation characteristics of four silages with three additive treatments ensiled in mini silos.

		Silage source (SS)					Statistics				
Item	Additive (A)	CS	PM	TC	S	A Mean	Effect	SE[c]	*df*	LSD[d]	P ≤[d]
pH	Control	3.65[a]	5.43[a]	3.68[a]	3.58[b]	**4.08[m]**	SS x A	0.03	84	0.1	0.001
	Pioneer	3.65[a]	5.36[a]	3.75[a]	3.78[a]	**4.13[m]**	SS	0.05	6	0.36	0.001
	Sun-Cure	3.66[a]	5.39[a]	3.70[a]	3.69[a]	**4.11[m]**	A	0.05	6	0.17	0.05
	Mean	**3.65[y]**	**5.39[x]**	**3.71[y]**	**3.68[y]**						
Component				% DM							
NH3	Control	0.04[a]	0.12[b]	0.19[a]	0.85[a]	**0.30[m]**	SS x A	0.04	83	0.15	0.001
	Pioneer	0.06[a]	0.31[a]	0.10[a]	0.82[a]	**0.32[m]**	SS	0.06	6	0.48	0.001
	Sun-Cure	0.05[a]	0.27[a]	0.17[a]	0.79[a]	**0.32[m]**	A	0.05	6	0.17	0.05
	Mean	**0.05[y]**	**0.23[y]**	**0.16[y]**	**0.82[x]**						
Lactic acid	Control	5.27[a]	0.11[a]	4.86[b]	7.21[b]	**4.36[m]**	SS x A	0.17	83	0.81	0.001
	Pioneer	5.55[a]	0.12[a]	6.41[a]	8.61[a]	**5.17[m]**	SS	0.31	6	2.61	0.001

	Sun-Cure	5.52[a]	0.17[a]	5.60[b]	7.05[b]	**4.58[m]**	A	0.27	6	0.93	0.05
	Mean	**5.45[a]**	**0.13[y]**	**5.62[x]**	**7.62[x]**						
Malic acid	Control	2.04[a]	0.04[a]	2.45[a]	3.80[a]	**2.08[m]**	SS x A	0.17	83	0.08	0.001
	Pioneer	1.92[a]	0.06[a]	2.31[a]	3.03[a]	**1.85[m]**	SS	0.12	6	1.1	0.001
	Sun-Cure	2.02[a]	0.04[a]	2.60[a]	3.31[a]	**1.99[m]**	A	0.11	6	0.04	0.05
	Mean	**2.02[y]**	**0.05[z]**	**2.45[xy]**	**3.38[x]**						
Volatile fatty acids											
Acetic	Control	1.03[a]	7.29[a]	1.56[a]	0.92[a]	**2.7[m]**	SS x A	0.32	83	0.89	0.05
	Pioneer	0.96[a]	5.70[b]	1.70[a]	0.82[a]	**2.29[m]**	SS	0.23	6	1.64	0.001
	Sun-Cure	1.03[a]	6.25[b]	1.98[a]	0.85[a]	**2.53[m]**	A	0.2	6	0.65	0.05
	Mean	**1.01[y]**	**6.41[x]**	**1.75[y]**	**0.86[y]**						
Butyric	Control	0.01[a]	1.08[b]	0.01[a]	0.01[a]	**0.28[m]**	SS x A	0.12	83	0.33	0.05
	Pioneer	0.05[a]	1.75[a]	0.02[a]	0.03[a]	**0.46[m]**	SS	0.12	6	0.94	0.001
	Sun-Cure	0.09[a]	1.25[b]	0.02[a]	0.03[a]	**0.35[m]**	A	0.1	6	0.33	0.05
	Mean	**0.05[y]**	**1.36[x]**	**0.02[y]**	**0.02[y]**						
Total VFA	Control	1.09[a]	8.61[a]	1.63[a]	0.97[a]	**3.08[m]**	SS x A	0.47	83	1.3	0.05
	Pioneer	1.09[a]	7.73[a]	1.77[a]	0.95[a]	**2.89[m]**	SS	0.27	6	2.25	0.001
	Suncure	1.18[a]	7.89[a]	2.07[a]	0.95[a]	**3.02[m]**	A	0.24	6	0.8	0.05
	Mean	**1.12[y]**	**8.08[x]**	**1.82[y]**	**0.96[y]**						
Lactic:Total VFA	Control	5.23[a]	0.02[a]	3.17[a]	7.70[b]	**4.03[m]**	SS x A	0.4	83	1.12	0.05
	Pioneer	5.35[a]	0.02[a]	3.92[a]	9.49[a]	**4.69[m]**	SS	0.27	6	2.23	0.001
	Sun-Cure	4.96[a]	0.02[a]	2.88[a]	7.66[b]	**3.88[m]**	A	0.23	6	1.2	0.01
	Mean	**5.18[y]**	**0.02[z]**	**3.32[y]**	**8.28[x]**						

[ab]Interior means in columns for additives within silage source with different letters differ ($P < 0.01$; Acetic, Butyric and Total VFA, Lactic:Total VFA, $P < 0.05$) .

[c]Two adjustments were made to SE for main effects: First, correction of interaction SE was obtained by computing the ratio of variance from the full model to variance of the 4 x 3 factorial, by taking the square root, and multiplying by the interaction SE. Second, SE adjusted to guard against differences not justified based on interaction means.

[d]The LSD = [SE multiplied by the square root of 2, product multiplied by *t* value, at the error degrees of freedom (*df*) and probability level]. The probability level was determined from analysis of variance from the reduced factorial such that differences reflect at a level

[m]Main effect means for additives were not different ($P > 0.10$).

[xyz]Main effect means for silage source with different superscript letters differ ($P < 0.001$).

Table 2-6. Fermentation characteristics of three silages with five additive treatments ensiled in mini silos.

		Silage source (SS)				Statistics				
Item	Additive (A)	PM	TC	S	A Mean	Effect	SE[d]	*df*	LSD[e]	P ≤[e]
pH	Control	5.43[a]	3.68[ab]	3.58[c]	**4.23[m]**	SS x A	0.03	105	0.11	0.001
	Corn grain	4.71[c]	3.64[bc]	3.71[ab]	**4.02[m]**	SS	0.08	8	0.66	0.001
	Pioneer	5.36[a]	3.75[a]	3.78[a]	**4.30[m]**	A	0.1	8	0.34	0.01
	Lact-A-Pro	5.19[b]	3.57[c]	3.66[bc]	**4.14[m]**					
	Sun-Cure	5.39[a]	3.70[ab]	3.69[ab]	**4.26[m]**					
	Mean	**5.22[x]**	**3.67[y]**	**3.68[y]**						
Component		% DM								
NH3	Control	0.12[b]	0.19[a]	0.85[a]	**0.39[m]**	SS x A	0.04	105	0.15	0.001
	Corn grain	0.12[b]	0.14[a]	0.83[a]	**0.36[m]**	SS	0.06	8	0.37	0.001
	Pioneer	0.31[a]	0.10[a]	0.82[a]	**0.41[m]**	A	0.07	8	0.22	0.05
	Lact-A-Pro	0.32[a]	0.12[a]	1.26[a]	**0.57[m]**					
	Sun-Cure	0.26[a]	0.17[a]	0.79[a]	**0.41[m]**					
	Mean	**0.22[y]**	**0.14[y]**	**0.91[x]**						
Lactic acid	Control	0.11[b]	4.86[c]	7.21[b]	**4.06[m]**	SS x A	0.17	105	0.8	0.001
	Corn grain	4.02[a]	5.80[ab]	6.08[c]	**5.30[m]**	SS	0.6	8	4.24	0.001
	Pioneer	0.12[b]	6.41[a]	8.61[a]	**5.05[m]**	A	0.77	8	2.51	0.05
	Lact-A-Pro	0.57[b]	6.08[ab]	7.82[ab]	**4.83[m]**					
	Sun-Cure	0.17[b]	5.60[bc]	7.05[b]	**4.27[m]**					
	Mean	**1.00[y]**	**5.75[x]**	**7.35[x]**						
Malic acid	Control	<0.01	2.45[a]	3.80[a]	**2.10[m]**	SS x A	0.17	105	0.48	0.05
	Corn grain	0.10[a]	2.60[a]	2.96[c]	**1.88[m]**	SS	0.1	8	0.72	0.001
	Pioneer	0.10[a]	2.31[a]	3.03[c]	**1.80[m]**	A	0.13	8	0.43	0.05
	Lact-A-Pro	<0.01	2.79[a]	3.78[ab]	**2.19[m]**					
	Sun-Cure	<0.01	2.60[a]	3.31[bc]	**1.98[m]**					

	Mean	**0.10^{z}**	**2.55^{y}**	**3.38^{x}**						
Volatile fatty acids										
Acetic	Control	7.29^{a}	1.56^{a}	0.92^{a}	**3.26^{m}**	SS x A	0.32	105	0.88	0.05
	Corn grain	5.04^{c}	1.23^{a}	0.67^{a}	**2.31^{m}**	SS	0.19	8	1.36	0.001
	Pioneer 1177	5.70^{bc}	1.70^{a}	0.82^{a}	**2.74^{m}**	A	0.25	8	1.17	0.01
	Lact-A-Pro	5.64^{bc}	1.44^{a}	0.98^{a}	**2.69^{m}**					
	Sun-Cure	6.25^{b}	1.98^{a}	0.85^{a}	**3.03^{m}**					
	Mean	**5.98^{x}**	**1.58^{y}**	**0.85^{y}**						
Butyric	Control	1.08^{c}	0.01^{a}	0.01^{a}	**0.37^{m}**	SS x A	0.12	105	0.56	0.001
	Corn grain	0.11^{d}	0.01^{a}	0.02^{a}	**0.05^{m}**	SS	0.31	8	1.45	0.01
	Pioneer	1.75^{b}	0.02^{a}	0.03^{a}	**0.60^{m}**	A	0.4	8	1.29	0.05
	Lact-A-Pro	3.62^{a}	0.02^{a}	0.03^{a}	**1.22^{m}**					
	Sun-Cure	1.25^{bc}	0.02^{a}	0.03^{a}	**0.43^{m}**					
	Mean	**1.56^{x}**	**0.01^{y}**	**0.02^{y}**						
Total VFA	Control	8.61^{ab}	1.63^{a}	0.97^{a}	**3.74^{m}**	SS x A	0.37	105	1.75	0.001
	Corn grain	5.26^{c}	1.26^{a}	0.78^{a}	**2.43^{m}**	SS	0.43	8	3.05	0.001
	Pioneer	7.73^{b}	1.77^{a}	0.95^{a}	**3.49^{m}**	A	0.56	8	1.8	0.05
	Lact-A-Pro	10.50^{a}	1.52^{a}	1.12^{a}	**4.36^{m}**					
	Sun-Cure	7.89^{b}	2.07^{a}	0.95^{a}	**3.64^{m}**					
	Mean	**7.99^{x}**	**1.65^{y}**	**0.95^{y}**						
Lactic:Total VFA	Control	0.02^{a}	3.17^{bc}	7.70^{b}	**3.63^{m}**	SS x A	0.4	83	1.12	0.05
	Corn grain	0.82^{a}	4.69^{a}	8.15^{b}	**4.55^{m}**	SS	0.27	6	2.23	0.001
	Pioneer	0.02^{a}	3.92^{abc}	9.49^{b}	**4.47^{m}**	A	0.23	6	1.2	0.01
	Lact-A-Pro	0.06^{a}	4.11^{ab}	7.34^{b}	**3.84^{m}**					
	Sun-Cure	0.02^{a}	2.88^{c}	7.66^{b}	**3.52^{m}**					
	Mean	**0.19^{z}**	**3.75^{x}**	**8.07^{y}**						

[abc]Interior means in columns for additives within silage source with different letters differ ($P < 0.01$; except malic acid and acetic acid ($P < 0.05$).

[d]Two adjustments were made to SE for main effects: First, correction of interaction SE was obtained by computing the ratio of variance from the full model to variance of the 3 x 5 factorial, by taking the square root, and multiplying by the interaction SE. Second, SE adjusted to guard against differences not justified based on interaction means.

[e]The LSD = [SE multiplied by the square root of 2, product multiplied by *t* value, at the error degrees of freedom (*df*) and probability level]. The probability level was determined from analysis of variance from the reduced factorial such that differences reflect at a level.

[m]Main effect means for additives were not different ($P > 0.10$).

[xyz]Main effect means for silage sources with different superscript letters differ ($P < 0.001$; butyric $P < 0.01$; lactic:total VFA $P < 0.05$).

Table 2-7. Intake and apparent digestion of four silages with or without an inoculant in growing beef steers.[a]

	Silage					Inoculant		
Item	CS	PM	TC	S	SE	NI	I	SE
DMI	kg/d							
Silage	5.4[b]	3.1[e]	4.6[c]	3.9[d]	0.21	4.3[b]	4.2[b]	0.15
Total diet	5.5[b]	3.3[e]	4.6[c]	4.2[d]	0.21	4.3[b]	4.4[b]	0.15
Apparent digestion	%							
DM	65.3[b]	57.9[c]	63.8[b]	60.0[c]	1.77	61.9[b]	61.6[b]	1.3
OM	67.0[b]	60.2[c]	65.7[b]	61.9[c]	1.65	64.0[b]	63.3[b]	1.6
CP	60.9[c]	65.9[b]	60.8[bc]	63.1[bc]	1.53	62.6[b]	62.7[b]	1.1

[a]Abbreviations: Silages, CS = corn; PM = pearl millet; TC = tropical corn; S = sorghum; SE = standard error; Inoculant applied at ensiling, NI = no inoculant; I = Sun-Cure™ inoculant.

[bcde]Means within main effects on same line with different superscript letters differ ($P < 0.01$). Least squares means adjusted for initial BW of steers.

Table 2-8. Interaction of silage source with inoculant applied at ensiling for apparent digestion of dietary ADF and NDF in steers.

	ADF digestion[a]		NDF digestion[b]	
Item[fg]	NI	I	NI	I
Apparent digestion	%			
Silage source				
Corn	56.3[cy]	47.0[ez]	51.1[cdy]	45.3[dz]
Pearl millet	46.0[dz]	54.5[cdy]	46.1[dz]	57.1[cy]
Tropical corn	57.1[cz]	58.2[cz]	57.8[cz]	58.9[cz]
Sorghum	52.3[cdz]	49.8[dez]	52.8[cdz]	52.6[cz]

[a]For ADF digestion silage source × inoculant interaction ($P < 0.05$).
[b]For NDF digestion silage source × inoculant interaction ($P < 0.05$).
[cde]Means within silage sources, within either ADF or NDF digestion with different letters differ ($P < 0.05$).
[yz]Means within inoculant treatments within either ADF or NDF digestion with different letters ($P < 0.05$).
[f]For different silage sources, NI vs I, SE = 2.29 for ADF digestion and 2.45 for NDF digestion.
[g]For inoculant treatments among silages, SE = 2.19 for ADF digestion and 2.34 for NDF digestion.

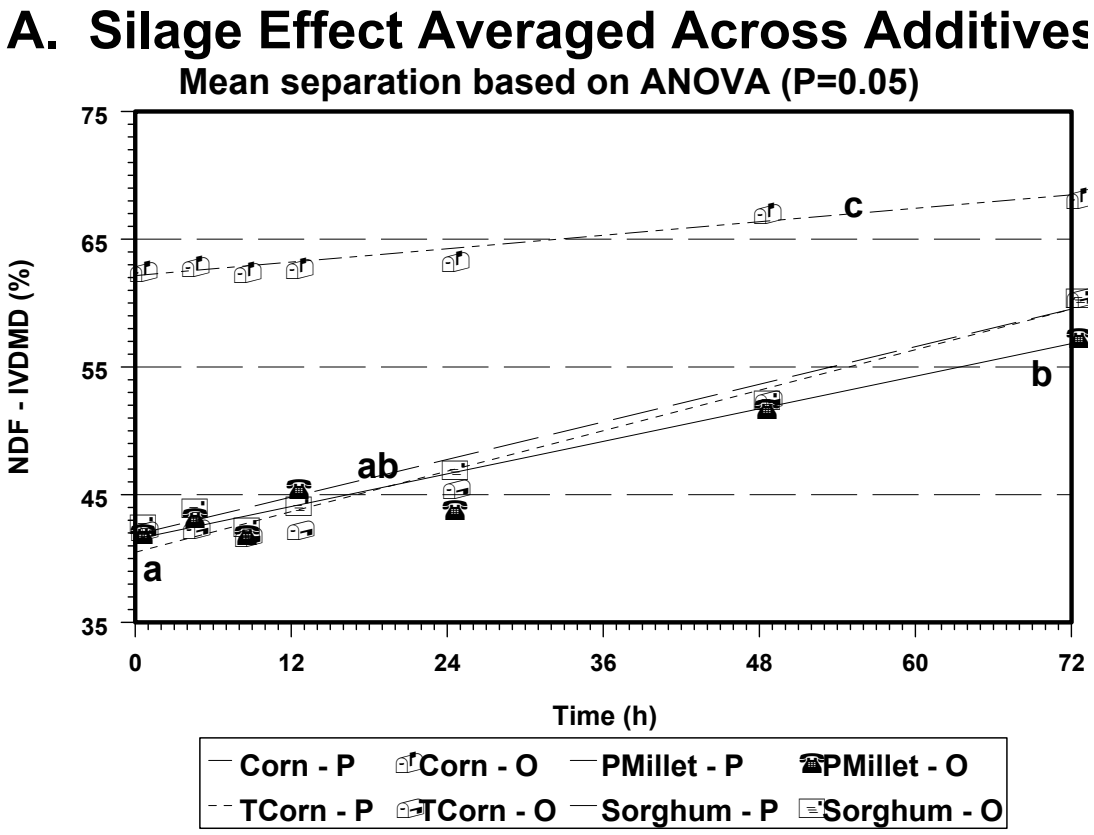

Figure 2.1. Effect of four silages with three ensiling additives on in vitro NDF digestion: 1A. Silage effect averaged across additives; 1B. Additive effects averaged across silages (a,b,c, P < 0.05).

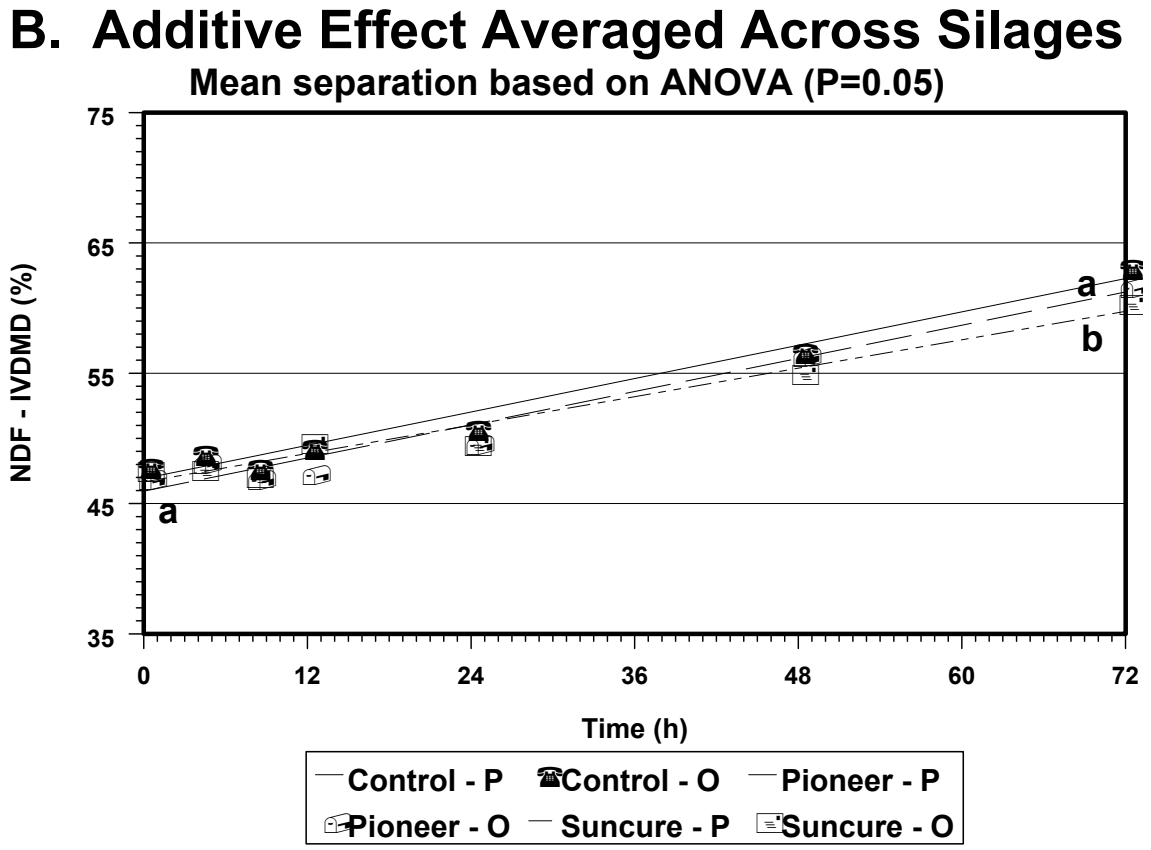

Figure 2.1. continued

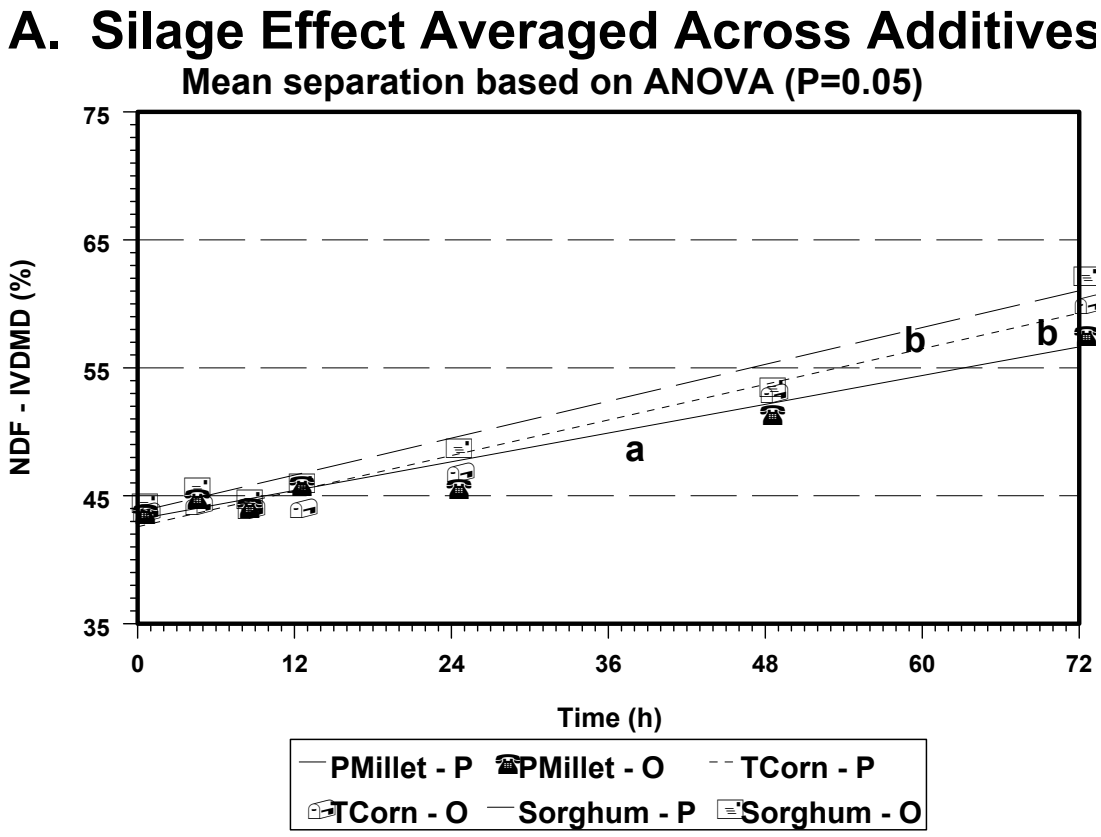

Figure 2.2. Effect of three silages with five ensiling additives on in vitro NDF digestion: 2A. Silage effect averaged across additives; 2B. Additive effects averaged across silages (a,b,c, P < 0.05).

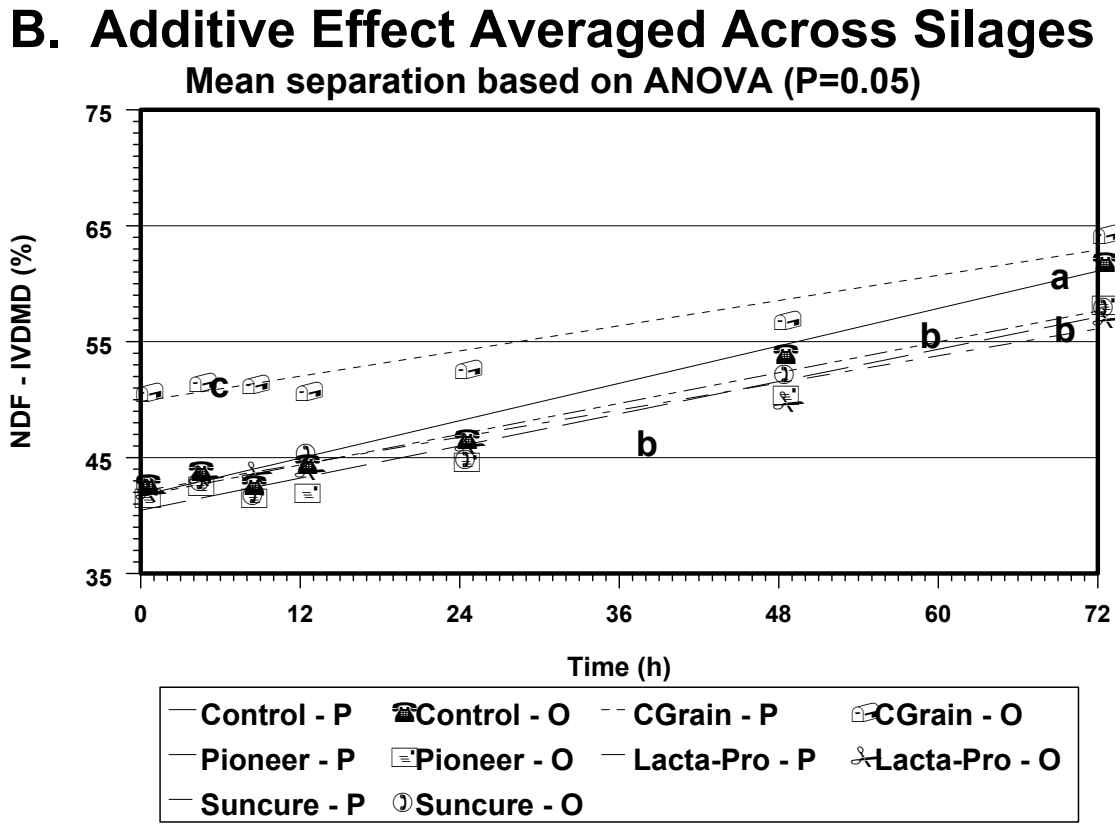

Figure 2.2. continued

CHAPTER 3

EFFECTS OF FOUR SILAGES WITH AN INOCULANT ON FEED QUALITY, DIGESTIBILITY AND PERFORMANCE OF GROWING CATTLE

Introduction

Hay and silages are often used as major feedstuffs for growing beef cattle after weaning in backgrounding systems, and in diets for growing beef and dairy replacement heifers. Temperate corn silage production in the southern U.S. usually involves corn varieties planted in early March and harvested in July. Alternatives are needed for silage production in mid-summer and autumn to allow for utilization of the land while producing crops that are moderate to high in energy to be fed to growing cattle or to beef and dairy herds. Some producers located in Florida, Georgia, and along the Gulf Coast have double-cropped tropical corn for silage following temperate corn silage harvested in July. Sorghum silage is an alternative crop that has moderate drought tolerance, but it usually has lower TDN than corn silage. Recent research has evaluated hybrid pearl millet grain fed to cattle as replacements for dietary corn and soybean meal (Hill et al., 1990; 1996; Mathis, 1993). Silages made from pearl millet grain hybrids and temperate corn silage were fed to growing steers, but resulted in lower performance on millet silage diets (Hill et al., 1999).

Silage additives have been used to increase quantity and quality of ensiled forages. Microbial inoculants are added to fresh chopped forage to rapidly reduce pH of the forage through enhanced microbial fermentation. There are conflicting theories on the effectiveness of inoculation and improvement in performance of cattle fed inoculated silages. El Hag et al. (1982) reported no improvement in feeding value (weight gain or feed efficiency) for yearling crossbred heifers in a feedlot study. In earlier studies by Bolsen (1978), additive application to ensiled forages had a wide range of effectiveness for silage quality and animal performance.

In our experiments we examined the differences in kinds of forage used for silage and effectiveness for meeting nutritional requirements, digestibility and actual performance of cattle. Further, we explored the additive effects of inoculated forages on performance and digestion of growing beef cattle.

Materials and Methods

Experiment 1

A feedlot experiment was conducted using steers and heifers fed each of four harvested silages (CS, PM, TC, and S) without inoculant (NI) or with Sun-Cure™ inoculant (I) applied at ensiling (Table 2-1; Chapter 2). Cattle in this experiment were weaned in September, 1999 at approximately 10 mo of age. The British and Charolais crossbred steers (n = 63; 282 ± 29.4 kg BW) and heifers (n = 64; 228 ± 25.9 kg BW) were ranked by BW and randomly assigned to eight treatments in a 4 x 2 factorial arrangement of treatment. Treatment main effects were silage source (SS) for the following forages: Temperate Corn (CS), Pearl Millet (PM), Tropical Corn (TC), Sorghum (S) with inoculant (Sun-Cure™) (I) or without inoculant (NI). One pen of steers and one pen of heifers were

assigned to each of the eight treatments. Steers and heifers were implanted with Synovex-S® or Synovex-H®, respectively, and received Totalon® (for removal of internal parasites; 2.5 ml/20.9 kg body weight; Schering-Plough Animal Health Corp., USA) on d 1 of the experiment. Cattle were housed in a feedlot, each pen (30.5 x 9.1 m) had sheltered feedbunks. Concrete flooring extended under one third of each pen, and two thirds of each pen was dirt.

When the 84-d feedlot experiment began in October, 1999, both ends of each silo bag were opened, with NI silage fed from one end, and I silage fed from the opposite end of each bag. Silages (Table 3-1) were fed free-choice once daily with added supplements (Table 3-2), which were formulated to balance CP and energy in the total mixed rations (TMR). Using initial silage analyses, corn and soybean meal were fed at different rates to meet growing cattle requirements (NRC, 1984). A compromise on energy and CP was made for steers and heifers for the feedlot. Based on preliminary analyses of the silages, more corn and less CP were fed in SUP-B, on PM and S diets; and more CP and lower corn grain levels were fed to cattle in SUP-A, on CS and TC diets. Silage and supplement were weighed into a mixer-feeder wagon equipped with electronic scales, and thoroughly mixed before feeding. Feed bunks were monitored to allow daily adjustments to amount of TMR offered. Water and minerals (Hi-Seven, W. B. Fleming Co., Tifton, GA; minimum (%), respectively, Ca, 16.0; P, 7.0; NaCl, 20; NaCl, 24.0 (maximum); F, 0.07 (maximum); Fe, 1.0; Cu, 0.012; Co,0.003; MN, 0.12; Mg, 1.0; Zn, 0.12; I, 0.003; S, 1.0; Se, 0.0015) were available ad libitum. Initial, mid-point, and final BW were means of two consecutive daily full weights.

The total DMI for each pen of feedlot cattle, during the experiment, was calculated. The added supplements were subtracted from the total intake for the 84-d experiment and then divided by 84 to obtain daily intake of silage. Pen total feed intake was then divided by number of cattle per pen. Total silage and TMR DMI were computed using DMI of silages and supplements. Cattle ADG was computed by subtracting initial BW from final BW and dividing by the number of days.

Experiment 2

An intake and digestion experiment was conducted using beef steers fed each of the four silages (CS, PM, TC, and S) that were treated with Sun-Cure™ inoculant (I) applied at ensiling (Table 2-1; Chapter 2). Steers in this experiment were weaned in September, 1999 at approximately 10 mo of age. The British and Charolais crossbred steers (n = 28; 259 ± 15.18 kg BW) were ranked by BW and assigned at random to four dietary treatments in a completely randomized design. Steers were implanted with Synovex-S®, and received Totalon™ (for removal of internal parasites; 2.5 ml/20.9 kg body weight; Schering-Plough Animal Health Corp., USA) on d 1 of the experiment. Collars were fitted for each steer to tether them in individual stalls, (1.22 x 2.02 m) equipped with rubber mats in a semi-enclosed barn. Seven steers were randomly assigned (based on BW) to each of the four dietary treatments, and the feeding experiment began in late November, 1999. During the 28-d experiment, the same inoculated silages and supplements in the TMR fed to feedlot steers in Exp. 1 were fed to the digestion experiment steers, since both Exp. 1 and 2 were conducted simultaneously. Chemical analyses of the four inoculated silages fed in the digestion and intake study are shown in Table 3-3. Feed refusals were measured daily and subtracted from total feed offered to

determine feed intake, and to allow daily adjustments of silage offered. Apparent digestibility of nutrients was determined using Cr as a marker. Chromic oxide (10 g/steer daily) was administered in gelatin capsules (d 18 - d 27). Fecal samples (12/steer) were collected at 8 h intervals from d 24 to d 28 on a schedule designed to reduce diurnal variation in extracted nutrients. A composite of fecal samples collected over time for each steer was made, and the dried samples were stored for chemical analyses.

Analyses

To determine nutrients in silages and in fecal samples from the digestion trial, all samples were dried at 60°C for 72 h and ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) using a 1 mm screen. Fecal samples were analyzed for Cr using the method of Brisson (1956). Dried and ground samples were then analyzed for CP by methods of the AOAC (1995). Diet and fecal sample NDF and ADF concentrations were determined by methods of Van Soest et al. (1991), and ADL was determined by the method of Van Soest and Wine (1968).

Statistical analyses were conducted for the feedlot trial and the steer digestion trial. Feedlot performance data were analyzed as a 4 x 2 factorial using a Mixed Model procedure of SAS (2000). The analysis of variance for initial BW only showed differences between steers and heifers, and it showed no effects of bias to any treatment combination. Therefore, ADG was adjusted for initial BW. The steer digestion trial data were analyzed as a completely random design using a Mixed Model procedure of SAS (2000), and least squares means were adjusted for weight classification of initial BW of steers.

Results and Discussion

Performance of cattle revealed no interactions of silage source with inoculant treatments (Table 4; $P > 0.10$). The I treatments did not affect any performance parameter ($P > 0.01$). Silage source affected ADG ($P < 0.01$) and DM/gain ($P < 0.01$), with highest ADG on CS, followed by TC and S, with lowest ADG recorded for PM. Conversion efficiency followed ADG patterns, with greatest efficiency on CS, followed by TC and S, and lowest efficiency on PM. Gain and efficiency of cattle was affected by DMI, because DMI was 17.5% higher for CS than PM ($P < 0.07$) and 14% higher for CS than TC, but DMI on CS was only 7% higher than S. The increased fiber content (ADF and NDF) of PM and S silages (Table 3-1) and decreased DM limited intake of these silages.

In earlier experiments Hill et al. (1999) fed either PM silages or CS to growing heifers for 38 d, resulted in significantly higher DMI and ADG in heifers fed CS diets. In their second experiment, growing steers were fed PM silage, PM silage with 0.5% cracked corn added at ensiling, or CS, with soybean meal added at 0.45, 0.45 or 0.68 kg/ steer daily, respectively, for the three silage treatments. The 56-d ADG was higher ($P < 0.05$) and DM/gain was improved ($P < 0.05$) for CS compared with either PM treatment. Addition of corn to PM at ensiling did not improve ADG or DM/gain above the levels for PM silage.

Limited research on sorghum and ensiled sorghum has been published in scientific journals. Although sorghum is adapted to extreme temperatures and drought, it has high lignin content. Lignin is the indigestible portion of the plant, a component of NDF, and reduces the DMI by increasing gut fill (Aydin et al., 1999). One study (Aydin et al., 1999) determined lignin content in standard sorghum silage compared with corn silage and a

bmr mutant sorghum silage. The reported value for lignin in standard sorghum silage was two times as large as lignin present in corn silage (lignin: S = 9.5%, lignin: CS = 4.4%). Lignin content in S in their study was similar to the ADL observed in our study (ADL: S = 5.0; ADL: CS = 2.84; Table 2-3). Aydin et al. (1999) fed Holstein cows diets containing standard sorghum, mutant sorghum, corn, or alfalfa silages that were formulated to equalize CP; therefore the differences were amount of lignin, ADF, and NDF. Although the values for the DMI were not different between silages, expressed as percent of BW, corn silage was highest and standard sorghum lowest, alfalfa and mutant sorghum were intermediate. Corn silage was superior to standard sorghum in this study and in our experiments. Tropical corn has a high DM yield potential, is resistant to many insects and disease, and can tolerate high temperatures. In our study CP and TDN were slightly lower, and ADF, NDF, and TDN were slightly higher for TC (I) compared with CS (I) (Table 3-3). However, intake by steers was lower ($P < 0.01$) for TC compared with CS and CP, ADF, NDF, and OM digestion of the TMR was similar for CS and TC (Table 3-5). These results agree with results of Monson et al., (1980), in which the growing TC plant had less DM in the ear than temperate corn plant; however by the time of harvesting there were no differences between corn cultivars. This confirms the advantages of growing TC in the late summer months because there may be equal quality of forage compared with temperate corn cultivars, plus TC exceeds the temperate corn in hardiness during this part of the growing season. Additionally, TC has higher TDN than S (Table 3-1), which is another potential late summer silage crop, and can therefore be considered a popular alternative crop for late summer.

Utley et al. (1997) and Johnson et al. (1981) reported greater NDF and ADF content in tropical corn silages than temperate corn silages, but higher apparent digestion of OM, ADF, and NDF in steers fed TC diets vs temperate CS diets. In the 84-d growth and DMI trial by Utley et al. (1997), steers fed the temperate CS diet had 16.9% higher DMI ($P < 0.01$) and 17.6% higher ADG ($P < 0.01$) than steers fed TC diets. The DMI of TC silage in our study was similar to that reported by Utley et al. (1997). They suggested that the increased fiber content (NDF and ADF in TC) resulted in greater gut fill which, decreased DMI for cattle fed tropical corn compared with temperate corn. The increased digestibility of TC may have resulted from lower DMI, and therefore the small amount of TC could remain longer in the gastrointestinal tract, providing time for complete digestion.

In our study, PM silages were higher in CP content (Table 3-1; 3-3) and had higher digestibility (Table 3-5), but increased fiber content of PM may have decreased DMI, resulting in lower DMI and performance of cattle. Data from the mini silos (Chapter 2; Table 2-3) indicated greater ADL content for PM than TC or S silages. Feeding PM silage diets containing high TDN and low CP supplements might improve utilization of PM silages, while additional protein sources in TC and S silage diets might improve performance of cattle fed those silages. The silage inoculant used in this experiment, did not affect cattle performance, but inoculants can often improve fermentation of some silages. Schneider et al. (1995) reported that the addition of lactic acid bacteria (inoculant) to wet brewer's grain caused more efficient fermentation because of rapid decline in pH via increased production of lactic acid and decreased production of acetic acid and butyric acid.

In Experiment 2, the TMR used in the feedlot experiment for the I treatments were individually-fed to steers. The DMI was highest for CS, intermediate for TC and S, and lowest for PM ($P < 0.01$; Table 3-5). The low DMI of PM may be related to the high fiber content plus the higher moisture content of PM silage (Table 3-3) compared with CS, TC, and S silages. Frequently, PM silages are low in DM content as reported by Hill et al. (1999) in a study comparing millet and corn silages fed to growing heifers.

In our study there were no significant differences between digestibility of DM, OM, ADF, and NDF of the silages (Table 3-5). The addition of the supplements (Table 3-3) equalized the digestibility coefficients for these variables for the different silages, and CP was the only variable affected by treatment. The CP digestibility was highest for PM ($P < 0.01$) followed by CS, TC and S, with the lowest digestibility. The higher quality of amino acid components in grain heads in PM silage may explain the increased digestibility of CP. Burton et al. (1972) reported that the amino acid composition of PM was higher than sorghum and wheat in tryptophan, threonine and valine. Similar increases in protein quality were observed by Hill et al. (1990; 1996). In addition, the lower digestibility observed for S might have occurred because of increased lignin content, as determined by Aydin et al. (1999) in a study of sorghum silage composition. In our companion study, the ADL concentrations in S and PM silages were higher compared with CS and TC silages from mini silos (Chapter 2; Table 2-3).

The four silages differed in chemical composition (Table 3-1; Table 3-3). The supplements (Table 3-2) were designed to equalize the differences in CP and energy available for the growing cattle in the feedlot and digestion experiment. In Table 3-5 all TMR resulted in similar digestibility, except for higher CP digestibility for PM. More

dry-rolled corn was added to the S and PM silages through the assigned supplement (Table 3-2; SUP-B) to provide additional energy. This may explain some of the decrease in DMI for cattle fed the S and PM silages (Table 3-6). The TMR contained enough energy supplied to meet the energy requirement based on NRC (1984) requirements for growing cattle, but depressed DMI on the PM diets was probably related most to higher fiber and ADL content of these silages. Even though OM and fiber digestibility coefficients were similar for all silages (Table 3-5), the reduced DMI in the feedlot and digestion experiments on the PM diets suggest that fiber content might be depressing intake, which in turn depresses performance (Table 3-4). Another possible reason for decreased intake of PM may be a consequence of the putrid smell of the silage. The calves may not find this silage very palatable.

Conclusion

The DMI of feedlot and digestion trial cattle were affected most by silage source in the TMR. In the feedlot, DMI was highest for CS, then TC, followed by S with lowest DMI on PM. In the intake and digestion experiments, DMI of the TMR was highest for CS (I), followed by PM (I), TC (I), and S (I) in Experiment 2. Adding the appropriate supplements to adjust energy and CP in the various diets resulted in similar digestibility of OM, ADF, and NDF in the TMR. A higher quality of CP in the PM silage made CP digestibility highest for the PM(I) TMR, intermediate for CS(I) and TC(I) and lowest for S(I). Addition of an inoculant did not increase DMI, ADG or DM/gain in the feedlot trial. Based on this observation, other methods of increasing animal performance on silage diets should be researched. If a starch source or energy source such as corn grain is added to silages at ensiling, nutrient content and digestibility should increase (Chapter 2).

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Table 3-1. Chemical analyses of silages with or without Sun-Cure inoculant fed to growing beef cattle.

Item[a]	CS	CSI	PM	PMI	TC	TCI	S	SI
Analyses[b]	% DM							
DM	28.1	29.3	21.0	20.8	25.9	27.3	22.2	23.6
CP	9.3	8.8	11.7	12.2	8.2	8.2	7.8	7.6
ADF	25.5	24.3	40.0	40.8	35.3	35.4	39.1	38.4
NDF	42.4	41.3	59.5	61.2	56.8	58.0	61.4	61.3
TDN[c]	69.8	68.7	55.3	56.3	66.2	66.0	56.0	55.8

[a]Acronyms: CS = corn silage; CSI = CS with inoculant; PM = pearl millet silage; PMI = PM with inoculant; TC = tropical corn silage; TCI = TC with inoculant; S = sorghum silage; SI = S with inoculant.
[b]Analyses based on six samples of each silage collected during feeding experiment.
[c]Total digestible nutrients (TDN) calculated from analyses.

Table 3-2. Composition of two dietary supplements (SUP) fed with different silage sources to cattle.

Item[a]	SUP-A Fed with CS and TC	SUP-B Fed with PM and S
Ingredient	As-fed basis, %	
Dry-rolled corn	44.40	76.00
Soybean meal	50.00	18.40
Trace mineralized salt	1.60	1.60
Calcium carbonate	2.80	2.80
Lasalocid premix[b]	1.10	1.10
Vitamin premix[c]	0.15	0.15
Chemical analyses	% of DM	
DM	89.5	89.0
CP	27.9	16.2
ADF	6.7	6.7
NDF	10.8	12.8
TDN	84.0	83.0

[a]In feeding trials, SUP-A fed in TMR for CS and TC silages at 1.25 kg/animal daily; and, SUP-B fed in TMR for PM and S silages at 2.27 kg/animal daily.
[b]Lasalocid mixed with finely ground corn, formulated to deliver 150 mg/animal daily in TMR.
[c]Vitamins A, D, and E provided at 24,000, 8,000, and 400 IU/animal daily; Se provided at 2.0 mg/d.

Table 3-3. Chemical analyses of silages with Sun-Cure inoculant fed to growing steers in digestion trial, Experiment 2.

Item[a]	CSI	PMI	TCI	SI
Analyses				
DM	30.0	20.8	27.7	23.1
CP	8.9	12.3	8.2	7.7
ADF	26.1	41.1	34.7	39.0
NDF	42.9	62.0	58.6	63.5
TDN	67.0	56.3	66.0	55.3

[a]Mean of analyses of three samples for each kind of silage obtained before mixing in TMR during the 28-d intake and digestion experiment.

Table 3-4. Performance of cattle fed four kinds of silage with or without Sun-Cure inoculant.

	Silage source					Inoculant		
Item[a]	CS	PM	TC	S	SE	NI	I	SE[d]
Initial BW, kg	253.4	252.9	258.7	254.9	5.04	255.7	254.2	3.58
84-d ADG, kg[b]	1.39	0.94	1.07	1.16	0.07	1.13	1.16	0.05
DMI, kg[c]	6.97	5.93	6.10	6.50	0.05	6.33	6.41	0.37
DM/gain[b]	5.02	6.37	5.66	5.64	0.14	5.71	5.60	0.20

[a]Silage source x inoculant interactions did not affect performance of cattle ($P > 0.10$).
[b]Silage source affected ADG and DM/gain ($P < 0.01$).
[c]Silage source affected DMI ($P < 0.07$).
[d]Addition of the inoculant did not affect ADG, DMI or DM/gain ($P > 0.10$).

Table 3-5 . Intake and apparent digestion of four silages treated with Sun-Cure™ inoculant at ensiling and fed with supplements to growing beef steers.

	Inoculated silage				
Item	CS	PM	TC	S	SE
DMI[a]	kg/d				
Silage	5.5[b]	3.3[e]	4.4[c]	3.7[d]	0.12
Total diet	6.6[b]	5.4[d]	5.5[cd]	5.7[c]	0.12
Apparent digestion[f]	%				
DM	66.3	64.5	63.3	67.0	1.64
OM	67.5	65.6	64.6	67.9	1.25
CP	62.1[c]	67.9[b]	61.4[c]	56.1[d]	1.37
ADF	56.1	56.4	53.7	56.3	2.22
NDF	49.1	51.6	52.2	55.9	2.36

[a]Weight classification categories of initial BW of steers did not affect DMI of silage or total diet ($P > 0.10$). Steer description (n = 28; 7/treatment; initial BW 259.04 ± 15.18 kg).
[bcde]Means on same line with different superscript letters differ ($P < 0.01$). Means for DM, OM, ADF, and NDF digestion for different silage sources were not different ($P > 0.10$).
[f]Initial steer BW classification categories affected apparent digestion coefficients ($P < 0.01$). Least squares means for digestion coefficients adjusted for initial BW classification categories.

CHAPTER 4

EFFECT OF *SACCHAROMYCES CEREVISIAE* ON IN VITRO MIXED RUMINAL MICROORGANISM FERMENTATION

Introduction

S. cerevisiae culture has been used as a dietary supplement in production ruminants for many years. However, interest in *S. cerevisiae* culture as a potential alternative to antimicrobial feed additives has increased within the past 10 to 15 yr. Some of the benefits associated with *S. cerevisiae* include increased DM and NDF digestion (Carro et al., 1992), increased initial rates of fiber digestion (Williams et al., 1991), and increased milk production in dairy cattle (Harris and Webb, 1990; Kung et al., 1997; Piva et al., 1993; Williams et al., 1991). In vitro experiments have also reported that, in some cases, *S. cerevisiae* culture favorably altered the mixed ruminal microorganism fermentation as well as stimulated lactate uptake and cellulose digestion by pure cultures of predominant ruminal bacteria (Callaway and Martin, 1997; Martin and Nisbet, 1992; Nisbet and Martin, 1991; Nisbet and Martin, 1993). Unfortunately, in vivo and in vitro effects of *S. cerevisiae* culture are not always consistent (Martin and Nisbet, 1992). Based on the research that has been done with *S. cerevisiae* culture, several models have been proposed regarding the stimulatory effects of yeast culture on the ruminal fermentation (Dawson, 1990; Lyons et al., 1993; Wallace, 1994).

Compared with other widely used feed additives (e.g., ionophores), little research has been conducted to evaluate the effects of microbial feed additives on the mixed ruminal microorganism fermentation or the growth and metabolism of predominant ruminal microorganisms. Because more *S. cerevisiae* products are becoming commercially available and because all commercially available microbial feed additives cannot be assumed to have the same effect on ruminal microorganisms, the objective of this study was to evaluate the effects of two live yeast products (Procreatin-7® and BIOSAF®, Saf Agri, Milwaukee, WI) on the in vitro mixed ruminal microorganism fermentation. Procreatin-7® is 100 percent active dry yeast with no cereal fillers with a minimum guarantee of 15 billion cfu per gram of product, whereas BIOSAF® consists of 100 percent yeast and a minimum guarantee of 8 billion cfu per gram of product. In addition, BIOSAF® is dried using a proprietary method which creates a "core" of live yeast surrounded by a "shell" of inactive yeast that provides protection to the live yeast core from heat and oxidation (Saf Agri, Milwaukee, WI). The effects of both *S. cerevisiae* feed supplements on the in vitro mixed ruminal microorganism fermentation of ground corn, soluble starch, alfalfa hay, and Coastal bermudagrass hay were examined in this study.

Materials and Methods

Ruminal contents were collected from an 800-kg ruminally fistulated Hereford steer maintained on a mixed forage pasture. The ruminal contents were obtained and squeezed through four layers of cheesecloth into an Erlenmeyer flask with an O_2-free CO_2 headspace. The flask was not disturbed for 30 min while being incubated in a 39°C water bath, permitting feed particles to rise to the top of the flask. Particle-free fluid from the flask was anaerobically transferred (20% vol/vol) to a medium (pH 6.5) containing 292 mg of K_2HPO_4,

240 mg of KH_2PO_4, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4,000 mg of Na_2CO_3, and 600 mg of cysteine hydrochloride per liter (Russell and Martin, 1984; Russell and Strobel, 1988). Particle-free fluid and medium were mixed and 40 mL transferred anaerobically to 160-mL serum bottles that contained either no substrate, 0.4 g of ground corn, 0.4 g of soluble starch, 0.4 g of alfalfa hay (NDF = 41.7%, ADF = 27.1%), or 0.4 g of Coastal bermudagrass hay (NDF = 64%, ADF = 27.6%). Weighed amounts of Procreatin-7® (**Y1**) or BIOSAF® (**Y2**) (Saf Agri, Milwaukee, WI) were added to achieve final concentrations of 0.35 and 0.73 g/L (Sullivan and Martin, 1999). These concentrations are consistent with current recommended feeding levels. Incubations containing only yeast were also run. Bottles were sealed (CO_2 atmosphere) with butyl rubber stoppers and aluminum seals to contain the gas pressure and placed in a 39ºC water bath for either 24 h (ground corn, soluble starch) or 48 h (alfalfa, bermudagrass) and periodically mixed every few hours.

After 24 h (ground corn, soluble starch) or 48 h (alfalfa, bermudagrass) of incubation, a gas sample (0.5 mL) was removed from each bottle and analyzed for hydrogen (H_2) and methane (CH_4) on a Gow Mac thermal conductivity series 580 gas chromatograph (Gow Mac Instrument, Bridgewater, NJ) equipped with a Porapak Q column (60ºC, 20 mL/min of N_2 carrier gas). The bottles were then uncapped, and the pH was measured immediately with a pH meter. Bottles then were emptied into centrifuge tubes, centrifuged (10,000 × *g*, 4ºC, 15 min) and the cell-free supernatant fluids stored at -20ºC. The organic acids in supernatant fluid samples were quantitated by HPLC using an organic acid column (Callaway and Martin, 1997).

To examine soluble components associated with both *S. cerevisiae* feed ingredients, a filter-sterilized filtrate was prepared by mixing 5 g of *S. cerevisiae* supplement in 50 mL of deionized water for 1 h at 25°C (Callaway and Martin, 1997). The slurry was then vacuum-filtered twice through a Whatman no. 1 filter (Whatman Lab Sales, Inc., Hillsboro, OR) and the resulting filtrate was filter-sterilized through a membrane filter (pore size, 0.45 μm).

To examine the effects of each yeast supplement on the rate of forage fiber digestion by mixed ruminal microorganisms, alfalfa hay and Coastal bermudagrass hay incubations were also conducted over time. Serum bottles were prepared as described above and incubated for 0, 24, or 48 h. After each time period, bottles were uncapped and poured into centrifuge tubes and centrifuged (10,000 × *g*, 4°C, 15 min). Pellets were resuspended in deionized water and poured back into the original serum bottles and stored at 4°C. Undigested residue was filtered through nylon bags (ANKOM Technology Corp., Fairport, NY) and then oven-dried (105°C) for 24 h to remove excess moisture and weighed. In vitro dry matter disappearance was calculated as original dry sample weight minus dry residue weight divided by the original sample weight. This value was then multiplied by 100 to derive IVDMD percentage. All fermentations were performed on duplicate days with two replicates per day (n = 4). Data were analyzed using a general linear model procedure (SAS, Version 5 ed.). All incubations were analyzed by fitting a model that contained *S. cerevisiae* culture dosage (0, 0.35, 0.73 g/L).

Results and Discussion

In the absence of added substrates, Y1 had no effect on final pH, H_2, butyrate, or the acetate:propionate ratio (Table 1). Both concentrations of Y1 increased ($P < 0.05$)

CH_4 concentrations, whereas concentrations of acetate and propionate numerically increased. Similar results were observed in the presence of Y2 (data not shown). Previous research showed that a filtrate of *S. cerevisiae* culture (Diamond V XP, Diamond V Mills, Inc., Cedar Rapids, IA) contained glucose, lactate, malate, formate, succinate, and aspartate (Callaway and Martin, 1997). When 5 g of both Y1 and Y2 were mixed with 50 mL of deionized water, fairly high concentrations of glucose, maltose, lactate, and succinate were detected in the resulting filter-sterilized filtrate (Table 2). However, based on the concentrations of *S. cerevisiae* supplement (0.35 g/L and 0.73 g/L) used in our mixed ruminal microorganism incubations, the concentrations of all carbon sources associated with the *S. cerevisiae* supplements would have been less than 0.15 m*M* in these fermentations. However, it is likely that fermentation of these carbon and energy sources as well as others (i.e., B vitamins, amino acids) by the mixed ruminal microorganisms account for the observed small increases in the concentrations of fermentation products in the absence of added substrates (Table 1). Similar results have been observed upon in vitro incubation of other *S. cerevisiae* feed supplements with mixed ruminal microorganisms in the absence of added carbon and energy sources (Martin et al., 1989; Sullivan and Martin, 1999).

To determine the effects of *S. cerevisiae* on fermentation of corn, mixed ruminal microorganisms were incubated with ground corn (0.4 g/40 mL of media = 10 g/L) (Table 3). As expected, final pH was lower and the concentrations of most fermentation products were much higher than the concentrations observed in the absence of carbohydrates (Table 3 vs Table 1). Both concentrations of both *S. cerevisiae* supplements had little effect on final pH or fermentation products (Table 3). Both Y1 and Y2 treatments tended to

numerically decrease acetate concentrations and this corresponded to a numerical decrease in the acetate:propionate ratio.

The effects of both *S. cerevisiae* supplements on the fermentation of soluble starch by mixed ruminal microorganisms are shown in Table 4. Final pH in these incubations was below 6.0 for all treatments. Both concentrations of both yeast supplements had little effect on final pH, H_2, and CH_4 concentrations. However, both concentrations of each *S. cerevisiae* supplement numerically increased concentrations of acetate and butyrate. The 0.73 g/L treatment increased ($P < 0.10$) propionate and both yeast treatments decreased ($P < 0.05$) the acetate:propionate ratio. In addition, all treatments numerically decreased lactate concentrations. Previous research has also reported that other *S. cerevisiae* supplements reduce ruminal lactate concentrations and it is believed that this response is due to the stimulation of lactate-utilizing ruminal bacteria (Callaway and Martin, 1997; Martin and Nisbet, 1992; Nisbet and Martin, 1991; Williams et al., 1991).

The effects of *S. cerevisiae* on the mixed ruminal microorganism fermentation of Coastal bermudagrass hay and alfalfa hay were determined (Tables 5 and 6). As expected, final pH remained above 6.0 and acetate concentrations were increased in the forage incubations compared to the ground corn and soluble starch incubations (Tables 5 and 6 vs Tables 3 and 4). There was no effect on final pH, volatile fatty acids, CH_4, H_2, or the acetate:propionate ratio with the addition of either yeast to these fermentations. These results are consistent with previous research with other *S. cerevisiae* supplements (Sullivan and Martin, 1999).

Previous research has shown that treatment with some yeast cultures increased the number of total and cellulolytic bacteria in the rumen and, in some cases, increased

cellulose degradation (Callaway and Martin, 1997; Dawson, 1990; Dawson et al., 1990; Newbold et al., 1995; Wiedmeier et al.,1987). Newbold et al. (1995) suggested that *S. cerevisiae* culture stimulated the rate rather than the extent of fiber digestion by ruminal microorganisms. In addition, others have reported that the stimulation of cellulose degradation by yeast culture is associated with a decreased lag time, which results in increased initial rates of digestion, but not in increased extent of digestion by ruminal microorganisms (Dawson, 1990; Williams et al., 1991). Therefore, experiments were conducted to examine the effects of Y1 and Y2 on IVDMD of alfalfa hay and Coastal bermudagrass hay (Figure 1). In vitro dry matter disappearance of both forages did increase over time. However, the addition of both concentrations of Y1 or Y2 had little effect on either the rate or extent of digestion of either forage by mixed ruminal microorganisms. Other studies have also reported no significant effect of yeast cultures on fiber digestion (Carro et al., 1992; Sullivan and Martin, 1999).

In conclusion, both Y1 and Y2 were able to stimulate the mixed ruminal microorganism fermentation in the absence of added substrates (Table 1). Both concentrations of each *S. cerevisiae* supplement numerically increased concentrations of acetate, propionate, and butyrate and decreased the acetate:propionate ratio in incubations that contained soluble starch (Table 4). However, in the presence of Coastal bermudagrass hay or alfalfa hay both yeast supplements had little effect on final pH, fermentation products (Tables 5 and 6), or IVDMD (Figure 1).

Implications

Few studies have been conducted to examine the effects of live yeast products on the in vitro mixed ruminal microorganism fermentation. Similar to the responses observed

with yeast culture products, both live yeast products used in this study tended to stimulate the ruminal fermentation in some instances. This small increase in fermentation end products is most likely associated with soluble nutrients associated with the yeast supplements. Even though both supplements had little effect on the ruminal fermentation, our in vitro experiments are unable to address other animal factors such as dry matter intake. Therefore, in vivo research is needed to determine if there might be a production response associated with both yeast supplements.

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Table 4-1. Effect of *Saccharomyces cerevisiae* (Y1) on in vitro mixed ruminal microorganism fermentation in the absence of added substrates.

	Yeast, g/L			
Fermentation product	0	0.35	0.73	SEM
pH	6.40	6.41	6.41	0.01
H_2, m*M*	0	0	0	0
CH_4, m*M*	0.55[a]	0.97[b]	1.51[c]	0.13
Acetate (A), m*M*	9.9	10.5	11.6	0.57
Propionate (P), m*M*	2.3	2.5	2.8	0.19
Butyrate, m*M*	3.7	3.5	3.9	0.35
A:P ratio	4.37	4.27	4.23	0.12

[a,b,c]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 4-2. Concentrations of carbon sources in *Saccharomyces cerevisiae* filtrates.

	Concentration, m*M*	
Carbon source	Y1	Y2
Glucose	15.4	6.9
Maltose	0.7	1.6
Malate	0	1.1
Succinate	1.0	1.4
Lactate	2.9	1.8

Table 4-3. Effects of Y1 and Y2 on in vitro mixed ruminal microorganism fermentation of ground corn.

		Yeast supplement				
		Y1, g/L		Y2, g/L		
Fermentation product	Control	0.35	0.73	0.35	0.73	SEM
pH	6.02	5.98	6.04	5.95	5.97	0.08
H_2, m*M*	2.6	2.0	1.5	1.6	1.7	0.78
CH_4, m*M*	7.5	8.0	7.1	8.1	7.8	0.81
Acetate (A), m*M*	11.6	11.0	10.9	9.8	10.3	1.35
Propionate (P), m*M*	6.3	6.7	6.4	5.7	6.4	0.88
Butyrate, m*M*	4.2	4.2	4.4	3.7	4.2	0.56
A:P ratio	1.84	1.63	1.8	1.73	1.64	0.08
Lactate, m*M*	0.53	0.86	0.70	0.64	0.41	0.59

Table 4-4. Effects of Y1 and Y2 on in vitro mixed ruminal microorganism fermentation of soluble starch.

		Yeast supplement				
		Y1, g/L		Y2, g/L		
Fermentation product	Control	0.35	0.73	0.35	0.73	SEM
pH	5.74	5.53	5.51	5.49	5.58	0.11
H_2, m*M*	0.2	0.3	0.3	0.3	0.3	0.09
CH_4, m*M*	6.6	5.9	6.6	6.8	7.0	1.49
Acetate (A), m*M*	9.8	10.6	13.6	10.0	13.4	1.81
Propionate (P), m*M*	5.8[a]	8.9[ab]	12.1[b]	9.1[ab]	12.8[b]	1.77
Butyrate, m*M*	2.1	4.5	5.2	3.4	5.2	1.19
A:P ratio	2.34[c]	1.20[d]	1.13[e]	1.09[e]	1.05[e]	0.08
Lactate, m*M*	4.58	2.06	0.15	1.40	0.14	3.02

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.10$).

[c,d,e]Means within a row lacking a common superscript letter differ ($P < 0.05$).

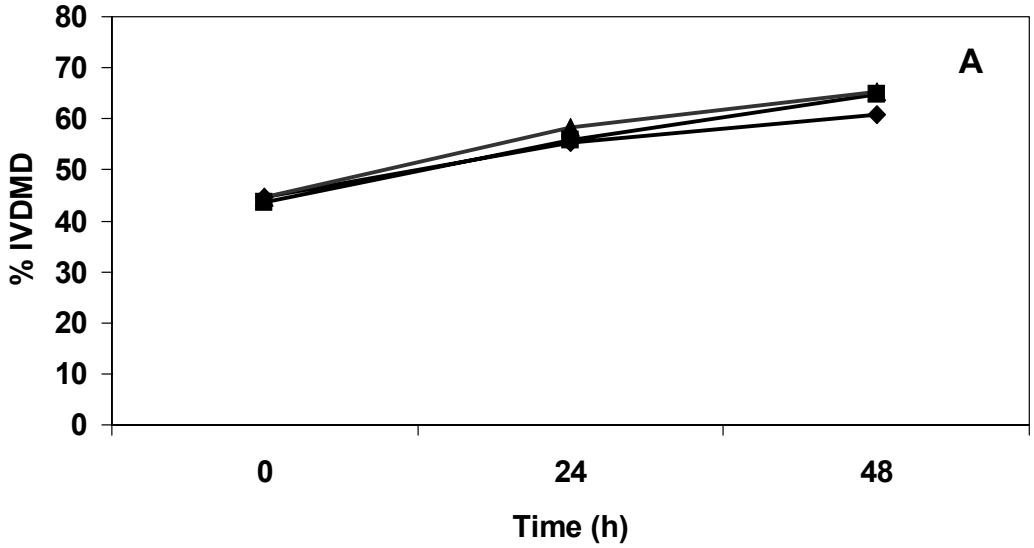
Table 4-5. Effects of Y1 and Y2 on in vitro mixed ruminal microorganism fermentation of Coastal bermudagrass hay.

		Yeast supplement				
		Y1, g/L		Y2, g/L		
Fermentation product	Control	0.35	0.73	0.35	0.73	SEM
PH	6.27	6.27	6.26	6.27	6.27	0.01
H_2, m*M*	0	0	0	0	0	0
CH_4, m*M*	6.2	5.8	6.3	6.2	7.3	0.42
Acetate (A), m*M*	20.1	19.6	20.7	20.8	20.2	0.83
Propionate (P), m*M*	6.6	6.8	7.1	7.5	7.3	0.42
Butyrate, m*M*	3.3	3.6	3.9	3.8	3.5	0.30
A:P ratio	3.32	3.20	3.24	3.04	3.13	0.11

Table 4-6. Effects of Y1 and Y2 on in vitro mixed ruminal microorganism fermentation of alfalfa hay.

		Yeast supplement				
		Y1, g/L		Y2, g/L		
Fermentation product	Control	0.35	0.73	0.35	0.73	SEM
pH	6.26	6.23	6.22	6.24	6.23	0.01
H_2, m*M*	0	0	0	0	0	0
CH_4, m*M*	6.2	6.7	6.6	5.9	6.3	0.31
Acetate (A), m*M*	21.9	21.4	21.7	21.6	20.9	0.59
Propionate (P), m*M*	8.6	7.9	8.6	8.9	8.8	0.38
Butyrate, m*M*	3.3	3.5	3.7	3.5	3.5	0.91
A:P ratio	2.76	3.05	2.79	2.71	2.66	0.18

Figure 4.1. Effect of Saccharomyces cerevisiae (Y1 and Y2) on in vitro dry matter disappearance of alfalfa hay (panel A = Y1; panel B = Y2) and Coastal bermudagrass hay (panel C = Y1; panel D = Y2). Treatments were control (?), 0.35 g/L of S. cerevisiae ( ), or 0.73 g/L of S. cerevisiae (_). Error bars represent standard error of the mean.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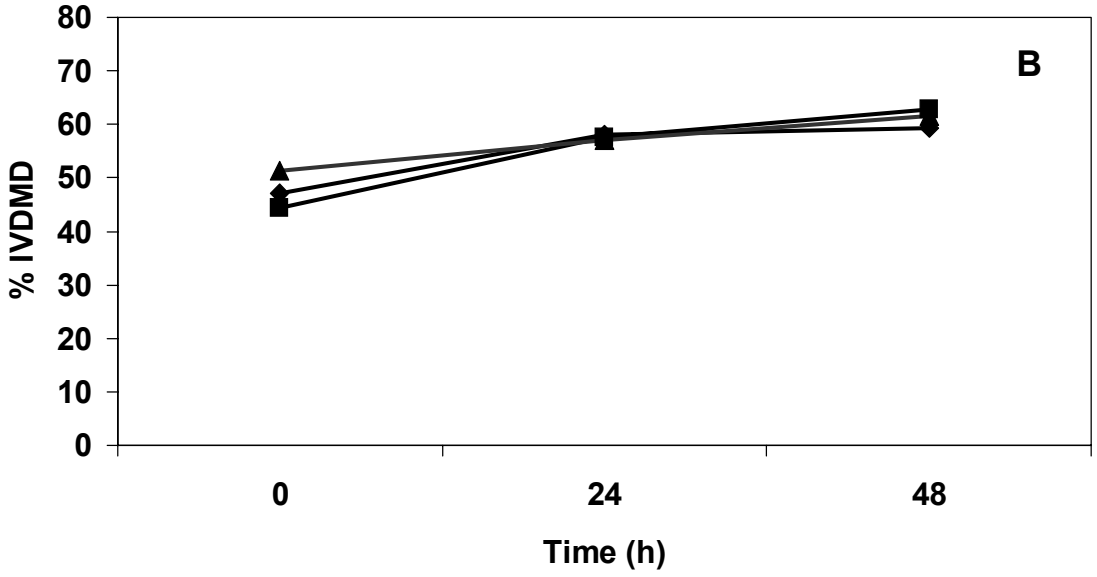
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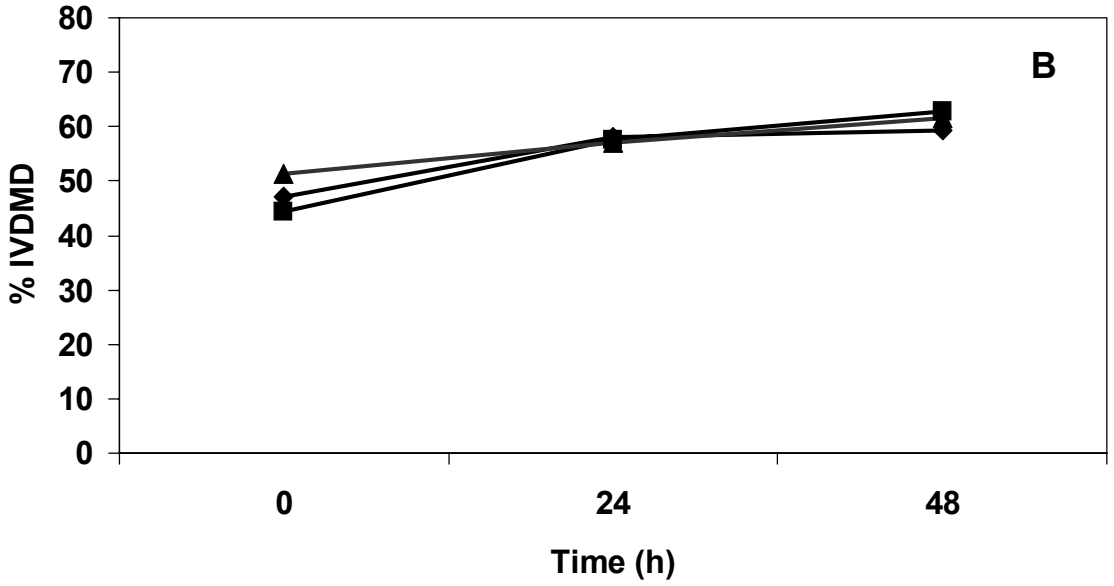
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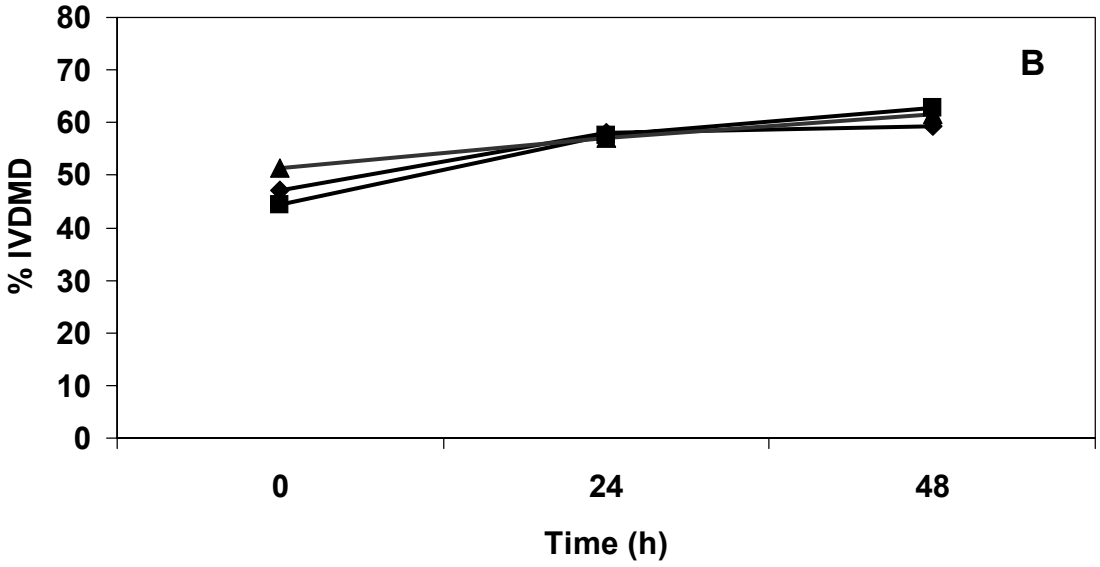
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CHAPTER 5

CONCLUSIONS

Our study indicated that PM was an inferior forage when ensiled compared with CS, TC and even S silages. Corn silage was the superior silage, although TC and S may be suggested for silage if a double and triple cropping system is being used. Tropical corn and S are typically more tolerant to many environment extremes. There was evidence of improved silage quality when a fermentable carbohydrate source was added in the form of corn grain. Corn grain provided a carbon source for the microorganisms to ferment which produce high concentrations of lactic acid. Lactic acid caused a rapid decrease in pH stabilizing the silage during storage. The addition of three microbial inoculants to the four kinds of silage at ensiling in mini silos had minimal effects on the ensiling process, and did not improve silage quality. Specifically, Sun-Cure inoculant decreased IVDMD and in vitro NDF disappearance in mini silos. Total tract digestibility of OM was greater for CS and TC than PM and S, but ADF and NDF digestion of PM was improved by the addition of an inoculant. Microbial populations present on the fresh chopped plants may have been adequate to ensile the forages, resulting in no additional improvement in silage fermentation or silage quality by the addition of commercial silage inoculants.

The DMI of feedlot and digestion trial cattle were affected most by silage source in the TMR. In the feedlot, DMI was highest for CS, then TC, followed by S with lowest DMI on PM. In the intake and digestion experiments, DMI of the TMR was highest for

CS (I), followed by PM (I), TC (I), and S (I) in Experiment 2. Adding the appropriate supplements to adjust energy and CP in the various diets resulted in similar digestibility of OM, ADF, and NDF in the TMR. A higher quality of CP in the PM silage made CP digestibility highest for the PM(I) TMR, intermediate for CS(I) and TC(I) and lowest for S(I). Addition of an inoculant did not increase DMI, ADG or DM/gain in the feedlot trial. Based on this observation, other methods of increasing animal performance on silage diets should be researched. If a starch source or energy source such as corn grain is added to silages at ensiling, nutrient content and digestibility should increase (Chapter 2).

Although not directly related to feeding ensiled forages, yeast cultures have been marketed as additives for improving the fermentation within the rumen as the inoculant have been marketed to improve the fermentation of forage within the silo. Previous research has shown that treatment with some yeast cultures increased the number of total and cellulolytic bacteria in the rumen and, in some cases, increased cellulose degradation (Callaway and Martin, 1997; Dawson, 1990; Dawson et al., 1990; Newbold et al., 1995; Wiedmeier et al.,1987). Newbold et al. (1995) suggested that *S. cerevisiae* culture stimulated the rate rather than the extent of fiber digestion by ruminal microorganisms. In addition, others have reported that the stimulation of cellulose degradation by yeast culture is associated with a decreased lag time, which results in increased initial rates of digestion, but not in increased extent of digestion by ruminal microorganisms (Dawson, 1990; Williams et al., 1991). Therefore, experiments were conducted to examine the effects of Y1 and Y2 on IVDMD of alfalfa hay and Coastal bermudagrass hay (Figure 1). In vitro dry matter disappearance of both forages did increase over time. However, the addition of both concentrations of Y1 or Y2 had little effect on either the rate or extent of

digestion of either forage by mixed ruminal microorganisms. Other studies have also reported no significant effect of yeast cultures on fiber digestion (Carro et al., 1992; Sullivan and Martin, 1999).

Both Y1 and Y2 were able to stimulate the mixed ruminal microorganism fermentation in the absence of added substrates (Table 1). Both concentrations of each *S. cerevisiae* supplement numerically increased concentrations of acetate, propionate, and butyrate and decreased the acetate:propionate ratio in incubations that contained soluble starch (Table 4). However, in the presence of Coastal bermudagrass hay or alfalfa hay both yeast supplements had little effect on final pH, fermentation products (Tables 5 and 6), or IVDMD (Figure 1).

The experiments conducted and then reported in this thesis re-enforce the need to study the effects of additives in vitro and in vivo. The results of our study with silage in mini silos showed that addition of inoculant improved NDF and ADF digestion for PM, but when PM was fed to cattle there was no increase in DMI or ADG. Although the theory that using microbial inoculants or yeast cultures may improve performance the actual test is whether the animal responds.

Table 5.1. Interaction means for chemical composition of four silages treated with Pioneer 1177 or Sun-Cure inoculants at ensiling in mini silos.

Item	Additive(A)	Silage source (SS)				A mean	Statistics				
		CS	PM	TC	S		Effect	SE[d]	*df*	LSD[e]	P ≤[e]
Component[a]		% DM									
Silage DM	Control	29.92[b]	19.04[b]	29.74[b]	24.18[b]	**25.72[m]**	SS × A	0.46	84	1.29	0.05
	Pioneer	29.38[b]	19.45[b]	28.07[c]	24.53[b]	**25.36[m]**	SS	0.32	6	2.26	0.001
	Sun-Cure	29.79[b]	19.22[b]	28.20[c]	24.14[b]	**24.34[m]**	A	0.27	6	1.07	0.05
	Mean	**29.70[x]**	**19.24[z]**	**28.67[x]**	**24.29[y]**						
Silage CP	Control	7.62[b]	9.80[c]	7.48[b]	6.86[b]	**7.94[m]**	SS × A	0.11	84	0.49	0.001
	Pioneer	7.77[b]	10.53[b]	7.55[b]	6.53[b]	**8.10[m]**	SS	0.21	6	1.46	0.001
	Sun-Cure	7.39[b]	10.51[b]	7.35[b]	6.58[b]	**7.96[m]**	A	0.18	6	0.69	0.05
	Mean	**7.59[y]**	**10.28[x]**	**7.46[y]**	**6.66[y]**						
Silage ADF	Control	21.48[b]	37.48[b]	33.44[b]	34.91[b]	**31.83[m]**	SS × A	0.34	84	1.23	0.01
	Pioneer	21.82[b]	36.46[b]	33.88[b]	34.48[bc]	**31.66[m]**	SS	0.33	6	2.41	0.001
	Sun-Cure	21.72[b]	36.88[b]	34.41[b]	33.48[c]	**31.62[m]**	A	0.29	6	1.14	0.05
	Mean	**21.67[z]**	**36.94[x]**	**33.91[y]**	**34.29[y]**						
Silage NDF	Control	39.36[b]	60.41[b]	60.47[b]	59.14[b]	**54.84[m]**	SS × A	0.61	84	1.71	0.05

	Pioneer	40.60[b]	59.51[b]	60.34[b]	59.00[b]	**54.86[m]**	SS	0.39	6	2.80	0.001
	Sun-Cure	40.11[b]	58.95[b]	61.00[b]	58.38[b]	**54.61[m]**	A	0.34	6	1.33	0.05
	Mean	**40.02[y]**	**59.62[x]**	**60.61[x]**	**58.84[x]**						
Silage ADL	Control	2.79[b]	5.68[b]	3.70[c]	5.31[b]	**4.37[m]**	SS × A	0.16	84	0.74	0.001
	Pioneer	3.02[b]	6.01[b]	4.49[b]	5.02[b]	**4.63[m]**	SS	0.16	6	1.16	0.001
	Sun-Cure	2.70[b]	5.73[b]	4.16[bc]	4.67[b]	**4.32[m]**	A	0.14	6	0.55	0.05
	Mean	**2.84[2]**	**5.81[x]**	**4.12[y]**	**5.00[xy]**						

[a]Abbreviations and terms: Additive = Pioneer 1177 or Sun-Cure inoculant; Control = No inoculant treatment of silage; SS = Silage source; A = Additive.

[bc]Interior means with different superscript letters differ (CP and ADL, P < 0.001; DM and NDF, P < 0.01; ADF P < 0.01).

[d]Two adjustments were made to SE for main effects: First, correction of interaction SE was obtained by computing the ratio of variance from the full model to variance of the 4 x 3 factorial, by taking the square root, and multiplying by the interaction SE. Second, SE adjusted to guard against differences not justified based on interaction means.

[e]The LSD = [SE multiplied by the square root of 2, product multiplied by *t* value, at the error degrees of freedom (*df*) and probability level]. The probability level was determined from analysis of variance from the reduced factorial such that differences reflect at a level

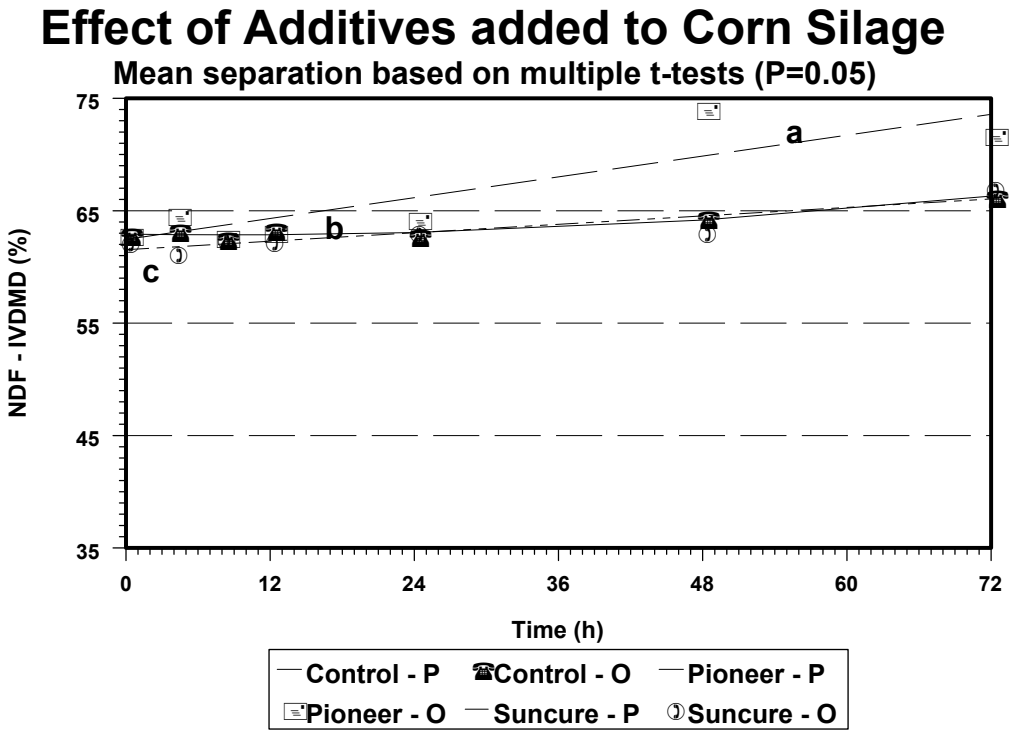
[m]Main effect means for silage additives were not different (P > 0.05) when tested over interaction.

[xyz]Main effect means for silage sources bearing different superscript letters differ (P < 0.001).

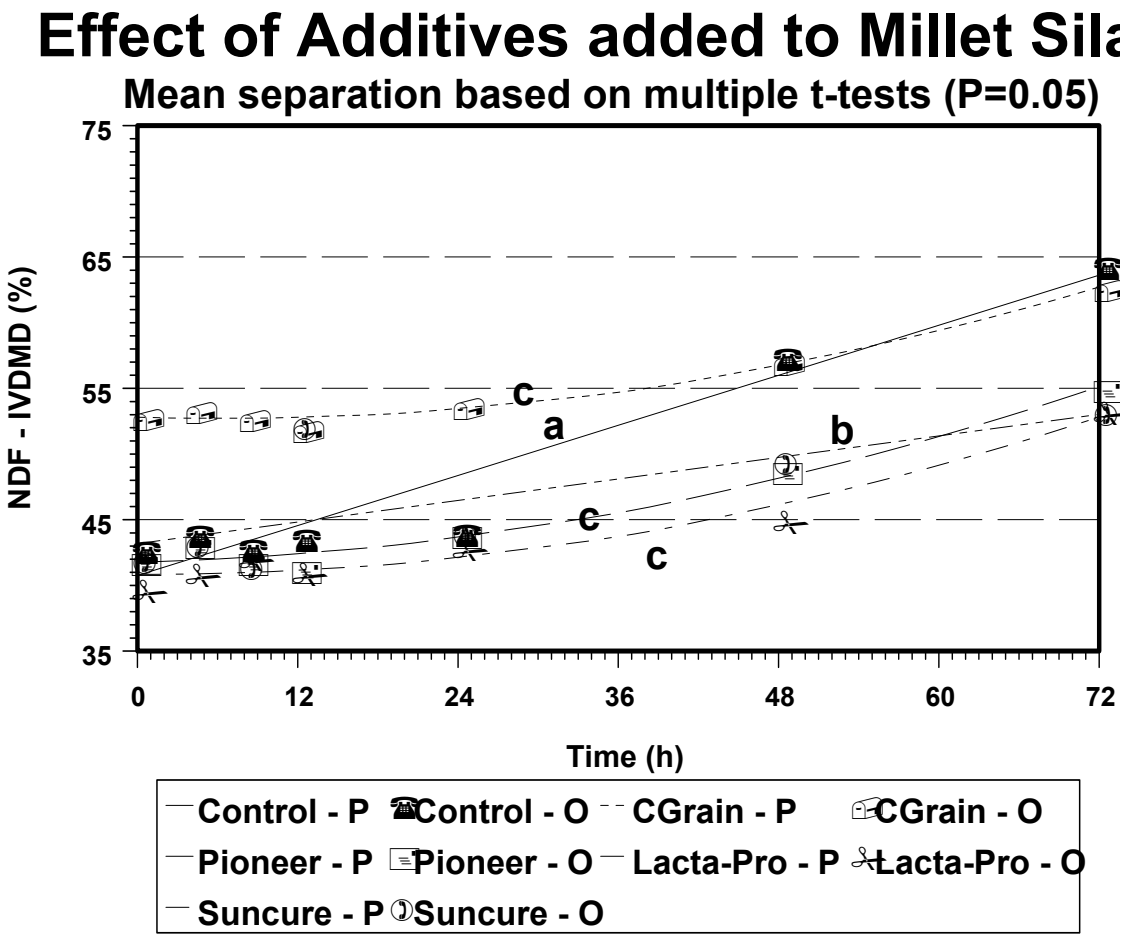
APPENDICES

Appendix A

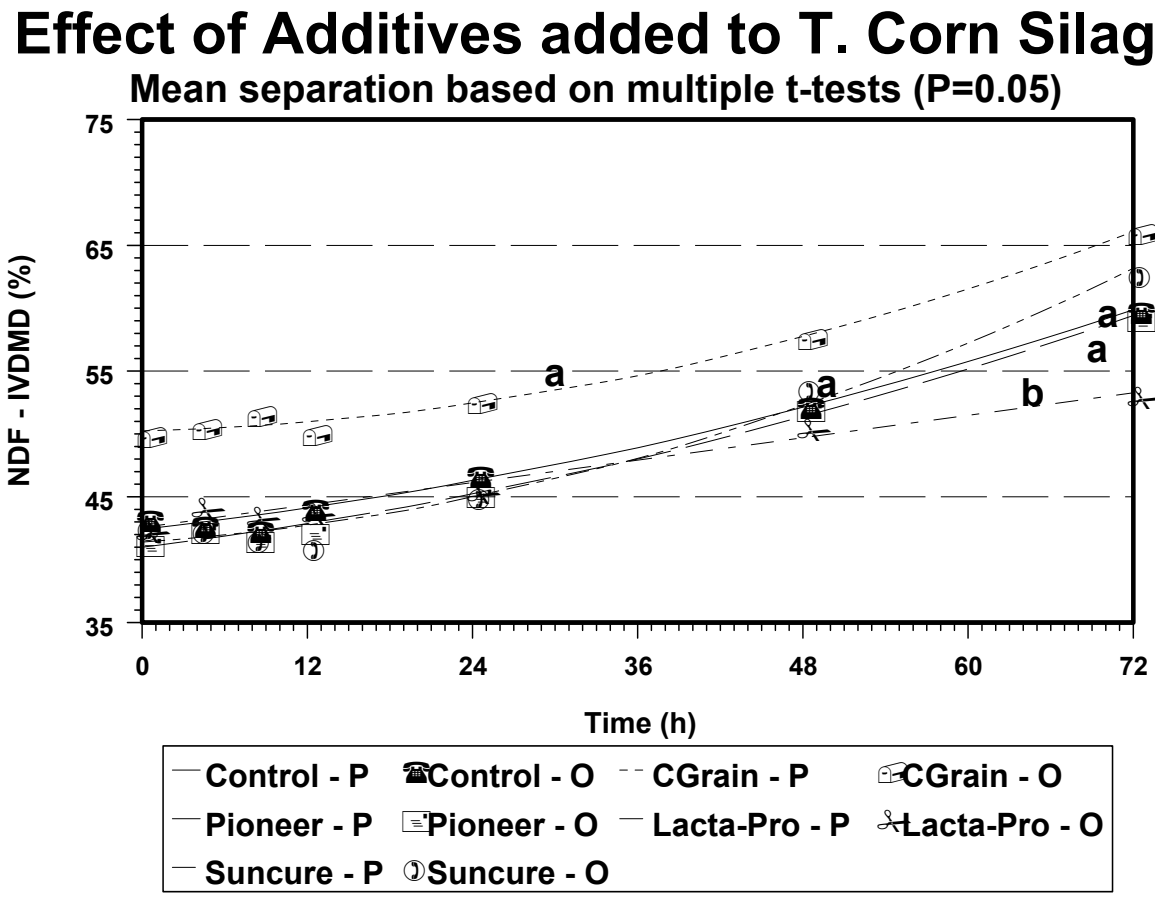
Appendix Figure 1. Effect of additives at ensiling on in vitro NDF digestibility of four silages over 72 h (observed values and prediction trends; a, b, c, $P < 0.05$).



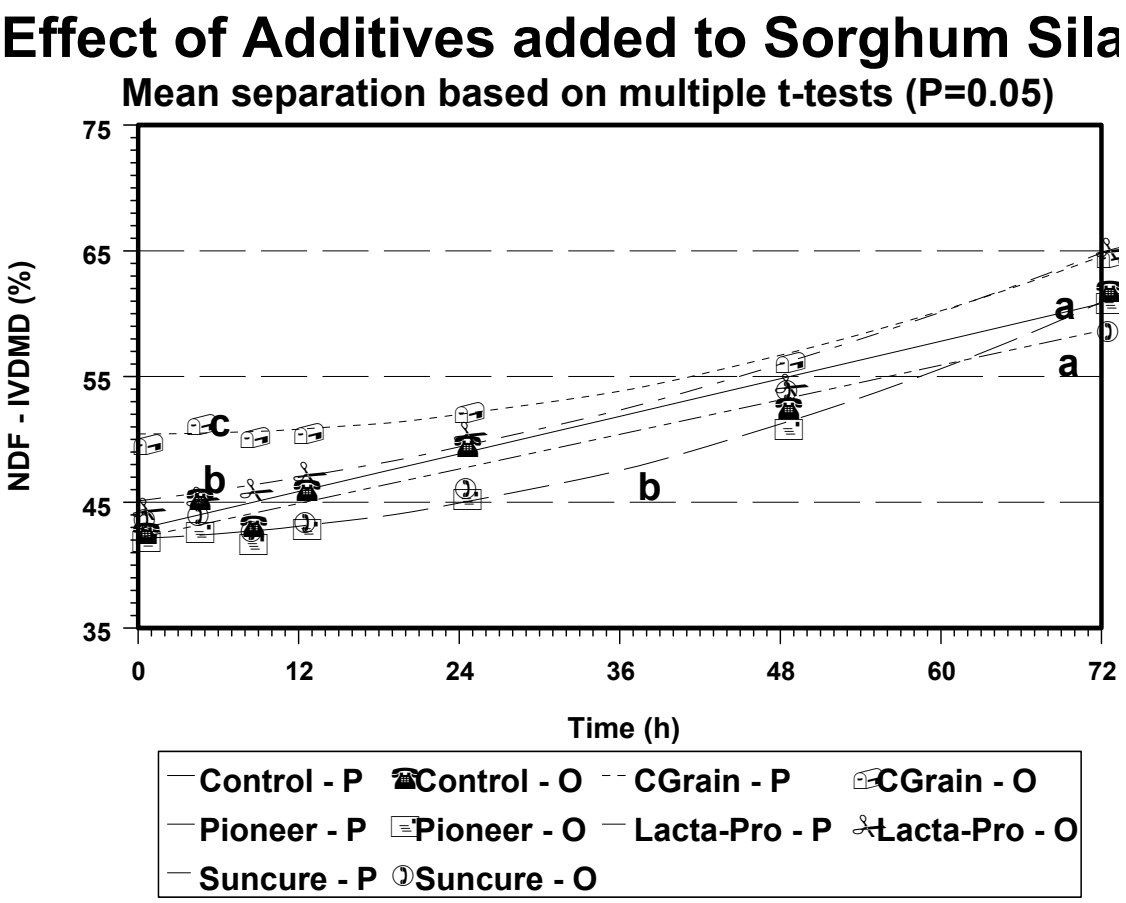
Appendix B


Effect of Additives added to Millet Sila
Mean separation based on multiple t-tests (P=0.05)
NDF - IVDMD (%)
75
65
55
45
35
0
12
24
36
48
60
72
Time (h)
c
a
b
c
c
Control - P
Control - O
CGrain - P
CGrain - O
Pioneer - P
Pioneer - O
Lacta-Pro - P
Lacta-Pro - O
Suncure - P
Suncure - O

Appendix C


Effect of Additives added to T. Corn Silag
Mean separation based on multiple t-tests (P=0.05)
NDF - IVDMD (%)
75
65
55
45
35
0
12
24
36
48
60
72
Time (h)
a
a
a
b
Control - P
Control - O
CGrain - P
CGrain - O
Pioneer - P
Pioneer - O
Lacta-Pro - P
Lacta-Pro - O
Suncure - P
Suncure - O

Appendix D


Effect of Additives added to Sorghum Sila
Mean separation based on multiple t-tests (P=0.05)
NDF - IVDMD (%)
75
65
55
45
35
0
12
24
36
48
60
72
Time (h)
c
b
b
a
a
Control - P
Control - O
CGrain - P
CGrain - O
Pioneer - P
Pioneer - O
Lacta-Pro - P
Lacta-Pro - O
Suncure - P
Suncure - O