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STUDIES ON THE EFFICACY OF A HOMOFERMENTATIVE LACTIC ACID-PRODUCING BACTERIAL INOCULANT AND COMMERCIAL PLANT CELL-WALL-DEGRADING ENZYME MIXTURES TO ENHANCE THE FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF FORAGES INSILED IN TEMPERATE AND TROPICAL ENVIRONMENTS

presented by

Abner Antonio Rodriguez-Carias

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Steven Kust
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STUDIES ON THE EFFICACY OF A HOMOFERMENTATIVE LACTIC ACID-PRODUCING BACTERIAL INOCULANT AND COMMERCIAL, PLANT CELL-WALL-DEGRADING ENZYME MIXTURES TO ENHANCE THE FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF FORAGES ENSILED IN TEMPERATE AND TROPICAL ENVIRONMENTS

By

Abner Antonio Rodríguez-Carías

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ABSTRACT

STUDIES ON THE EFFICACY OF A HOMOFERMENTATIVE LACTIC ACID-PRODUCING BACTERIAL INOCULANT AND COMMERCIAL, PLANT CELL-WALL-DEGRADING ENZYME MIXTURES TO ENHANCE THE FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF FORAGES ENSILED IN TEMPERATE AND TROPICAL ENVIRONMENTS

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A series of studies were conducted to address the hypothesis that addition of silage additives (microbial inoculant or enzymes) improved the ensiling characteristics and aerobic stability of forages ensiled in temperate and tropical environments. In experiment one, a two year study was conducted to evaluate the effect of a microbial inoculant and a commercial enzyme preparation on the ensiling characteristics and aerobic stability of forage sorghum ensiled after 90 d of growth in temperate and tropical environments. In both locations, the microbial inoculant alone or in combination with enzyme enhanced the ensiling characteristics of forage sorghum as evidenced by a decrease in pH; an increase in lactic acid producing-bacterial population and lactic acid content. However, it had either a negative or no effect on the aerobic stability of the resulting silage. Results from experiment two indicated that inoculation also improved the fermentation characteristics, but did not reduce the aerobic deterioration of forage sorghum ensiled at 90 and 110 d of growth in a tropical environment. In experiment three, addition of a microbial inoculant plus

enzymes had a positive effect on the fermentation characteristics of Johnson grass ensiled after two regrowth periods in a tropical environment. Findings from experiment four indicated that under in vitro conditions, cell-wall disappearance in forage sorghum and Johnson grass harvested in a tropical climate is increased when treated with enzyme application rates greater than recommended, but activity of the enzyme complex differed between forage species. In experiment five, the NDF disappearance from forage sorghum harvested in a temperate environment, treated with 5 commercial enzyme mixtures and applied at different rates were evaluated. Enzyme preparations differed in their ability to degrade the NDF fraction of forage sorghum, but application rates greater than recommended did not improve NDF disappearance. Results from experiment six indicated that addition of enzyme mixtures did not consistently affect the fermentation characteristics and carbohydrate content of forage sorghum ensiled in a temperate environment.

In memory of G.M.J. Horton

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INTRODUCTION

Conservation of feedstuffs by ensiling is a major practice used in many livestock operations. In temperate environments, ruminant feeding systems are based on the production of forage, grain, or cereal silages during the warmer seasons to be utilized as ingredients in growing-finishing rations for beef cattle or in total mixed rations (TMR) for dairy cattle during winter months. This management system provides a consistent forage supply of uniform quality thereby, providing for a more constant feeding program throughout the year.

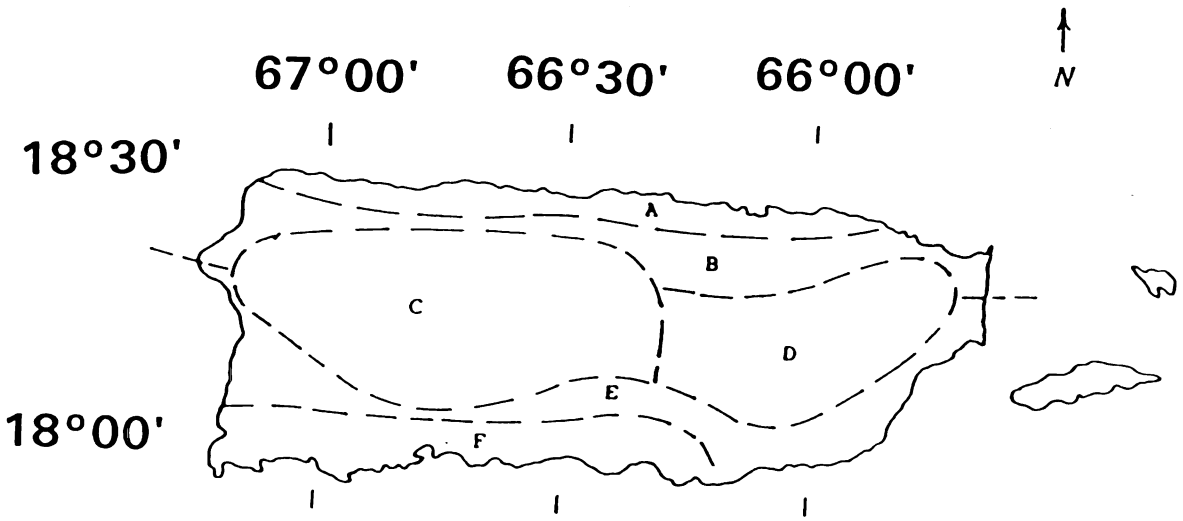
In tropical regions, due to the socio-economic conditions which prevail, ruminant production depends largely on the efficient management and utilization of pasture forage species. However, because of the typical environmental conditions, beef and dairy production experience marked seasonal fluctuations in forage supply and quality.

Puerto Rico is an island lying between the Atlantic Ocean and the Caribbean Sea, located on the latitude 18°N and longitude 66°W and measures about 160 kilometers from east to west, and 56 kilometers from north to south. The weather of Puerto Rico is classified as tropical. Temperature changes

are small, and the seasons are not clearly defined (Capiel and Calvesbert, 1976). The minimum and maximum annual temperatures are 19.3°C and 30°C, respectively, with an average temperature of 24.7°C. The island is divided into six climatic regions (Figure I-1) based on the amount of precipitation. However, in all climatic regions, two seasons are observed, a dry season which begins in December and ends in July, and a rainy season which begins in August and ends in November (Figure I-2).

Animal production represents 59.0% of the gross annual agricultural income with the dairy and beef industries ranking first and third, respectively. Currently, island production contributes 24% of the total beef consumption with the remaining beef (76%) being imported from Central America and the continental United States. Beef production is based on grazing systems, which are limited by fluctuations in forage quality and quantity throughout the year, resulting in a annual pattern of weight gain in the wet season, and weight loss in the dry season. With this production system, it requires 36 months to reach market weight.

The dairy industry utilizes TMR based on imported concentrates and forages produced locally. All fresh milk used for human consumption is produced on the island. The feeding system during the dry season which coincides with short days, is characterized by the excessive use of concentrates, poor grazing conditions, and low quality hay. This results in an increased cost of production, greater incidence of metabolic disorders (e.g. acidosis), and



Legend:

A - North Coastal
B - Northern Slopes
C - Western Interior
D - Eastern Interior
E - Southern Coastal
F - South Coastal

Figure I-1. Climatic regions of Puerto Rico

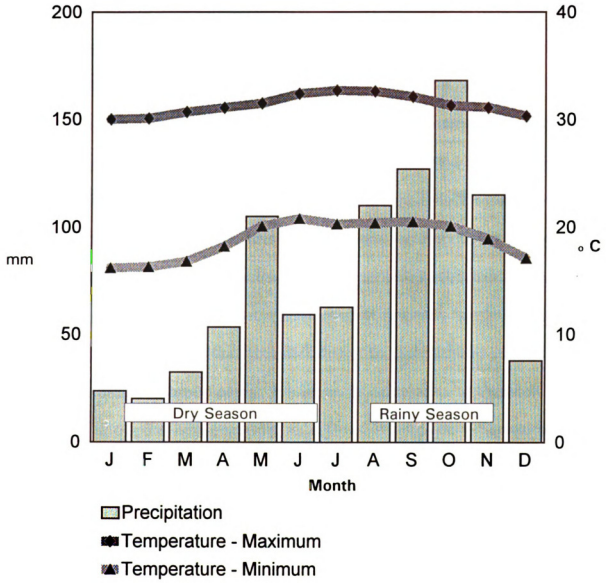


Figure I-2. Typical monthly precipitation and temperature patterns of Puerto Rico

decreased fat content of the milk.

In addition to the problems associated with lack of forage quality and quantity during the dry season, another major problem is the demand for land by industrialization on a limited landbase. Therefore, there is an urgent need to develop intensive or semi-intensive feeding systems that optimize the use of shrinking land resources to produce the needed forages. The introduction of silage as a major feed component into feedlot diets for finishing beef cattle, and into total mixed rations for dairy cows utilizing high yielding species (e.g. forage sorghum) or aggressive native species (e.g Johnson grass) represent a viable alternative to intensify forage production. Land with limited agricultural potential should be dedicated to pastures for growing cattle and brood cows. However, in contrast to temperate climates, in tropical environments, production of silage as a method of conservation has not been extensively practiced because of problems associated with the ensiling of tropical grasses (Catchpoole and Williams, 1969; Catchpoole and Henzell, 1971). Problems which limit silage - making practice in the tropics are the low concentration of water soluble carbohydrate found in the forages (Van Soest, 1994), and a lack of epiphytic homofermentative lactic acid bacterial population (McDonald et al., 1991). In addition to the problems associated with ensiling of tropical grasses, the aerobic stability of tropical silage is not well documented. Currently, the effectiveness of lactic acid bacterial inoculants and plant cell-

wall degrading enzymes to improve ensiling of forages harvested in temperate and subtropical environment has been variable. In a review of five years of published studies evaluating bacterial inoculants, Muck and Bolsen (1992) indicated that silage fermentation was improved as evidenced by lower pH, shifting fermentation end-products to a predominance of lactic acid, or reducing the levels of ammonia-nitrogen in 66% of the studies. Henderson et al. (1982) reported that adding .4% cellulase to grass and legume forage (fresh material) resulted in marked cellulose hydrolysis. However, the use of enzyme additives on alfalfa ensiled at three moisture contents did not improve the silage fermentation (Jaster and Moore, 1990). The effects of lactic acid bacteria inoculation on aerobic stability of silage in temperate regions has shown mixed results. In a study of microbial inoculant addition to corn silage, stability was improved (Wolth, 1989) whereas in other studies no response (Schaefer et al., 1989) or a negative response (Rust et al., 1989) have been observed. Effects of enzyme mixtures on aerobic stability of silages are not well documented.

In tropical environments, there is limited information regarding the use of microbial inoculants and enzymes on the resulting fermentation, and aerobic stability of silage. Because of the warm and humid environment prevailing in tropical areas, the composition of the plant material and epiphytic microflora are different than in temperate environments. Consequently, it is difficult to extrapolate the effectiveness of silage additives from one

environment to another. Therefore, studies to evaluate the use of microbial inoculants and enzymes on fermentation of forages ensiled in tropical areas are required. In this dissertation, two questions were addressed; 1.) Do silage additives improve the fermentation of forages ensiled in temperate and tropical environments, and; 2.) what factors limit or enhance their effectiveness?.

The specific objectives of this dissertation were:

1. To evaluate the effects of a homofermentative lactic acid bacterial inoculant and a plant cell wall-degrading enzyme on the organic acid contents, pH, microbial succession, fermentation end-products, and carbohydrate content of forage sorghum ensiled in temperate and tropical environments.
2. To determine the effect of the silage additives (enzymes and microbial inoculant) on the aerobic stability of the resulting silage.
3. To study the differences in effectiveness of microbial inoculant and enzymes when forage sorghum is ensiled at two stages of maturity in a tropical environment.
4. To evaluate ensiling of Johnson grass treated with silage additives at two regrowth periods as a potential forage source.
5. To determine the effect of a commercial enzyme preparation applied at different rates on cell wall disappearance from

Johnson grass and forage sorghum harvested in a tropical environment.

6. To compare the effects of enzyme preparations applied at different rates on NDF disappearance from forage sorghum harvested in temperate environments.
7. To determine the differences in activity of different commercial enzyme mixtures applied at different rates on the fermentation characteristics of forage sorghum ensiled in a temperate environment.

In order to accomplish these objectives, Chapter 1 of this dissertation reviews pertinent information related to the fermentation phases, microbiology, biochemistry, and aerobic stability of the ensiling process. In addition, Chapter 1 includes a review of the literature associated with the effects of climate on forage quality, characteristics of tropical silages, and utilization of silage additives to enhance silage fermentation, with emphasis in lactic acid-producing bacterial inoculants and plant cell wall-degrading enzyme preparations. Objectives 1 and 2 were accomplished with a two year study that evaluated the effects of a microbial inoculant and enzyme preparation on ensiling characteristics (Chapter 2) and aerobic stability (Chapter 3) of forage sorghum ensiled in temperate and tropical environments. The possible differences in effectiveness of silage additives when forage sorghum is ensiled at two stages of maturity in a tropical

environment (Objective 3) is reported in Chapter 4. Chapter 5 describes the benefits of a microbial inoculant and enzymes to preserve Johnson grass as a silage (Objective 4). The in vitro evaluations of commercial enzyme preparations on cell wall disappearance of forages harvested in either environment (Objectives 5 and 6) are presented in Chapters 6 and 7. In Chapter 8, a study compares the different enzyme preparations on fermentation characteristics of forage sorghum ensiled in a temperate environment (Objective 7) . A summary of the findings and conclusions from all the studies is presented in Chapter 9. Raw data tables for each chapter, and literature cited are presented in the two appendices.

The general hypothesis of this work is that utilizing a homofermentative lactic acid bacterial inoculant and plant cell wall-degrading enzymes will improve the fermentation characteristics and aerobic stability of forages ensiled in temperate and tropical environments.

CHAPTER 1

Effect of climate on forage quality

In any livestock operation, it is well recognized that animal performance is highly dependent on forage quality. Forage is any vegetative plant material in a fresh, dried, or ensiled state that is fed to livestock (Jergens, 1993). The nutritive value of forages is primarily determined by its chemical composition, which result from the distribution of photosynthetic resources into the various plant tissues (Van Soest, 1994; Figure 1-1). The interaction between the plant and environment determines the extent of structural components in the vegetative material, and subsequently its nutritive value. The structural components (e.g. cellulose, hemicellulose, and lignin) are distributed in the primary and secondary plant cell wall and are inversely related to forage quality. Other plant components such as soluble cell content, including proteins, sugars, fats, starch and pectin, are positively related to nutritive value (Figure 1-2).

The effect of environment on the chemical composition of forages and subsequent nutritive value was initially defined by Minson and McLeod (1970). Differences in plant composition have been attributed to light

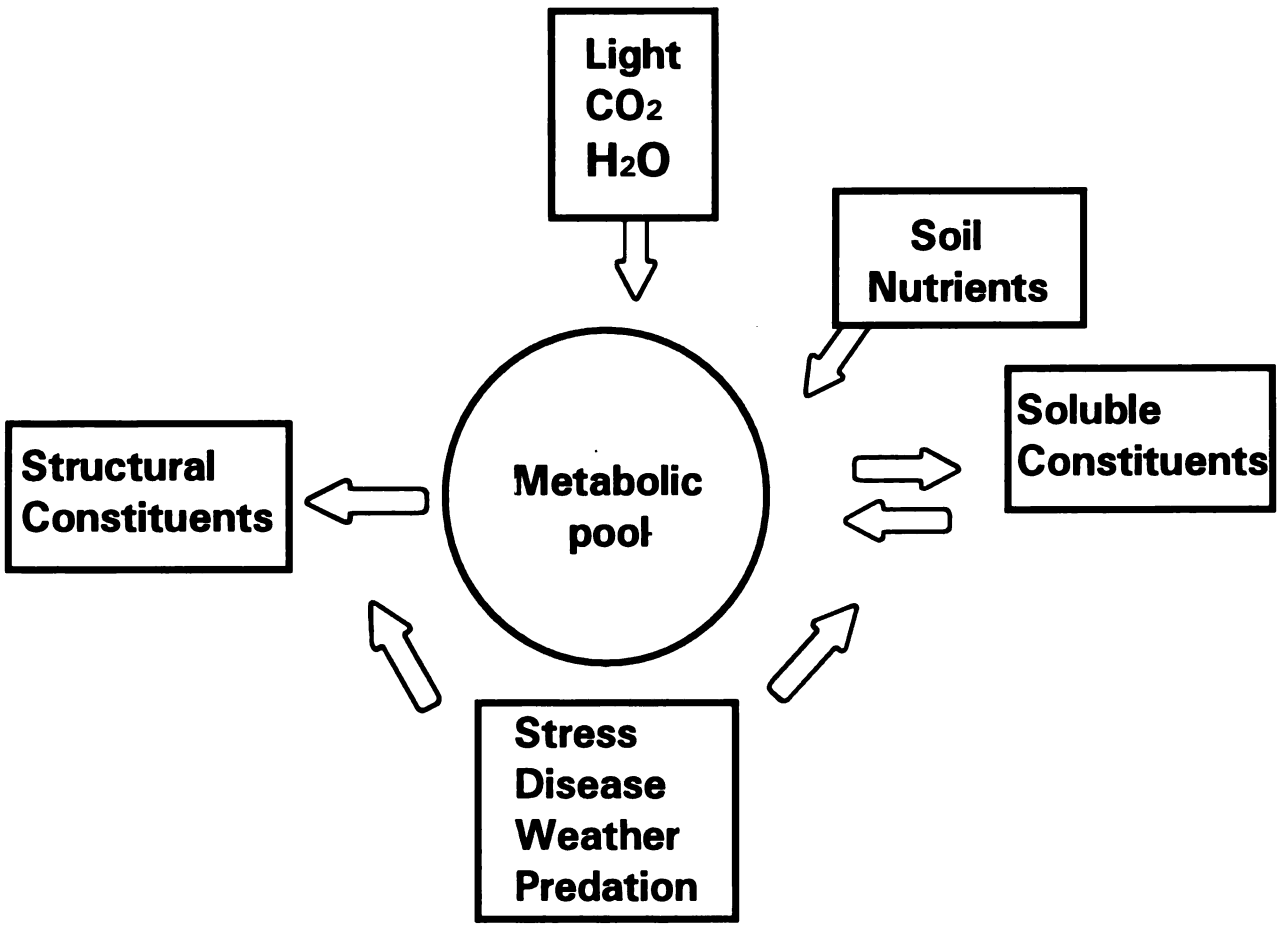


Figure 1-1. Relation of environmental factors to plant metabolic components (adapted from Van Soest, 1994)

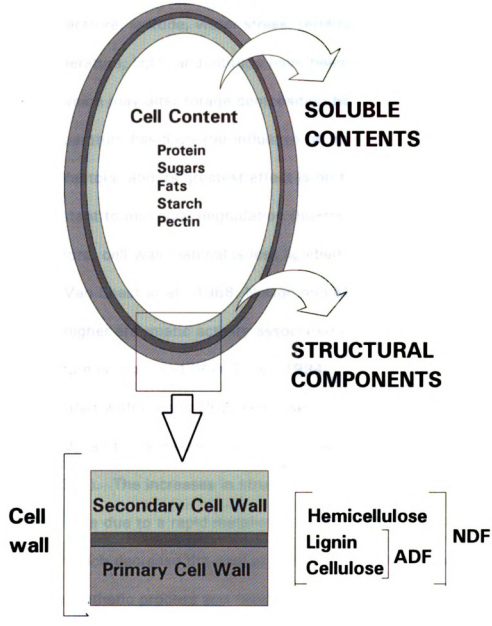


Figure 1- 2. Resistant structures and reserve components in vegetative material

intensity, temperature, latitude, water stress, fertilization and soil. However, temperature, light, and latitude have been described as the three major factors which may alter forage composition between environments. Generally, temperature has a greater influence on forage quality than other environmental factors, and its greatest effect is on the accumulation of structures resistant to microbial degradation (Buxton and Fales, 1994). At lower temperature, cell wall material is less lignified than at higher temperatures (Van Soest et al., 1968; Nelson and Moser, 1994). This results from a higher enzymatic activity associated with lignin biosynthesis when temperature is increased (Van Soest, 1994). Elevated temperatures are also associated with greater NDF, cellulose, and silica (Henderson and Robinson, 1982), and a decrease in non-structural carbohydrates (Vough and Marten, 1971). The increases in structural carbohydrates and decrease in cell solubles, are due to a rapid metabolic activity in the plant at higher temperatures, which decrease the pool of metabolites within the cell. The elevated photosynthetic process and resulting end-products result in a more rapid accumulation of structural components (Van Soest, 1994). In addition, amino acids and protein synthesis from sugars are greater as temperature increases. As a result, nitrogen supply is increased and sugar content decreased.

The effect of light is exerted directly on metabolism through photosynthesis and is influenced by total amount of light received, light intensity and day

length (Van Soest, 1994). In temperate environments, plant growth occurs mainly during the summer. Long photoperiods result in high forage quality because of greater photosynthetic activity of the plant and increased soluble sugar contents (Wilson, 1982; Deinum, 1984). Conversely, in tropical climates, plants growth under a relatively constant day length and high temperatures, resulting in forages that tend to be of lower quality. Plant nutrients are metabolized at a faster rate than they are produced. The metabolic adaptations associated with the warm, long dark periods promote respiration. Elevated temperatures increase lignification and decrease the nutritive value of tropical forages (Crowder and Chheda, 1982; Van Soest, 1994).

The effect of latitude on plant development is closely related to photoperiod. In temperate environments, the higher latitudes have longer day lengths and less variation in density of solar radiation, whereas, in tropical climates located in the lower latitudes daylengths are shorter, but flux density is greater. Increments in solar radiation have been associated with higher reserve contents in the plant (Bathurst and Mitchell, 1958; Melvin and Sutherland, 1961; Smith, 1973). However, the higher temperatures and characteristic shorter day length override the positive effect of solar radiation on plant composition in tropical environments.

The effect of water, fertilization, and soil on plant development have been extensively documented (Minson, 1990; Buxton and Fales, 1994, Van

Soest, 1994), and their effects are variable between environments and within geographical regions.

Silage Quality

In any conservation method for forage, quality is defined by recovery of nutrients, and production of a nutritive feedstuff for livestock (Barnett, 1954; McCullough, 1978; Van Soest, 1994). Silage quality, has traditionally been associated with the type of fermentation that occurs. Characteristics of a successful fermentation include high lactic acid content, low pH, and stimulation of greater animal intake and performance. Conversely, silages with high concentration of butyric, and acetic acids, have shown a negative correlation with animal performance (Wilkinson et al., 1976; McCullough, 1978; Shaver et al., 1985) and are considered to be of poor quality. Good quality silage can be described as the plant material that has a pH of less than 4.2, lactic acid greater than 1.5 g/100g DM, acetic acid between .5 and .8 g/100g DM, butyric acid less than .1 g/100 g DM (Breirman and Ulvelsi, cited by McCullough, 1978), and ammonia-nitrogen less than 8% of the total nitrogen (McCullough, 1978). Criteria associated with high quality silage and the losses associated with the ensiling process (e.g. plant enzymes respiration, microbial metabolism, and effluent losses) have been extensively reviewed (Muck, 1988; McDonald et al, 1991; Woolford, 1992; Van Soest, 1994).

Microbiology of Silage

Silage fermentation is characterized by a very heterogenous microbial population. The major microbial groups associated with the ensiling process include; lactic acid bacteria, Enterobacteriaceae, yeasts and molds, and endospore-forming bacteria. However, other microbial groups such as acetic acid bacteria, propionic acid bacteria, and listeria, have also been identified as part of the silage microflora.

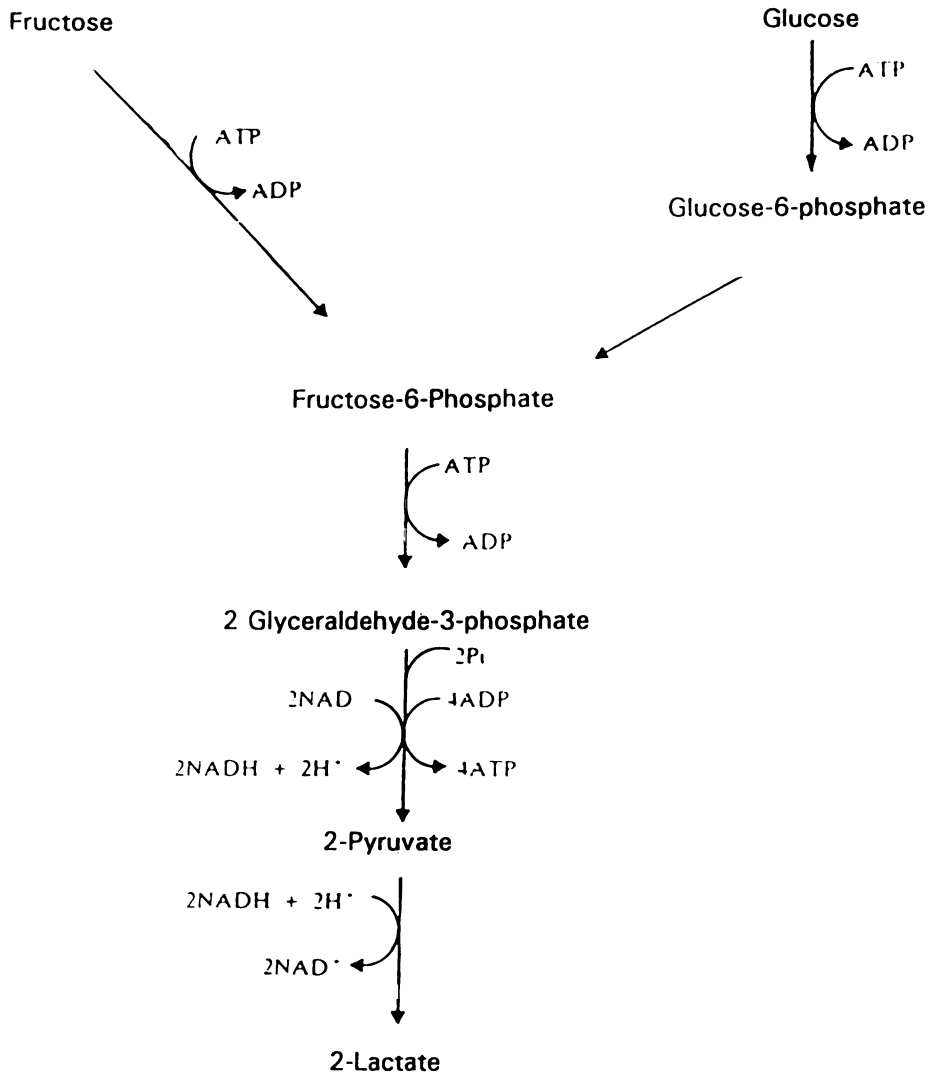
Lactic Acid Bacteria

The lactic acid bacteria are described as gram-positive, microaerophilic, asporogenous, sometimes motile, and capable of fermenting sugars (Buchanan and Gibbons, 1974; London, 1976). They are also classified based on cell morphology (e.g. cocci, rods) and the fermentation pathway utilized for sugar metabolism (heterofermentative and homofermentative). The last classification is the most important from a silage fermentation standpoint. During the ensiling process, both homofermentative and heterofermentative lactic acid-producing bacteria are present (Table 1.1). The homofermentative bacteria are the desired type because they degrade one mole of hexose (e.g. glucose, fructose) to two moles of lactic acid (Figure 1-3), resulting in more efficient production of lactic acid and subsequent decrease in pH. The heterofermentative lactic acid bacteria are

Table 1-1. Species of lactic acid bacteria found in silage

Homofermentative	Heterofermentative
Lactobacillus	Lactobacillus
L. acidophilus	*L. brevis*
L. casei	*L. buchneri*
L. coryniformis	*L. fermentum*
L. curvatus	*L. viridescens*
L. plantarum	
L. salivarus	
Streptococcus	Leuconostoc
S. bovis	*L. mesenteroides*
Pediococcus	
P. acidilactici	
P. cerivisiae	
P. pentosaceus	
Lactococcus	
L. lactis	
Enterococcus	
E. faecalis	
E. faecium	

(Adapted from McDonald et al., 1991)



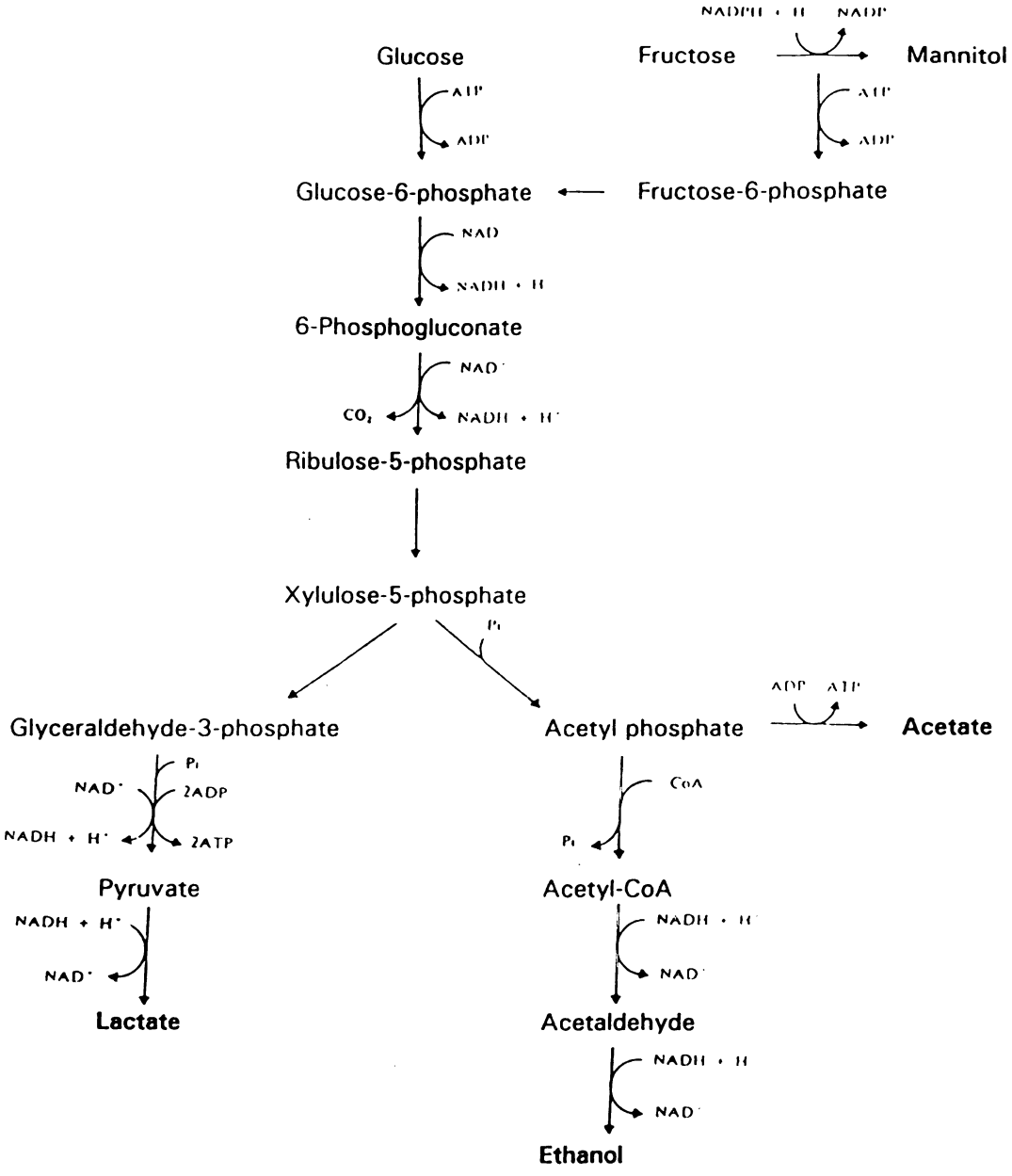
Sum: Hexose + 2ADP + 2Pi = 2 Lactate + 2ATP + $2H_2O$

Figure 1-3. Fermentation of glucose and fructose by homofermentative lactic acid bacteria (Adapted from McDonald, et al 1991)

less desirable because many of their fermentation products (e.g. acetic acid, ethanol, mannitol; Figure 1-4) that result from the metabolism of sugars, have dissociation constant (pKa's) greater than lactic acid, and their accumulation tends to result in silage with higher pH. Carbon dioxide is also produced by most heterofermentative lactic acid bacteria, resulting in carbon losses. Homolactic bacteria normally metabolize sugars by the Emden-Meyerhoff-Parnas pathway; whereas heterofermentative bacteria use the hexose monophosphate pathway (Buchanan and Gibbons, 1974). Both homofermentative and heterofermentative lactic acid bacteria can degrade pentoses (e.g. xylose, arabinose) yielding lactate and acetate as fermentation products (Figure 1-5). Some lactic acid bacterial strains are capable of degrading other organic acids, such as citric, malic, lactic and acetic acids (Bryan-Jones, 1969; Cited by McDonald et al, 1991; Figure 1-6), and both types have been shown to deaminate and decarboxylate amino acids and possess some proteolytic activity (Brady, 1976; Beck, 1978).

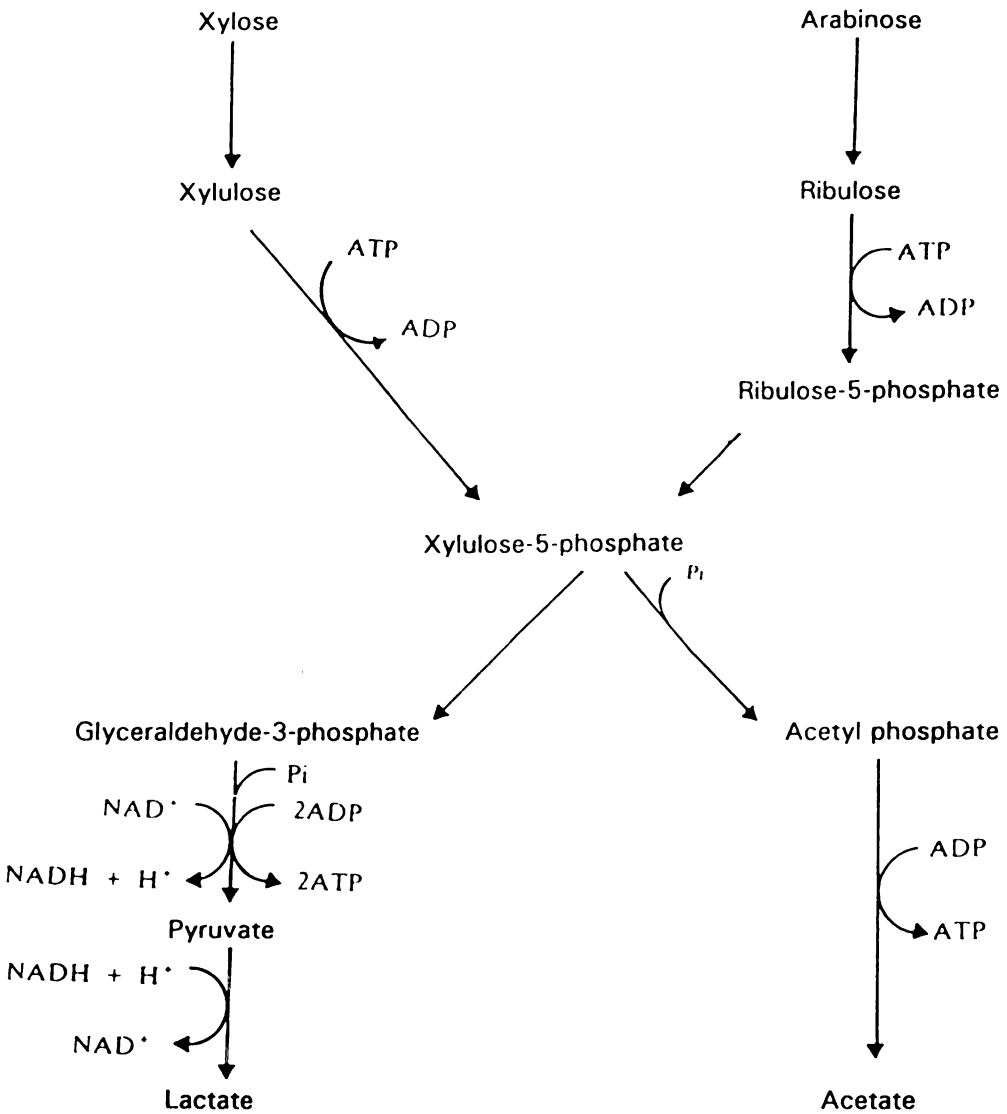
Enterobacteriaceae

The enterobacteriaceae are gram negative, rod-shaped bacteria, that are facultatively anaerobic, asporogenous, capable of fermenting sugars, and usually found at early stages of ensiling (Beck, 1978). Enterobacteriaceae have been found to occur at levels of 10^5 - 10^6 cfu/g of fresh material in alfalfa, (Bolsen et al, 1992) and grasses (Rauramaa, 1987b), but their



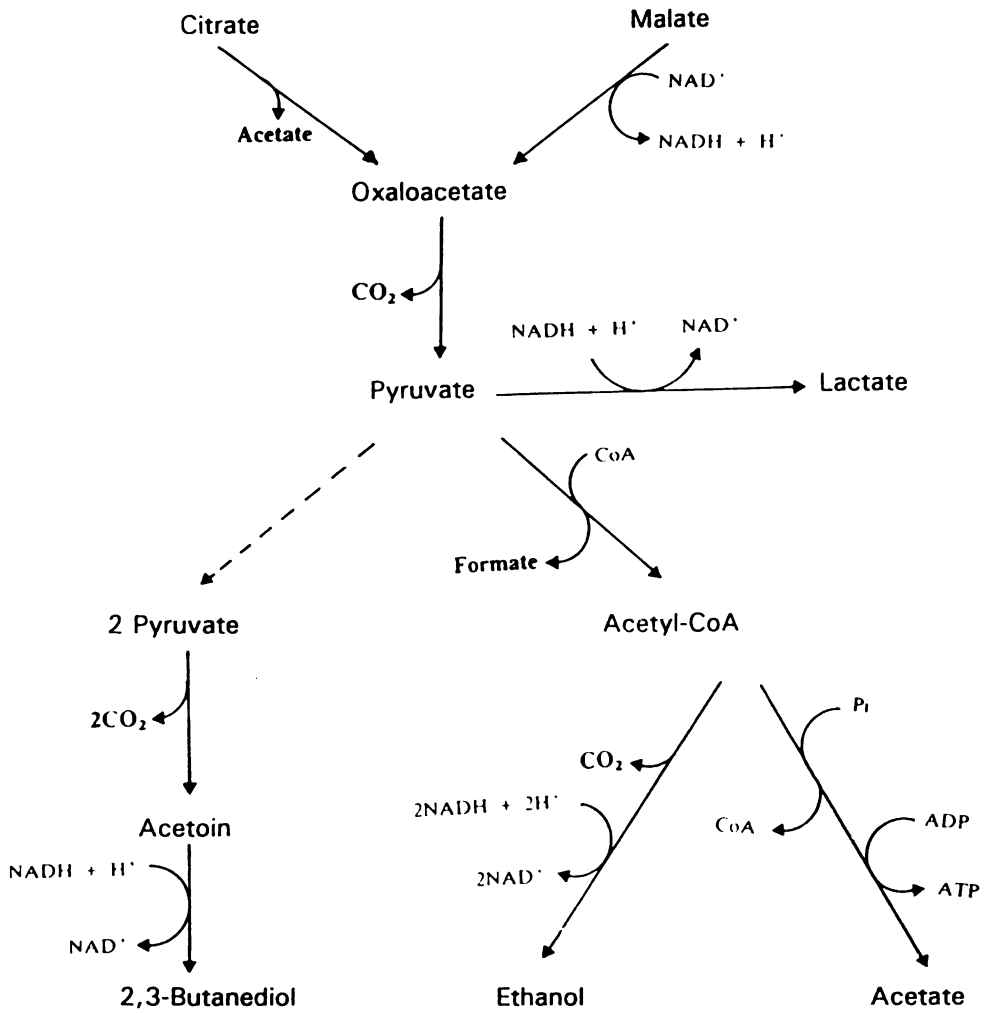
Sum: Glucose + ADP + Pi = Lactate + Ethanol + CO_2 + 2ATP + $2H_2O$
3 Fructose + 2ADP + 2Pi = Lactate + Acetate + 2 Mannitol + CO_2 + 2ATP + H_2O
Glucose + 2Fructose + 2ADP + 2Pi = Lactate + Acetate + 2Mannitol + CO_2 + 2ATP + H_2O

Figure 1-4. Fermentation of glucose and fructose by heterofermentative lactic acid bacteria (adapted from McDonald, et al 1991)



Sum: Xylose (or Arabinose) + 2ADP + 2Pi = Lactate + Acetate + 2ATP + $2H_2O$

Figure 1-5. Fermentation of pentoses by homofermentative and heterofermentative lactic acid bacteria (Adapted from McDonald, et al 1991)



Sum: Citrate + ADP + Pi = 2 Acetate + Formate + CO_2 + ATP
2 Citrate + 2H = 2 Acetate + 2,3-Butanediol + $4CO_2$
2 Citrate + ADP + Pi = 3 Acetate + Lactate + $3CO_2$ + ATP
Citrate + 4H = Acetate + Ethanol + Formate + CO_2
Malate = Lactate + CO_2
2 Malate = 2,3-Butanediol + $4CO_2$ + 2H
Malate + ADP + Pi = Acetate + Formate + CO_2 + 2H + ATP
Malate + 2H = Ethanol + Formate + CO_2

Figure 1-6. Fermentation of citrate and malate by homofermentative and heterofermentative lactic acid bacteria

presence have been associated with poor quality silage (Östling and Lindgren, 1991). The main fermentation products of Enterobacteriaceae from hexose metabolism are acetic acid and ethanol. Other fermentation products produced in lesser amount include lactate, succinate, and 2,3-butanediol (Buchanan and Gibbons, 1974; Beck 1978).

Yeasts and Molds

Yeasts and molds are classified within the fungi kingdom. Yeasts grow as single cells, and have been classified by their ability to ferment sugars and degrade lactic acid (sedimentary or pellicle). Hexoses are the main sugars utilized by yeast, however, some yeast utilize pentoses (e.g. xylose, ribose), starch, alcohols, and organic acids (Pelczar and Reid, 1972). Yeasts are not inhibited by low pH levels reached during ensiling, and under anaerobic conditions may utilize a great variety of substrates including organic acids and ethanol (Woolford, 1976). Studies have demonstrated that yeasts are capable of producing lactic acid from sugar (Woolford, 1976). However, their presence in silage is not desirable because they tend to utilize carbon sources inefficiently (i.g. increase ethanol production which results in silages that are susceptible to aerobic deterioration).

Molds grow as multicellular filamentous colonies, are not common in the early stages of ensiling, and their presence is generally associated with deterioration after exposure to air (McDonald et al., 1991). The major end-

products of respiration by yeasts are lactate, acetate and ethanol, and have been shown to compete with lactic acid bacteria for substrates (Rauramaa et al., 1987b). The activity of molds are usually secondary to the activity of yeasts (Ohyama and McDonald, 1975; Ohyama et al., 1975b), and are implicated in the production of toxins (e.g. aflatoxin, zearalenone) and other products that may be detrimental to animals (Beck, 1978).

Endospore-forming bacteria

Clostridia and bacillus are endospore-forming bacteria that have been isolated from silage. Clostridia have been classified into two major groups according to substrates utilized or end-products produced. Saccharolytic clostridia are associated with silage that does not reach a pH below 4.2 , and will degrade carbohydrates to butyric acid and carbon dioxide (Woolford, 1984). Accumulation of butyric acid causes a increase in pH which will favor the growth of proteolytic clostridia. The presence of clostridia during the ensiling process is undesirable because they act against preservation by destroying lactic acid, increasing pH, and increase protein degradation. Some strains of clostridia are able to produce propionic acid by reduction of lactic acid. Bacilli are microorganisms that have not received as much attention as the clostridia, but are associated with poor quality silage. These bacteria are aerobic or facultatively anaerobic, and ammonia has been shown to be an end-product of their metabolism

(Buchanan and Gibbons, 1974).

Other microorganisms associated with the ensiling process

Acetic acid- and propionic acid-producing bacteria, and Listeria are other microorganisms that have been associated with the ensiling process. Acetic acid-producing bacteria are more active when pH is over 5.0 which usually occurs in the initial stages of aerobic deterioration of poorly fermented silage. These bacteria produce acetic acid from ethanol, and carbon dioxide and water from acetic and lactic acids. Propionic acid-producing bacteria have been shown to be part of the silage microflora. This bacteria may utilize lactate or sugar as substrate and their fermentation products mainly include propionic acid, acetic acid, and carbon dioxide. Propionic acid-producing bacteria have been tested to enhance the aerobic stability of silage (Dawson, 1994).

Listeria are microorganisms found frequently in silage, but at relatively low numbers (Gray and Killinger, 1966). Their importance is related to metabolic diseases rather than silage fermentation. In forage ensiled under strictly anaerobic conditions and well fermented, listeria population is absent due to their susceptibility to low pH.

Source of microorganisms in the ensiling process

Epiphytic microflora are found on the standing crop prior to or immediately

after harvest, and represent the main source of microorganisms for the ensiling process. Aerobic bacteria, Enterobacteriaceae, and yeasts and molds seem to be the most predominant organisms on the standing fodder plants (Lindgren et al., 1985). However, in view of their importance during silage fermentation, most of the research has been focused on the presence of lactic acid-producing bacterial populations. The occurrence of epiphytic microflora can range from no detectable levels to 10^4-10^6 cfu/g of fresh material (Fenton, 1987), and may be influenced by environmental and plant factors. J.A. Rooke (1990) reported that lactic acid-producing bacteria were higher in the second cutting of perennial ryegrass (*Lolium perenne)*. Fenton (1987) and Muck (1989) showed that the epiphytic, lactic acid-producing bacterial population was positively associated with air temperature and length of wilting, but negatively correlated with drying rate. Epiphytic microflora present on alfalfa plants were shown to be higher at elevated temperatures during the growing season, but little effect due to cutting number, plant maturity, or wilting was observed (Lin et al., 1992a). In addition to epiphytic microflora, soil and machinery have been identified as a source of microorganisms for the ensiling process. Endospore-forming bacteria (i.e. clostridia, bacillus) are generally not defined as part of the epiphytic microflora, but are considered to be soil contaminants (Rauramaa et al., 1987b). Stirling and Whittenbury (1963) observed an increase in bacterial populations on plant material during the harvesting process, while

Fenton (1987) reported that passage through the farm machinery served to inoculate the forage prior to ensiling.

Biochemistry of the Silage Fermentation

The growth of microorganisms during the ensiling process requires the presence of an aqueous environment. Most substrates used by microorganisms are water soluble, low molecular weight, and readily absorbed by the microorganisms. There are three major chemical groups that serve as substrates during the ensiling process; carbohydrates, organic acids, and nitrogenous compounds.

Carbohydrates in the plant play an important role in intermediate metabolism, energy transfer and storage, and plant structure. Carbohydrates may be classified in two main categories; structural and non-structural. Structural carbohydrates constitute the plant cell-wall and are important in the structural integrity of individual cells, tissues and organs (Hatfield, 1989). The main components of structural carbohydrates include cellulose, a linear polymer of D-glucose units joined by ß-(1-4) bonds, hemicellulose comprised by mixtures of different monosaccharides (e.g. xylan, arabinose, glucose), and pectins which consist of chains of methylglucuronic acid, interspersed with glucose, galactose, and arabinose. Cellulose is not degraded during the ensiling process and only small amounts of hemicellulose are degraded by enzymes present in the plant.

Fructans and starch are the major non-structural, storage carbohydrates found in plants. Fructans are composed entirely of fructose molecules, and are not normally metabolized during silage fermentation. However, they are the major storage carbohydrates of temperate grasses. Starch is formed by polymers comprised of α-D-glucose units, and occur in two forms in the plant; amylose and amylopectin, which differ in molecular weight, type of bonding, and water solubility. Starch is not fermented by microorganisms normally found in silages (Woolford, 1984), however, it is the main storage carbohydrate in tropical grasses. Glucose, fructose, and sucrose are also non-structural carbohydrates found in plants. Glucose and fructose are simple sugars classified according to their functional group (aldehyde or ketone) when in the open-chain form, and sucrose is a disaccharide composed of glucose and fructose. These carbohydrates are highly soluble in cold water and easily hydrolyzed in weak acid. Collectively, they comprise the fraction measure as water soluble carbohydrates (WSC) and represent the main energy source for microorganisms during silage fermentation. Smaller concentrations of arabinose, xylose, ribose, galactose, and mannose are also present in the WSC fraction of forages (McIlroy, 1967; Volenec and Nelson, 1984). In the silage fermentation, a distinct preference for fructose, glucose, arabinose, and xylose over other water soluble, storage and non-fermentable carbohydrates has been shown (Salisbury, et al, 1949).

Organic acids and their salts play an important role in the ensiling process. The content of organic acids in plants range from 2.0 to 6.0% of the DM (Van Soest, 1994), and are associated with the buffering capacity of silages (Playne and McDonald, 1966). Citric, malic, and succinic are the prevalent organic acids in grasses. In tropical environments, grasses also contain oxaloacetic acid (an intermediate in the tricarboxylic cycle and other biochemical pathways in C-4 plants), which have been shown to have a strong buffering capacity, and may be associated with the inability to achieve a low pH in tropical silages. Organic acids are readily degraded by lactic acid-producing bacteria to end-products with lower disassociation constants such as 2,3-butanediol, formate, acetate, ethanol, and carbon dioxide (Beck, 1978; McCullough, 1978).

Nitrogenous compounds present in the fresh forage include protein, amino acids, amides, amines, chlorophyll, ureides, nucleotides, and low molecular weight peptides (Edwards and McDonald, 1987). Nitrogenous compounds have been implicated as a source of buffering capacity in forages (Playne and McDonald, 1966), and are positively correlated with ammoniacal nitrogen content of silage (Rauramaa et al., 1978a).

Phases of the ensiling process and storage

Silage production has been divided into 6 phases according to the microbiological, biochemical, and environmental changes occurring during

the ensiling process (Barnett, 1954; McCullough, 1978; Figure 1-7).

Phase 1. The aerobic or respiration phase. Begins when the forage is placed into the silo and ends when all oxygen in the ensiled material is depleted. At the onset of the ensiling process, plant enzymes hydrolyze carbohydrates (monomers and polymers), proteins, and small amounts of organic acids to carbon dioxide, water, and heat (Wylan, 1954). However, their action does not seem to play a major role in degradation of these substrates when compared with the further action of microorganisms (Woolford, 1984). During this respiration phase temperature is increased and pH is decreased. Exponential growth of facultative and obligate obligate lactic acid-producing bacteria is initiated, and the number of enterobacteriaceae may increase reaching populations as high as 10^8 to 10^9 cfu\g of fresh material (Gibson et al, 1958). Oxygen utilization should be rapid to minimize the growth of aerobic bacteria, yeasts and molds, and certain clostridia.

Phase 2. Anaerobic phase. Begins when the oxygen is depleted. Generally temperature of the silage mass which peaks in

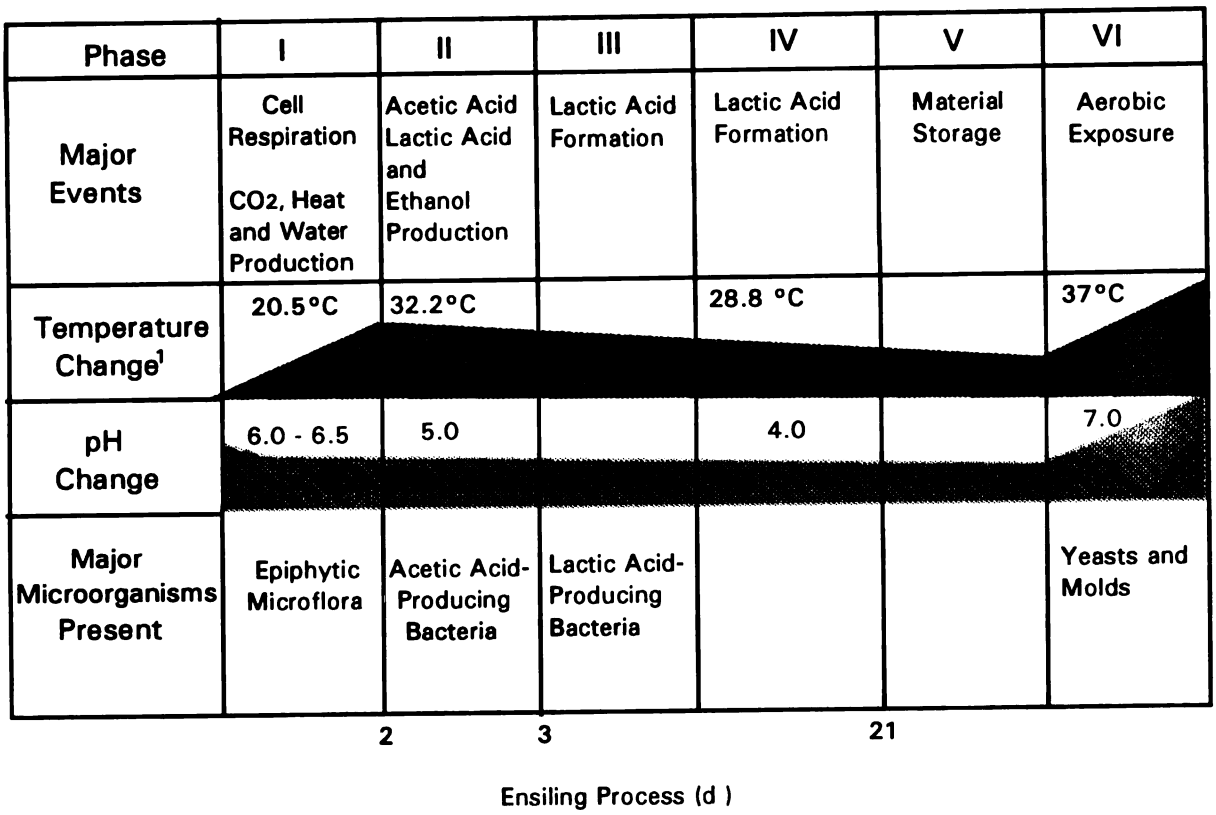


Figure 1-7. Phases of silage fermentation and storage
[1] Silage temperature is a function of ambient temperature
(Adapted from McCullough, 1987)

phase I, is declining. At this point, anaerobic bacteria begin the fermentation process and convert water soluble carbohydrates to acetic acid, lactic acid, ethanol, and carbon dioxide. Exponential growth of lactic acid-producing bacteria continues. This phase is usually short in duration and merges into phase III.

Phase 3. Lactic acid accumulation phase. It is the continuation of the anaerobic stage and early phase of lactic acid accumulation. Enterobacteriaceae bacteria decline in activity after the first three to seven days of ensiling. The lactic acid-producing bacterial population may peak during this phase. Lactic acid-producing bacteria produced acid rapidly and reduce the pH to levels inhibitory to most of the other microorganisms (Lindgren et al., 1988; Pettersson, 1988). The end result is a domination of the fermentation by LAB. This exponential growth of lactic acid-producing bacteria is one of the most important factors for a successful ensiling process.

Phase 4. Stable phase. Lactic acid-producing bacteria continue to ferment carbohydrates into acids but at a much slower

rate. The lactic acid bacterial population begins to decline (Gibsons et al., 1958).

Phase 5. Material Storage Phase. The first four phases in the ensiling process usually are completed within the first 17 to 21 d. If the production of lactic acid is insufficient and the pH in the ensiled material is greater than 4.2, a proliferation of saccharolytic clostridia may occur. The organisms convert WSC into butyric acid which is not an efficient mechanism to conserve carbon. Silage with high levels of butyric acid tends to have higher pH and end-products from proteolysis. If sufficient lactic acid is produced, spores of clostridia will not germinate because conditions are not favorable for vegetative growth. Changes in the homofermentative - heterofermentative ratio of lactic acid-producing bacteria may occur in this phase with dominance of heterofermentative strains due to their greater tolerance to lactic acid (Beck, 1978). Some yeast may survive under high acidic conditions.

Phase 6. Feedout phase. Silage is being fed out from the storage structure, and exposed to aerobic conditions.

It is generally assumed that silages which have higher levels of lactic acid are best because of better carbon preservation and bunk stability. However, exceptions do exist. Woolford (1984) concluded that poor quality silage could be associated with large LAB populations, and good quality silage could be prepared from silage with high numbers of clostridia. Several factors such as crop moisture, packing density of the ensiled crop, ambient temperature at harvest and during ensiling, and crop maturity influence the success of silage fermentation.

Aerobic stability

Exposure of ensiled material to air may lead to silage deterioration. The microbiological and biochemical changes that occur when silage is exposed to aerobic conditions have been the focus of many studies (Ohyama et al., 1975a, 1975b; Woolford, 1990). Aerobic spoilage usually occurs on the exposed surfaces of the silage mass, and is commonly identified by appearance of white or multicolored colonies of organisms (e.g. molds), that grow when temperatures are above 37°C, and pH is above 5.0 (Muck and Pitt, 1993). The increases in temperature and pH result from the metabolism of organic acids and residual, water-soluble carbohydrates by yeast and molds, and aerobic bacteria to carbon dioxide and water (Honing and Woolford, 1979). Extensive aerobic deterioration results in decreased dry matter recovery and loss of valuable nutrients. Fermentation products

produced during ensiling are metabolized and loss of these acids may account for 35% of dry matter losses associated with the ensiling process (Henderson et al., 1979; Woolford, 1990).

The probability of aerobic spoilage in silage is influenced by moisture content, temperature during storage, length of storage, oxygen infiltration during storage, packing conditions, number of microorganisms and substrates available during the period of aerobic exposure, presence of oxygen and carbon dioxide, forage species, and concentrations of fermentation acids (Ohyama et al., 1975; Pitt, 1986; Muck, 1988).

Characteristics of tropical silage

In tropical environments, ensiling of grasses results in lower quality silage as compared to temperate environments (McDonald et al., 1991). Ensiling of tropical forages have been associated with fermentations characterized by low lactic acid accumulation, and high ammonia, acetic acid and pH (Catchpoole and Henzell, 1971; Aguilera, 1975; Pantitharatne et al., 1986). Tjandraatmadja et al. (1990) demonstrated that *Lactobacillus plantarum*, a predominant lactic acid-producing bacterial strain found in forages ensiled in temperate environments, was also the primary lactic acid-producing bacteria present in silage made from forage sorghum (*Sorghum bicolor* cv. Sugardrip), hamil grass (*Panicum maximum*), pangola grass (*Digitaria decumbens*), and setaria (*Setaria sphalaceta*) in a tropical environment.

However, McDonald et al. (1991) reported that silage with high percentages of acetic acid seem to be deficient in lactic acid-producing bacteria, and that other microorganisms (e.g. Enterobacteriaceae) may predominate in the silage fermentation. Silage with high concentrations of acetic acid also have been associated with low feeding value (Wilkinson et al., 1976) and poor dry matter recovery (Hamilton et al., 1978). In addition, ruminant animals use acetate less efficiently than propionate under most circumstances (Van Soest, 1994).

In the tropics, the low concentration of readily-available, fermentable carbohydrates found in forages is another problem associated with poor ensiling characteristics. Wilkinson (1983) reported from a study of 231 silages made from temperate crops, that a concentration of water soluble carbohydrates of 3% was necessary to provide a stable, low pH, lactic acid dominated, well-preserved silage. However, tropical forages generally contain lower levels of fermentable carbohydrates, and during ensiling, are more susceptible to more extended fermentation periods as compared to temperate species (Catchpoole and Henzell, 1971; Wilson and Ford, 1973; Noble and Lowe, 1974). Ojeda and co-workers (1987) reported that typical, tropical grasses (e.i. *Digitaria decumbens*, *Cynodon dactylon*, and *Chloris gayana*), have 2 to 3 percentage units less water soluble carbohydrate contents than typical temperate grasses (Perennial ryegrass, Orchardgrass, Timothy). In more recent studies, Tjandraatmadja et al. (1994a), reported

that the water-soluble carbohydrate contents of three tropical grasses (hamil grass, *Panicum maximum*; pangola grass, *Digitaria decumbens*; setaria, *Setaria sphalaceta*) was insufficient to promote a strong lactic acid fermentation.

Silage additives

The utilization of silage additives to improve the ensiling process has been the subject of numerous studies. Silage additives can be subdivided into various categories based on the mechanisms of action which include: stimulants, fermentation inhibitors, aerobic deterioration inhibitors, nutrients, and absorbents (Table 1-2). It is important to emphasize that silage additives do not ensure a better silage if good management practices at ensiling (i.e. filling rapidly, good material compaction) are not followed. Currently, utilization of lactic acid-producing bacterial inoculants, and plant cell wall-degrading enzymes are the two most popular methods to improve silage fermentation. Addition of nitrogen (NPN, ammonia), and additional carbohydrate sources (molasses) are also utilized to some extent.

Lactic acid-producing bacterial inoculants

The use of lactic acid bacterial inoculants as starter cultures to enhance silage fermentation was first studied by French workers in sugar beet pulp at the beginning of this century (Watson and Nach, 1960). Currently, nine

Table 1-2. Classification of silage additives

Fermentation stimulants

Bacterial Cultures:
Lactic acid bacteria

Carbohydrate Source[1]:
Glucose
Sucrose
Molasses
Cereals
Whey
Beet pulp
Citrus pulp
Potatoes
Degrading enzymes

Fermentation Inhibitors

Acids
Mineral acids
Formic acid
Acetic acid
Lactic acid
Benzoic acid
Acrylic acid
Glycollic acid
Sulphamic acid
Citric acid
Sorbic acid

Other
Formaldehyde
Paraformaldehyde
Glutaraldehyde
Sodium nitrate
Sulpur dioxide
Sodium metabisulphite
Ammonium bisulphite
Sodium chloride
Antibiotics
Carbon dioxide
Carbon bisulphite
Hexamethylenetetramine
Bronopol
Sodium hydroxide

Aerobic deterioration inhibitors
Lactic acid bacteria
Propionic acid
Caproic acid
Sorbic acid
Piramicin
Ammonia

Nutrients[1]
Urea
Ammonia
Buiret
Minerals

Absorbents
Barley
Straw
Sugar beet pulp
Polymers
Bentonite

Adapted from McDonald, et al. (1991)
[1] Most substances listed under carbohydrate sources can be also listed under nutrients

criteria have been described to define the characteristics of a desirable microorganisms to be used as starter cultures (Seale, 1986; Muck, 1988). Desirable microorganisms must have the following characteristics:

1. The lactic acid-producing bacteria must growth vigorously and be able to compete with and preferably dominate other organisms.
2. It must possess a homofermentative pathway in order to produce the maximum amount of lactic acid from hexose sugars immediately available.
3. It must be acid tolerant and capable to producing a final pH of at least 4.0. Preferably it should be able to produce this low pH as rapidly as possible in order to quickly inhibit the activities of other microorganisms.
4. It must be able to ferment glucose, fructose, sucrose, fructans, and preferably, pentose sugars.
5. It must not produce dextran from sucrose or mannitol from fructose.
6. It should have no action on organic acids.
7. It should possess a growth temperature range extending to 50°C.
8. It should be able to grow in material of low moisture content as might arise when wilted material is ensiled.
9. It should have no proteolytic activity.

If the microorganism to be utilized has all the outlined characteristics, then changes expected during the ensiling process include; a shift in the LAB from heterofermentative to homofermentative, an increase in the ratio of lactic acid to other fermentation products (e.g. organic acids, ethanol), increase in rate of pH decline, reduction of proteolysis, and increase in dry matter recovery.

The most frequently used Genus and species of lactic acid-producing bacteria in microbial inoculants are *Lactobacillus plantarum*, *Pediococcus cerevisiae*, and *Enterococcus faecium* (Mahanna, 1993). However, other species of bacteria (e.i. *Pediococcus acidilactici*, *Lactobacillus curvatus*, *Lactobacillus xylosus*, and *Streptococcus faecium*) have also been evaluated. Even though these strains seem to accomplish the nine criteria that characterize a desirable microbial inoculant, their effects on silage quality have been variable. This variable response may result from plant, management, and other microbiological factors that influence the microbial culture activity. The number and type of epiphytic microflora have been mentioned as the most critical factor that determines the success of a microbial inoculant (Muck, 1991). It is desirable to have lactic acid-producing bacteria added at least 10 times the epiphytic bacteria counts to compete and dominate the natural microflora, and have a positive effect on silage fermentation and animal performance (Satter, 1991; Muck and Bolsen, 1992). Water-soluble carbohydrate content of the crop also has a great impact on microbial activity. Bacteria utilize sugar for growth, and if

insufficient sugar is available then a decrease in microbial inoculant activity would be expected. The growth rate and environmental adaptability of the bacterial strain utilized, rate of application, synergistic or antagonistic effects between bacterial strains when used in combination, and the specificity of lactic acid-producing bacteria to forage species to be ensiled may also influence the microbial inoculant activity. It has been shown that individual strains of lactic acid-producing bacteria differ in their ability to ferment substrates, and grow at various moisture levels and temperatures (Dennis, 1989; Hill 1989). In addition, many different species exist within each Genus and it is estimated that as many as 5000 strains exist within each specie (Soderlund, 1988).

Most studies utilizing microbial inoculants have been performed in temperate environments. Inoculants have been applied as monoculture, biculture or mixtures of three or more microorganisms. Whole corn plant and alfalfa have been the crops most extensively studied, but the effectiveness of microbial inoculants on grasses, cereals, and grass-legume silages have been also investigated. *Lactobacillus plantarum* seems to be the lactic acid-producing bacterial species most utilized. Kung et al., (1993) reported that a microbial inoculant containing *Lactobacillus plantarum* improved the ensiling characteristics in corn silage, but the same bacterial specie had little benefit on corn silage fermentation patterns in another study (Wittenberg et al., 1983). Studies using bicultures of lactic acid-producing bacteria in whole plant corn showed greater dry matter recovery and less

residual soluble carbohydrate concentrations when the crop was treated with a microbial inoculant containing *Pediococcus acidilactici* and *Lactobacillus xylosus* (Cleale, IV. et al., 1990). In another study, a biculture composed of *Lactobacillus plantarum* and *Enterococcus faecium* did not improve the ensiling characteristics of whole corn plant silage (Bolsen et al.,1992). Seale et al. (1986) demonstrated desirable changes during the silage fermentation of high moisture corn as compared with little benefit in whole corn plant silage when both crops were treated with *Pediococcus acidilactici* and *Lactobacillus xylosus*. In grasses, a microbial inoculant containing *Lactobacillus plantarum* and *Pediococcus acidilactici* improved the ensiling characteristics of ryegrass silage, as evidenced by faster acidification, increased lactic acid content, and reductions in acetic acid and proteolysis (Heron et al., 1988). In alfalfa, an early decrease in pH and greater lactic acid-producing bacterial population was observed when silage was treated with an inoculant containing *Lactobacillus plantarum* and *Enterococcus faecium* (Phillip et al., 1990). Kung et al., (1984) observed an increase in lactic acid content in alfalfa ensiled at three different dry matter concentrations when treated with a bacterial inoculant that contained *Lactobacillus plantarum*, *Lactobacillus brevis* and *Pediococcus acidilactici.* Shockey and Borgen (1991) compared the effectiveness of a microbial inoculant containing *Lactobacillus plantarum* and *Streptococcus faecium*, and a fermentation inhibitor (NaCl) on fermentation characteristics of alfalfa silage, and found that inoculated alfalfa had a faster decrease in pH, more

effective inhibition of clostridial organisms, and greater lactic acid content than alfalfa silage treated with only NaCl. In grass-legume silage treated with *Lactobacillus plantarum* and *Streptococcus faecium*, Harrrison and co-workers (1989) observed an accelerated pH decline, and reduction in content of ammonia-N, which supports the concept that the population of lactobacilli shift during fermentation from a heterofermentative to selected strains of homofermentative lactic acid-producing bacteria.

In other studies, inoculants containing starch-degrading bacteria have been studied to increased substrate availability for acid production. Jones et al. (1991) reported that application of *Streptococcus bovis* as a microbial inoculant decreased pH and ammonia nitrogen in low dry matter alfalfa silage, but no degradation of starch was observed. Other strains of starch-degrading bacteria (e.i. *Lactobacillus amylophilus* and *Lactobacillus amylovorus)* have also been studied but the results have been variable (Fitzsimons and O'Connel, 1994). However, there are no studies that evaluated a microbial inoculant containing starch-degrading bacteria in a crop with high starch content (e.g. some tropical forages).

A limited number of studies in subtropical environments have evaluated the effect of microbial inoculants on the ensiling process. Wilted coastal bermudagrass inoculated with *Lactobacillus plantarum* and *Streptococcus faecium* had lower pH, acetic acid, and ammonia concentrations, and greater lactic acid content and in vitro organic matter degradability than silage without inoculant (Umaña et al, 1991). A decreased in pH in

bermudagrass ensiled as large round bales and treated with a bacterial inoculant was reported by Dawson (1989). In South Africa, Figueiredo and Marais (1994) showed that addition of Lactobacillus plantarum or a biculture containing *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* did not increased lactic acid content, but decreased ammonia content in kikuyu grass (*Pennisetum clandestinum*).

The changes in fermentation end-products due to addition of a microbial inoculant may benefit animal performance. Reductions in acetic acid and ethanol should improve silage palatability and improve intake (McCullough, 1978). Higher levels of lactic acid is beneficial as lactic acid is fermented to propionate in the rumen. Propionate is used more efficiently by the animal than ethanol and acetic acid (Van Soest, 1994). However, in a recent review (Muck, 1993) only 25 % of the scientific studies showed a benefit on animal intake and average daily gain due to the use of a microbial inoculant. In meat producing animals, inoculation of whole plant corn silage increased dry matter intake in heifers, but average daily gain was similar (Clearle et al. 1989). Feed conversion efficiency of beef steers fed inoculated whole corn plant silage or high moisture corn were similar to controls (Schaefer et al, 1989). In dairy cattle, incresed milk production was observed when a mixture of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) was treated with a microbial inoculant (Martinsson, 1992). Mayne (1993) reported that perennial ryegrass (*Lolium perenne*) silage inoculated with a bacterial starter culture did not increase

milk yield, but there was a tendency for increases in milk fat and protein concentrations. In small ruminants, an increase in apparent digestibility of fiber and a tendency for improved nitrogen retention in lambs fed lucerne treated with a bacterial inoculant were observed by Phillips et al. (1990). Conversely, no differences in digestibility in lambs fed corn ensiled with microbial inoculant were reported by Whittenberg et al. (1983). In a subtropical environment, voluntary dry matter consumption by heifers fed either control or treated round bale bermudagrass silage treated was similar (Dawson, 1989). There is a limited information on the use of microbial inoculants in tropical forages and the resulting effects on dry matter intake and animal performance.

Higher accumulation of lactic acid during fermentation due to inoculation may result in lower pH and inhibit growth of undesirable microorganisms, improving the stability of the resulting silage. However, utilization of microbial inoculants to improve aerobic stability of silage has shown mixed results. Wolth, (1989) reported that inoculation of corn silage improved aerobic stability as compared to control silage as evidenced by lower temperature after 96 h of aeration, but Rust, et al. (1989) showed that inoculating corn silage appeared to lower the stability of corn silage upon exposure to air. A possible explanation for the instability of inoculated silages, may be a higher ratio of lactic acid/short chain fatty acids (e.g. acetic acid, propionic acid). Short chain fatty acids have been shown to have antimycotic activity, and may inhibit the growth of yeasts and molds,

and other microorganisms responsible for aerobic deterioration of silages (Moon, 1983). Additionally, there may be more residual WSC in the silage mass at time of exposure that allows spoilage organisms to grow.

Plant cell wall degrading-enzymes preparations

The two main goals for use of plant cell wall-degrading enzymes as silage additives are to increase the content of soluble sugars during the ensiling process, and to improve the digestibility of organic matter of the resulting silage. Most commercial enzyme preparations contain cellulases and hemicellulases, and their action on plant carbohydrates result in hydrolysis of cellulose and hemicellulose to pentoses and hexoses. Enzyme preparations containing amylases are also available. A positive response to the enzyme preparation may reduce the NDF and ADF content of the ensiled material, and may cause significant changes in fermentation, DM recovery, and animal performance. However, the effects of plant cell wall degrading enzymes on silage fermentation have been variable. The effectiveness of enzymes may be influenced by plant, environmental, management and microbiological factors. van Vuuren et al. (1989) reported that enzyme preparations are more effective in degradation of the cell wall in immature forages. Enzyme preparations generally have pH and temperature optimums that are not typically found in silages (Sheperd and Kung, Jr., 1994), and require application rates that would not be economically feasible (Hopking and Bass, 1987; Kung Jr. et al., 1991). Enzyme activity of the various

commercial preparations are expressed in different units which makes it difficult to compare different sources. Additionally, there is a large variation in efficacy of the various preparations (Sheperd et al., 1995). The microbial source of the enzymes may also influence its effectiveness. Different fermentation patterns were observed in timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), and red clover (*Trifolium platense*) silage treated with cellulase derived from different fungal preparations (Selmer-Olson, 1994). Bertin et al. (1985) found that enzymes derived from *Aspergillus niger* had more hemicellulolytic activity than enzymes from *Trichoderma viride*, whereas Henderson and McDonald (1977) reported more active enzymes derived from *Trichoderma viride* than *Aspergillus niger*. The majority of studies evaluating plant cell-wall degrading enzymes have been performed in temperate environments using forages with a low sugar content (i.e. grasses, alfalfa). In a current review (1985 to 1992), enzyme additives reduced the fiber content of grass silage in 80% of the studies reported (Muck, 1993). In alfalfa, however, the success rate was only 50 %. Leatherwood, et al. (1959) reported that addition of enzymes increased cellulose hydrolysis and decreased pH in alfafa silage. Conversely, little effect of enzyme additives on the ensiling characteristics of alfalfa ensiled at three moisture contents were reported by Jaster et al. (1990). In another study, cellulase increased cellulose hydrolysis in a grass-alfalfa-clover silage, but did not change the fermentation pattern (Henderson et al., 1982).

An increase in fiber degradability or a reduction in fiber content would be expected to enhance animal performance. Consequently, if enzymes reduce fiber content and improve its degradability one would expect the resulting silage to have a greater energy value. However, in a summary of 59 trials using beef cattle, only 15% of the cases showed improved degradability by enzyme products (Muck, 1993). In lactating animals, an enzyme-treated corn silage did not improve dry matter intake, and slightly reduced daily milk production during early lactation (Chen et al., 1994). Conversely, grass-legume silage treated with a commercial enzyme resulted in an increase in dry matter intake, milk production, and protein and fat content of the milk. In sheep, a decreased in DM intake and digestibility were observed when timothy silage was treated with an enzyme preparation containing cellulase and hemicellulase (Narasimhalu, et al.,1992). In contrast to this observation, sheep fed second-cut alfalfa silage treated with enzymes had a greater DM intake than sheep fed with untreated alfalfa (Fredeen and McQueen, 1993).

Utilization of plant cell wall-degrading enzymes have shown positive (Jaakkola et al., 1991) or little effect (Stokes, 1992) on aerobic stability of silages. The effects of enzyme products on the ensiling characteristics, animal performance and aerobic stability of forages ensiled in tropical and subtropical climates is limited.

Microbial inoculant and enzyme mixtures

When forages lack adequate amounts of water-soluble carbohydrates to serve as substrates for microbial growth and possess low number of epiphytic lactic acid-producing bacteria, utilization of a microbial inoculant in combination with enzymes would seem to be beneficial for the ensiling process. Most studies using microbial inoculant and enzymes have shown a positive response during the ensiling process, but conflicting results have been observed on animal performance.

In silage fermentation studies, no differences in the ensiling characteristics in corn and grass-legume silage were observed when treated with a enzyme mixture containing cellulase, α-amylase and protease, but a decreased pH and an increased lactic acid-producing bacterial populations were obtained when the enzyme complex was combined with a microbial inoculant (Grant et al, 1994). Sheperd et al. (1995) reported that additives containing combinations of lactic acid-producing bacteria and enzymes improved the fermentation characteristics and reduced fiber content in alfalfa silage. In another study, a combination of lactic acid-producing bacteria and enzymes negatively affected DM intake, but increased milk production in dairy cattle (Stokes, 1992). Fermentation characteristics, ruminal degradation of silage DM and NDF, or total tract digestibility in early lactation cows were not improved when grass-legume and corn silages were treated with a enzyme-inoculant mixtures (Chen et al. 1994). Conversely, perennial ryegrass (*Lolium perenne*) treated with enzyme-inoculant mixtures was more

digestible than control silage when fed to lactating dairy cows (Smith et al., 1993). The effect of microbial inoculant and enzyme mixtures on the ensiling characteristics, animal performance, and aerobic stability of tropical forages is limited.

Other silage additives

Nutrients, fermentation inhibitors, and absorbents are examples of other products that have been evaluated as silage additives. Various nutrient sources have been added to silage to increase the supply of available energy for the growth of lactic acid-producing bacteria, or increase the nitrogen content of the silage (urea, ammonia). In a temperate environment, adding glucose did not show a beneficial effect on the ensiling characteristics of ryegrass silage (Heron et al., 1988), but alfalfa ensiled with glucose or fructose had a lower pH, more protein-N, less ammonia-N, and a greater increase in lactic acid-producing bacterial numbers than in alfalfa ensiled without the carbohydrate source (Seale et al., 1986). Addition of ammonia prior to ensiling increased lactic acid content and decrease pH in alfalfa silage after 50 d of fermentation (Kung et al., 1984). Ensiling of grasses with molasses in the subtropical environment of Florida, has shown to decrease pH, increase lactic acid content, and increase in vitro organic matter degradability (Becker et al., 1970; Umaña et al, 1991). In tropical climates, added molasses to pangola grass silage (*Digitaria decumbens*), hamil grass (*Panicum maximum* cv. Hamil), and setaria (*Setaria sphacelata*

cv. Kuzungula) increased lactic acid content, but no differences in final pH, or ammonia-N were observed (Tjandraatmadja et al., 1994a). However, in other studies addition of molasses has improved the silage fermentation in tropical environments (Castel and Watson, 1985; Tjandraatmadja, 1994b). As a silage additive, molasses lost its popularity probably because of the difficulty in applying it, and any beneficial effects molasses had on fermentation were limited to environments where temperatures in silage exceeded 30°C (Lanigan, 1961).

Utilization of fermentation inhibitors (e.g. formic acid, formaldehyde) is a popular method to inhibit the growth of undesirable microflora, and reduce proteolysis, and has been shown to be an effective method to decrease aerobic deterioration (Jones et al, 1974; Raeker et al, 1992). However, use of these additives is becoming less attractive because of the caustic nature of acid on workers and equipment, and problems associated with feed refusal by animals.

CHAPTER 2

A TWO YEAR STUDY ON THE EFFICACY OF SILAGE ADDITIVES TO ENHANCE THE ENSILING OF FORAGE SORGHUM IN TEMPERATE AND TROPICAL ENVIRONMENTS. 1. FERMENTATION CHARACTERISTICS.

Abstract

A two year (1993 -1994) study was conducted to determine the effects of silage additives (enzymes and microbial inoculant) on the ensiling characteristics of forage sorghum ensiled in temperate and tropical environments. In both years, forage sorghum was harvested at 90 d of growth at Michigan State University, East Lansing, and at the Lajas Agricultural Experiment Station, University of Puerto Rico. In both years at each location, forage was chopped into 2.5 cm pieces, assigned to one of four treatments; no additive (control), enzymes (.1% of fresh weight), inoculant (10^6 cfu/g of fresh material), and enzymes plus inoculant, and placed into PVC silos. Three silos per treatment were opened after eight ensiling periods (0, 1, 3, 7, 14, 21, 40, and 100 d), and analyzed for

plant organic acid contents (citric, malic, succinic and oxaloacetic), pH, microbial succession (lactic acid bacteria, enterobacteriaceae, yeasts and molds, and lactate assimilating yeast), silage fermentation end-products (lactic, acetic, propionic and butyric acids, and ethanol), and water soluble carbohydrates (glucose, fructose, galactose, xylose, and arabinose). Structural carbohydrates (NDF, ADF, cellulose and hemicellulose) were determined after 0, 40 and 100 d. Microbial ecology and ensiling characteristics of forage sorghum ensiled in temperate and tropical environments varied between years. In both years at each location, malic and succinic acids were the major organic acids metabolized during the fermentation process, lactic acid was the principal fermentation end-product, and glucose and fructose were the predominant water soluble carbohydrates utilized. Silage additives did not affect the degradation of organic acids, in either environment. Forage sorghum treated with microbial inoculant alone or in combination with enzyme in the temperate environment decreased pH at 3, 7, and 14 d of fermentation, and increased lactic acid bacterial population after 7 d and lactic acid content after 14 d as compared to silage without the inoculant. In the tropical area, silages containing microbial inoculant had lower pH over the entire ensiling period, and higher lactic acid bacterial populations after 3 d and higher lactic acid content after 14 d in comparison to control silage or silage treated only with enzymes. Neither silage additive influenced other microbial groups or other

fermentation end-products. Forage sorghum ensiled in the temperate environment and containing a microbial inoculant had lower glucose content over the entire ensiling period, but greater fructose from d 1 to d 100 d of ensiling. In the tropical environment, inoculated forage sorghum had lower glucose after 3, 7 and 14 d post-ensiling, but higher fructose content over the entire ensiling period as compared to silages without microbial inoculant. Silage additives did not have a major influence in other WSC or structural carbohydrate contents, regardless of environment.

Introduction

The importance of the water soluble carbohydrate (WSC) content in forages prior to ensiling, and the presence of a sufficient lactic acid bacterial population (LAB) are necessary to ensure a good quality silage is well documented (Woolford, 1984; McDonald et al., 1991). Lactic acid bacteria use WSC as the energy source to produce lactic acid, causing an early decrease in pH and reducing the growth of undesirable microorganisms such as enterobacteriaceae and fungi (Muck and Bolsen, 1992). In temperate environments, the combined use of a LAB inoculant and an enzyme mixtures to increase the number of desirable organisms and to increase the availability of WSC has been the subject of several studies (Kung et al., 1990; Bolsen et al., 1992, Sheperd et al., 1995). However, the results have been inconsistent. Corn (O'Leary et al., 1985; Cleare et al., 1990),

sorghum (Ely et al., 1982; Sanderson, M.A., 1993), and alfalfa (Bolsen et al., 1992) silages treated with a microbial inoculant have been shown to increase LAB populations early in the fermentation period, resulting in silage with higher lactic acid content and lower pH. However, after an extended fermentation period, the end-products in corn silage (Wittenberg et al., 1983) and alfalfa silage (Shockey et al., 1985) treated with a bacterial inoculant containing *Lactobacillus plantarum* were similar to the control silage.

An increase in cellulose content was observed in grass-alfalfa-clover silage treated with a cellulase preparation, but fermentation end-products were similar (Henderson et al., 1982). Additionally, an enzyme preparation did not improve the fermentation characteristics of barley and vetch silage (Kung et al., 1990). In tropical environments, ensiling of forages results in less accumulation of lactic acid, higher pH, and lower LAB population than temperate environments (McDonald et al., 1991). It has been suggested this results from warmer climate, less WSC, higher buffering capacity, and fewer epiphytic LAB. However, there is limited information regarding the utilization of microbial inoculants or enzymes to enhance the ensiling characteristics of tropical forages. The objective of this experiment was to evaluate the efficacy of a commercial enzyme preparation and a homofermentative LAB inoculant to enhance the fermentation process of forage sorghum ensiled in two different climatic environments.

Experimental Procedure

The study was conducted over a two year period (1993 and 1994) at Michigan State University, East Lansing (84°29'39 west longitude, 42°41'50 north latitude, elevation 265 m) and at the Lajas Agricultural Experiment Station, University of Puerto Rico (67°00'00 west longitude, 18°00'00 north latitude, elevation 100 m). In both years at each location, forage sorghum (Hi Energy Hybrid II, Hereford, TX) was harvested at 90 d of growth and chopped mechanically into 2.5 cm pieces. Temperature (minimum and maximum), and precipitation were monitored daily during the forage growing season. In Puerto Rico, forage was manually harvested and placed through a commercial forage harvester in 1993, whereas the sorghum was cut and chopped with a commercial forage harvester in 1994. A commercial forage harvester was used in both years at the East Lansing site. Chopped forage was analyzed for DM (55°C for 72 h), ash (550°C for 12 h), total-N (AOAC, 1990), buffering capacity (Playne and McDonald, 1966), organic acid content, water soluble and structural carbohydrates, and epiphytic microbial populations. Prior to ensiling, vegetative material was treated with one of four treatments; no additive (control), enzymes (Viscozyme™L, Novo Nordisk Bioindustrials, Inc. Farnham, Survey, UK), bacterial inoculant (Ecosyl™, Zeneca Bioproducts, Farnham, Survey, UK), and enzymes plus bacterial inoculant. The enzyme additive consisted of a multi-enzyme preparation containing arabinase, cellulase, ß-glucanase,

hemicellulase, and xylanase and was applied at .1% of fresh material. The bacterial inoculant, consisted of a homofermentative strain of *Lactobacillus plantarum* applied at 10^6 cfu/g of fresh plant material. Treatments were applied to weighed portions (1.6 kg) of forage sorghum, manually mixed, and packed into PVC laboratory silos. Laboratory silos were fitted with release valves to allow gas escape and maintained at room temperature (20-23°C in Michigan, and 27-30°C in Puerto Rico) until opened. The control treatment received a similar amount of water as the sorghum treated with additives. In both years and at each location, triplicate silos from each treatment were opened at 0, 1, 3, 7, 14, 21, 40, and 100 d post-ensiling and analyzed for plant organic acid content, pH, microbial succession, silage fermentation end-products, and water soluble carbohydrates. Fifty g of forage from each silo at each sampling day were placed into 450 ml of distilled water (w/v) and homogenized for 5 min with a Stomacher apparatus (Tekmar 3500, Tekmar, Cincinnati, OH). Homogenates were strained through eight layers of cheesecloth and analyzed for pH with a pH meter fitted with a combination electrode (Fisher Scientific, Pittsburgh, PA) that was standardized from pH 4 to 7 using commercial buffers (Curtis Mattheason, Wodale, IL). For microbial succession determinations, tenfold dilutions of the clarified, extract homogenate were prepared for each sample in sterile peptone solution (.1%) and enumerated for lactic acid bacteria, enterobacteriaceae, yeasts and molds, and lactate assimilating yeast. Plates

for all microbial groups were poured with selective media and manually enumerated after respective incubation (Table 2-1) periods using a digital colony counter. Organic acids (citric, malic, oxaloacetic, and succinic acids) found in plant material and silage fermentation end-products (lactic, acetic, propionic, and butyric acids, and ethanol) were determined by ion exchange-exclusion HPLC analysis (Biorad aminex HPX-87H) following the general procedures of Canale et al., (1984). Mobile phase consisted of .005 N H_2SO_4 at a flow rate of .9 ml/min. Column temperature was maintained at 65°C by an external column heater (Waters Millipore). Three ml of the clarified homogenate from each silo were filtered through 2 μm ion chromatography syringe filters (Gelman Acrodisk, 25mm, Ann Arbor, MI) into 4 ml HPLC sample vials (National Scientific, Atlanta, GA). Filtered samples were stored at -20°C until analysis. Fifteen μl of the filtered samples was injected by an autoinjector (Water WISP 712) and quantified with a refractive index detector (Waters 410 refractive index detector). Peak heights were quantified by a commercial HPLC software program (Turbochem 3, PE Nelson) and compared to known individual standards for respective organic acids and fermentation end-products. Water soluble carbohydrates were also determined by ion exchange-exclusion HPLC (Biorad aminex HPX-87P) except that 20 μl of the filtered sample was injected, the mobile phase was millipore water at a rate of .6 ml/min and the column temperature was maintained at 85°C. Structural carbohydrates;

Table 2-1. Selective media and incubation period of microorganisms enumerated

Microbial Group	Selective Media[a]	Incubation Period (d)
Lactic Acid Bacteria	Rogosa SL agar	2
Enterobacteriaceae	Violet red agar (supplemented with 5% glucose)	1
Yeasts and Molds	Rose Bengal agar (supplemented with chloramphenicol)	7
Lactate-Assimilating yeast	Yeast nitrogen base agar (supplemented with .01% lactic acid)	3

[a] Difco Laboratories, Detroit, MI

NDF, ADF, hemicellulose (calculated as the difference between NDF and ADF), and cellulose (calculated as the difference between ADF and lignin), were determined at 0, 40 and 100 d post-ensiling by the procedures of Goering and Van Soest (1970) and Van Soest et al. (1991 Method A). Statistical analysis was performed within environment as a completely randomized design with a 2 (years) by 4 (silage additives) by 8 (ensiling period) factorial arrangement of treatments (Steel and Torrie, 1978) using the General Linear Model subroutine of SAS (1990). The Anova models for organic acid contents, pH, microbial group, fermentation end-products, and water soluble carbohydrates were as follows:

$$Y_{ijkl} = \mu + A_i + B_j + (A*B)_{ij} + C_k + (A*C)_{ik} + (B*C)_{jk} + (A*B*C)_{ijk} + E_{ijkl}$$

Where:

Y_{ijkl} = Individual response variable measured (e.g. pH, microbial group)

μ = Overall mean

A_i = Effect of year

B_j = Effect of silage additive

$A*B_{ij}$ = Interaction of year by silage additive

C_k = Effect of day of ensiling

$A*C_{ik}$ = Interaction of year by day of ensiling

$B*C_{jk}$ = Interaction of silage additive by day of ensiling

$A*B*C_{ijk}$ = Interaction of year by silage additive by day of ensiling

E_{ijkl} = random residual error

Bonferroni-t test was used for mean separation (SAS, 1990). The model for structural carbohydrate hydrolysis was similar except that only three ensiling periods (d 0, 40 and 100) were utilized.

Results and Discussion

Environmental Data and Forage Characteristics

In both years at each location, typical environmental conditions prevailed during the growing season. In the East Lansing site, average temperature was 16.42°C (13.45 - 24.81°C) in 1993 and 16.76°C (13.27 - 26.13°C) in 1994, with a total precipitation of 258 mm in 1993 and 533 mm in 1994 (Figure 2-1). In the tropical environment, average temperature was 26.04°C (18.65 - 32.07°C) in 1993 and 24.19°C (16.54 - 31.84°C) in 1994. Total precipitation during the growing season was 372 mm in 1993, and 136 mm in 1994 (Figure 2-2). As expected, in both years a greater temperature and lower total precipitation was observed in the tropical environment. In Puerto Rico, the growing season included months corresponding to the dry period, which may explain the low rainfall observed during the experiment. However, the forage was periodically

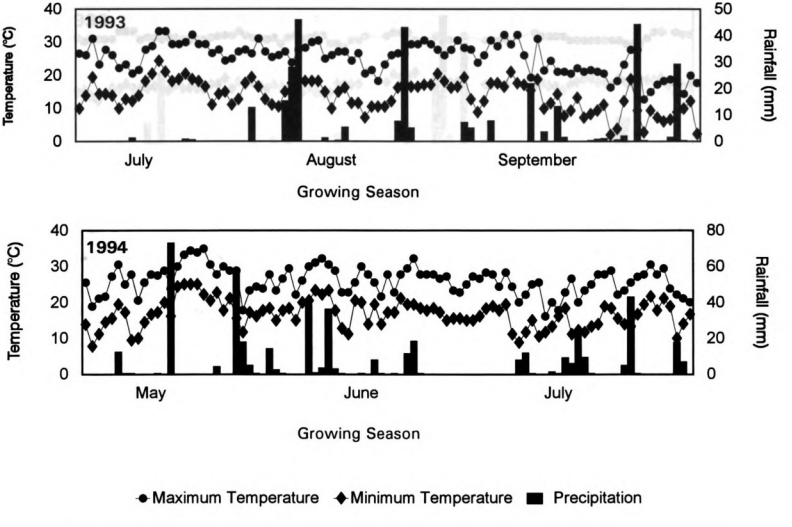


Figure. 2-1. Temperature and precipitation during the growing season in the temperate environment of Michigan (Source: Department of Horticulture, MSU)

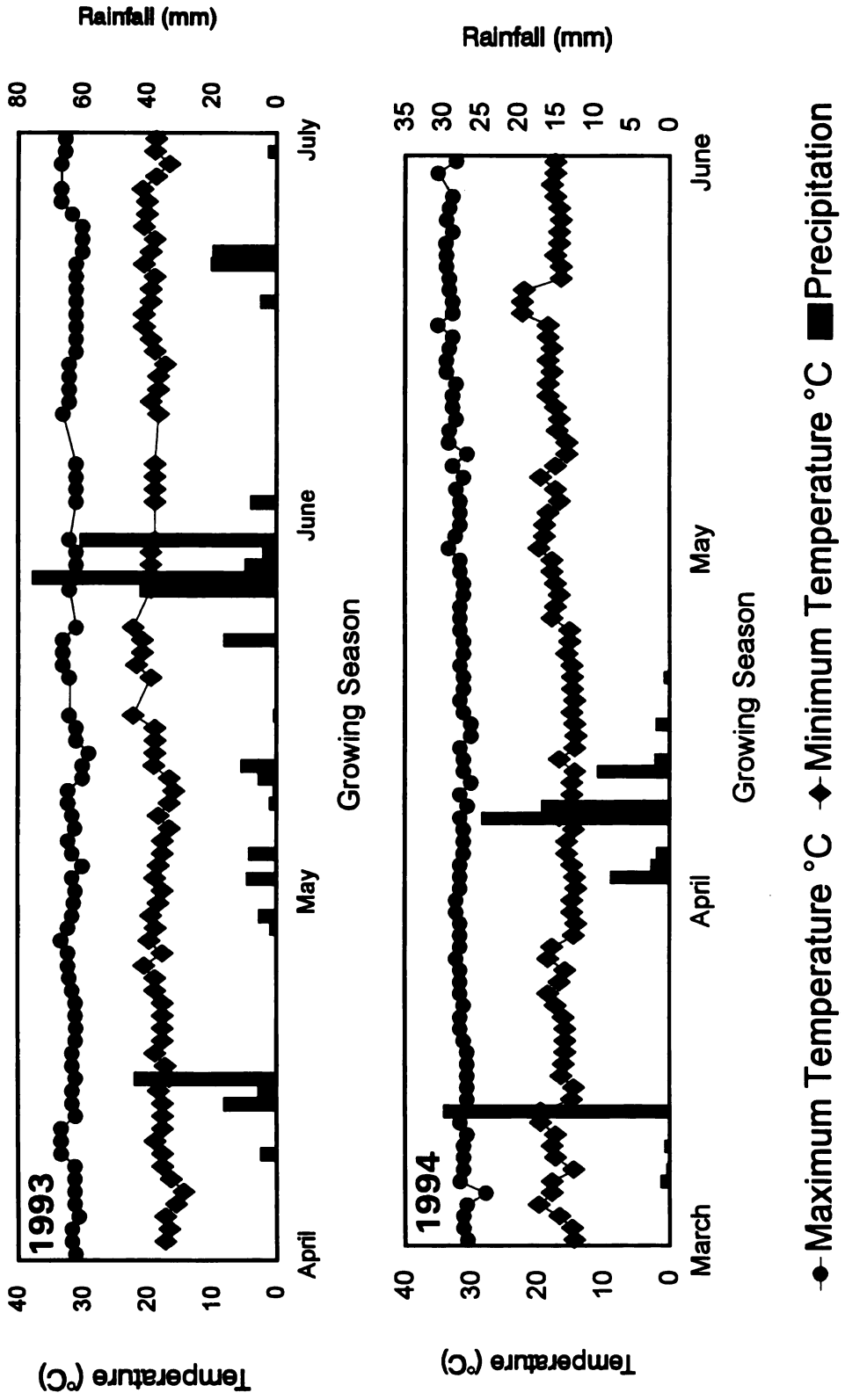


Figure. 2-2. Temperature and precipitation during the growing season in the tropical environment of Puerto Rico. (Source: Lajas Agricultural Experimental Station)

irrigated to maintain a normal growth.

Initial chemical characteristics of forage sorghum (Table 2-2) utilized in this experiment are in agreement with values previously reported for grasses harvested in temperate and tropical environments (Vicente-Chandler et al, 1983; Van Soest, 1991). Forage sorghum harvested in the temperate climate had lower DM, structural carbohydrates, lignin, pH, and buffering capacity than forage sorghum harvested in the tropical environment. Organic matter and total-N were similar regardless of climate. In both locations, glucose and fructose were the major WSC found in forage sorghum, and only small concentrations (< 1% DM) of galactose and pentoses (e.i. xylose, arabinose) were detected. Forage sorghum harvested in the temperate environment had greater glucose and fructose concentrations than forage sorghum in the tropical area, but lower xylose content. Galactose and arabinose contents were similar regardless of environment. The concentrations of malic and succinic acids of forage sorghum were greater than citric and oxaloacetic acids, in both environments. In contrast to cool season-C_3 plants, citric and oxaloacetic acids (intermediates in the tricarboxylic cycle and other biochemical pathways indigenous to C-4 plants) have been previously extracted from warm season grasses (Van Soest, 1994). The buffering capacity of these acids are strongest within pH range of 4.0 to 6.0 (Playne and McDonald, 1966) and have been associated with the greater buffering

Table 2-2. Characteristics of forage sorghum prior to ensiling in temperate and tropical environments

	Environment							
	Temperate				Tropical			
Item	1993	SD[a]	1994	SD	1993	SD	1994	SD
Chemical Composition								
DM, g/100 g DM	19.13	1.31	20.04	1.30	25.56	0.89	23.75	0.54
OM[b]	92.93	0.76	92.41	0.77	92.57	0.94	93.08	1.02
NDF[b]	56.41	1.80	54.51	3.22	66.09	1.61	66.50	2.53
ADF[b]	34.64	2.54	35.66	2.84	37.22	1.03	39.99	2.42
Hemicellulose[b]	21.76	1.24	24.20	0.98	28.84	0.87	26.30	0.80
Cellulose[b]	29.51	2.51	28.14	2.69	30.11	2.02	32.50	2.43
Lignin[b]	4.51	0.62	4.45	0.96	6.01	0.42	5.54	0.77
Glucose[b]	5.31	0.32	6.41	0.58	3.28	0.08	2.93	0.17
Fructose[b]	4.83	0.50	6.30	0.57	2.63	0.20	3.25	0.30
Galactose[b]	0.06	0.01	0.17	0.03	0.07	0.01	0.20	0.05
Xylose[b]	0.07	0.01	0.06	0.01	0.37	0.13	0.67	0.06
Arabinose[b]	0.07	0.02	0.16	0.04	0.06	0.03	0.09	0.02
Total-N[b]	1.02	0.10	1.12	0.12	1.01	0.10	1.04	0.08
pH	5.40	0.05	5.16	0.09	5.56	0.32	5.61	0.29
Buffering capacity[c]	18.09	1.11	19.16	0.94	24.18	1.80	25.27	2.75
Organic Acids								
Citric[b]	0.34	0.23	0.53	0.15	0.64	0.17	0.70	0.20
Malic[b]	2.49	0.17	4.76	0.56	4.48	0.65	4.62	0.40
Oxaloacetic[b]	0.69	0.14	1.09	0.09	0.86	0.35	1.19	0.17
Succinic[b]	2.40	0.50	1.58	0.07	1.74	0.61	3.21	0.65
Epiphytic Microflora[d]								
Enterobacteriaceae	4.80	.177	4.50	.770	7.01	.407	5.47	.955
Lactic acid bacteria	3.46	.365	3.28	.347	4.10	.477	3.30	.696
Yeasts and molds	2.92	.547	5.28	.345	6.11	.320	3.14	.442
Lactate assimilating yeast	2.05	.674	2.86	.237	2.55	.197	2.75	.406

[a] Standard deviation
[b] g/100 g DM
[c] meq/ 100 g DM
[d] cfu/g of fresh material

capacity for forages harvested in tropical environments (McDonald et al., 1991). Forage sorghum is a warm season-C_4 grass native to tropical climates. However, in this experiment when forage sorghum was harvested at the same chronological age as in the temperate environment, total organic acid concentrations were lower than in a tropical environment. Epiphytic enterobacteriaceae, lactic acid bacteria, and lactate assimilation yeast populations were similar in chopped forage sorghum harvested in temperate environment, regardless of year, but yeast and mold populations were higher in 1994 than in 1993. In the tropical environment, enterobacteriaceae, lactic acid bacteria, and yeast and mold populations were higher in 1993 than in 1994, but lactate assimilating yeast counts were similar. All four epiphytic microbial groups enumerated in chopped forage prior to ensiling were higher in sorghum harvested in the tropical environment.

In this experiment, identical forage species and length of growing season were utilized in both years at each location. However, variation in the initial chemical composition, organic acid content, and epiphytic microflora were observed within and across locations. This is a common problem encountered in research with biological materials. The variation in chemical composition of forage sorghum between environments may be attributed to the differences in temperature, latitude, solar radiation, and photoperiod. The effects of environmental factors on chemical composition of forages

have been extensively reviewed (Minson and McLeod, 1970; Buxton and Fales, 1994; Van Soest, 1994). Experiments also have shown that the occurrence of epiphytic microflora may be influenced by environmental conditions within and between geographical areas (Henderson, 1972; Muck, 1989; Lin et al., 1992b); which may explain the variability in epiphytic microbial populations found in this experiment.

Temperate Silage

There was a significant interaction for day of ensiling between years for all organic acids in forage sorghum ensiled in the temperate area (Table 2-3). In both years, concentration of malic acid decreased ($P<0.01$) the initial 40 d post-ensiling, then remained constant the last 60 d of fermentation. Succinic acid decreased ($P<0.01$) as the length of ensiling period increased. A general trend for the fermentation of citric and oxaloacetic acid was not observed. These two acids remained mainly constant throughout the fermentation process, but lower concentrations were seen after 100 d post-ensiling as compared to the initial citric and oxaloacetic acids content found in vegetative material prior to ensiling. In this experiment, all four organic acids disappeared in forage sorghum silage, but a greater and more consistent degradation of malic and succinic acids was observed as compared to citric and oxaloacetic acids.

In both years, pH of forage sorghum silage decreased ($P<0.01$) through the

Table 2-3. Effects of year and day of ensiling on organic acid content of forage sorghum ensiled in a temperate environment

Organic acid , g/100 g DM	Day	Year		SEM[a]	Probability		
		1993	1994		Y[b]	D[c]	Y*D[d]
Citric	0	0.34[g]	0.53[a]	.034	.007	.001	.001
	1	0.28[gh]	0.36[g]				
	3	0.40[f]	0.40[f]				
	7	0.59[a]	0.42[f]				
	14	0.60[a]	0.44[f]				
	21	0.63[a]	0.42[f]				
	40	0.47[f]	0.41[f]				
	100	0.23[h]	0.28[h]				
Malic	0	2.49[a]	4.76[a]	.131	.001	.001	.001
	1	1.68[f]	4.14[a]				
	3	1.42[f]	3.70[f]				
	7	1.45[f]	2.62[g]				
	14	1.08[g]	1.39[h]				
	21	0.94[g]	1.18[h]				
	40	0.67[h]	0.59[i]				
	100	0.69[h]	0.57[i]				
Oxaloacetic	0	0.69[g]	1.09[a]	.056	.221	.001	.001
	1	0.57[h]	0.69[g]				
	3	0.89[a]	0.81[f]				
	7	0.75[af]	0.83[f]				
	14	0.83[fg]	0.86[f]				
	21	0.92[a]	0.80[f]				
	40	0.70[g]	0.74[g]				
	100	0.56[h]	0.46[h]				
Succinic	0	2.37[a]	1.58[a]	.075	.001	.001	.001
	1	1.52[fg]	0.71[f]				
	3	1.71[f]	0.55[g]				
	7	1.57[fg]	0.68[fg]				
	14	1.65[fg]	0.68[fg]				
	21	1.48[g]	0.72[fg]				
	40	1.31[g]	0.59[fg]				
	100	0.81[h]	0.39[h]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year by day of ensiling
[afghi] Means with unlike superscripts in the same year and organic acid heading differ ($P<0.01$)

initial 14 d of fermentation, and reached a pH value of 4.2 after 3 d post-ensiling (Table 2-4); which is the pH needed for a stable silage (Woolford, 1984). After 14 d post-ensiling, a slight increase in pH the last 60 d of fermentation was observed in forage sorghum ensiled in 1993, but pH remained constant throughout the ensiling period in 1994. The final pH of ensiled material is highly influenced by the buffering capacity of the forage (Woolford, 1984; McDonald et al., 1991). In this experiment, higher buffering capacity and total organic acid content (5.92 vs. 7.96 DM % in 1993 and 1994, respectively) were found in forage sorghum harvested in 1994 than in 1993, but pH values were lower throughout the fermentation process. This result may indicate a more active microflora associated with degradation of organic acids in material ensiled in 1994 or differences in the fermentation end-products. Initial pH values were also different between years (5.40 in 1993, and 5.18 in 1994). In this experiment, pH was measured 2 and 4 h after the material was harvested in 1993 and 1994, respectively. These differences in initial pH may also influence the final pH values of the silages during the fermentation process. In both years, the lactic acid bacterial population followed the same general trend, reaching maximum level after 7 d of fermentation and slightly decreasing thereafter. A similar pattern in lactic acid bacterial populations was observed in corn and alfalfa silages by Seale et al. (1986); who associated this decrease in

Table 2-4. Effects of year and day of ensiling on pH and microbial succession of forage sorghum ensiled in a temperate environment

Item	Day	Year		SEM[a]	Probability		
		1993	1994		Y[b]	D[c]	Y*D[d]
pH	0	5.40[f]	5.18[f]	.014	.001	.001	.001
	1	4.60[g]	4.32[g]				
	3	3.94[h]	3.57[h]				
	7	3.77[i]	3.45[i]				
	14	3.58[k]	3.49[i]				
	21	3.56[k]	3.52[i]				
	40	3.61[jk]	3.50[i]				
	100	3.66[j]	3.50[i]				
Microbial Group[e]							
Lactic acid bacteria	0	4.32[k]	4.17[i]	.087	.001	.001	.001
	1	7.28[j]	7.90[h]				
	3	7.56[h]	8.54[g]				
	7	8.20[f]	8.90[f]				
	14	7.76[g]	8.50[g]				
	21	7.34[i]	7.70[j]				
	40	7.15[j]	7.25[j]				
	100	7.10[j]	6.99[k]				
Enterobacteriaceae	0	4.80[g]	4.50[g]	.182	.001	.001	.001
	1	5.89[f]	5.34[f]				
	3	2.85[i]	5.05[f]				
	7	1.66[j]	3.81[h]				
	14	3.01[h]	2.30[j]				
	21	2.59[i]	2.54[i]				
	40	2.43[i]	2.50[j]				
	100	2.72[i]	2.48[i]				
Yeasts and molds	0	2.92[h]	5.28[f]	.140	.001	.001	.004
	1	3.39[g]	5.31[f]				
	3	3.67[g]	4.44[g]				
	7	3.22[g]	4.81[g]				
	14	4.20[f]	4.69[g]				
	21	4.47[f]	4.85[g]				
	40	4.34[f]	4.68[g]				
	100	4.32[f]	4.61[g]				
Lactate assimilating yeast	0	2.05[hi]	2.80[h]	.218	.001	.001	.001
	1	2.90[g]	3.13[gh]				
	3	2.16[hi]	3.39[fg]				
	7	2.28[h]	3.40[fg]				
	14	1.82[i]	3.16[gh]				
	21	2.80[g]	2.96[h]				
	40	4.14[f]	3.75[f]				
	100	4.26[f]	3.16[h]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year by day of ensiling
[e] cfu/ g of fresh material
[fghik] Means with unlike superscripts in the same year and item heading differ ($P<0.01$)

lactic acid bacteria with the increase in acidity at later stages of the fermentation process. In both years, enterobacteriaceae population decreased ($P<0.01$) during the first 14 d of fermentation, then remained constant. Previous studies have demonstrated that enterobacteriaceae populations decrease early in the silage fermentation period, and have little significance during the last stages of the ensiling process (Beck, 1978; Muck and Bolsen, 1992). A different pattern in yeast and mold and lactate assimilating yeast populations was observed between years. In 1993, yeast and mold populations were higher ($P<0.01$) after 14 d of fermentation. This rise in yeast and mold counts after 14 d post-ensiling, may be attributed to an increase in lactate assimilating yeast during the last two thirds of the fermentation period. In 1994, yeast and mold counts decreased ($P<0.01$) during the first 3 d of fermentation, then remained constant after 100 d post-ensiling, and a trend for increased lactate assimilating yeast population was not observed. In this experiment, yeast, mold, and lactate assimilating yeast populations were not inhibited by the high acidic conditions found in the ensiled material. These results are in agreement with Henderson et al. (1972); who found high yeast and mold and lactate assimilating yeast populations in low pH silage treated with formic acid.

In both years, acetic acid production increased ($P<0.05$) over the ensiling period (Table 2-5). A greater acetic acid content during the fermentation

Table 2-5. Effects of year and day of ensiling on fermentation end-products of forage sorghum ensiled in a temperate environment

Fermentation End-product , g/100 g DM	Day	Year		SEM[a]	Probability		
		1993	1994		Y[b]	D[c]	Y*D[d]
Acetic acid	0	0.22[J]	0.28[J]	.066	.001	.001	.030
	1	1.06[I]	0.79[I]				
	3	1.20[h]	0.94[h]				
	7	1.40[g]	1.14[g]				
	14	1.71[f]	1.38[f]				
	21	1.79[f]	1.38[f]				
	40	1.73[f]	1.52[e]				
	100	1.89[e]	1.54[e]				
Lactic acid	0	0.59	0.58	.303	.085	.001	.142
	1	4.31	4.89				
	3	6.47	6.27				
	7	8.05	7.82				
	14	9.44	9.33				
	21	9.12	10.42				
	40	8.32	8.38				
	100	6.43	7.13				
Propionic acid	0	0.00[G]	0.00[F]	.001	.001	.001	.001
	1	0.00[G]	0.00[F]				
	3	0.01[F]	0.00[F]				
	7	0.01[F]	0.00[F]				
	14	0.01[F]	0.01[E]				
	21	0.02[E]	0.00[F]				
	40	0.01[F]	0.01[F]				
	100	0.02[E]	0.01[E]				
Butyric acid	0	0.00[D]	0.00[F]	.001	.001	.001	.004
	1	0.01[F]	0.00[F]				
	3	0.01[F]	0.00[F]				
	7	0.01[F]	0.00[F]				
	14	0.02[E]	0.01[E]				
	21	0.01[F]	0.01[E]				
	40	0.01[F]	0.01[E]				
	100	0.01[F]	0.01[E]				
Ethanol	0	0.19[J]	0.18[G]	0.07	.001	.001	.001
	1	0.52[I]	0.50[F]				
	3	1.20[G]	0.54[EF]				
	7	1.62[E]	0.61[E]				
	14	1.42[F]	0.55[F]				
	21	1.45[F]	0.69[E]				
	40	1.22[G]	0.49[F]				
	100	1.12[H]	0.56[E]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year by day of ensiling
[EFGHIJ] Means with unlike superscripts in the same year and fermentation end-product heading differ ($P<0.01$)
[efghi] Means with unlike superscripts in the same year and fermentation end-product heading differ ($P<0.05$)

process was observed in 1993 than in 1994; which may be responsible to the higher pH found in forage sorghum ensiled in 1993. Acetic acid has a disassociation constant (pKa = 4.76) greater than other major end-products produced in the fermentation process (e.g. lactic acid, pKa = 3.86), and its presence has been associated with high pH silages (Woolford, 1984; McDonald et al., 1991). Lactic acid was the major fermentation end-product associated with the ensiling of forage sorghum. No significant interaction between year and day of ensiling for lactic acid content was observed. However, forage sorghum ensiled in 1994 tended ($P<.08$) to have greater lactic acid content than sorghum ensiled in 1993. In both years, lactic acid production increased through 21 d post- ensiling, then decreased slightly. Numbers of lactic acid producing bacteria found in this experiment increased at an exponential rate during the first 7 d of ensiling. This increase in number of lactic acid bacteria correspond to the decrease in pH and increase in lactic acid content. In both years, small amounts of propionic and butyric acids were found in forage sorghum silage, being greater ($P<0.01$) in 1993 than in 1994. However, concentration of these fermentation end-products were below .1% DM; which is the minimum concentration required to play a significant role in the fermentation process (McDonald et al., 1991).

Ethanol production increased ($P<0.01$) during the first 7 d post-ensiling in forage sorghum ensiled in 1993, then decreased slightly ($P<0.01$) the last

two-thirds of the fermentation period. In 1994, ethanol content increased ($P<0.01$) through 3 d post-ensiling, then remained constant thereafter. All microorganisms associated with ethanol production were higher in forage sorghum ensiled in 1994 than in 1993, but ethanol content was lower. There appears that more metabolically active microbial population associated with ethanol production (e.g. enterobacteriaceae, heterofermentative lactic acid bacteria, and yeasts and molds) were present in forage sorghum ensiled in 1993.

There was a significant interaction for year and length of ensiling on glucose and fructose content in forage sorghum silage. In both years, glucose and fructose were rapidly degraded the first 7 d post-ensiling (Table 2-6). This decrease in glucose and fructose content corresponds to the maximum LAB counts and maximum pH decrease found in this experiment. After 7 d post-ensiling, degradation of both sugars continued, but at a slower rate. Enterobacteriaceae population, which is well known for production of acetic acid at the early stages of the fermentation period, decreased after 7 d post-ensiling. The increase in acetic acid and decrease in lactic acid at the later stages of the fermentation may indicate a shift in the lactic acid bacterial population from homofermentative to heterofermentative. Additionally, the greater glucose content over fructose observed after 7 d post-ensiling may indicate that acetic acid producing microorganisms had a fructose preference, and increased their metabolic activity during the later

Table 2-6. Effects of year and day of ensiling on water soluble carbohydrate contents of forage sorghum ensiled in a temperate environment

Carbohydrate, g/100 g DM	Day	Year		SEM[a]	Probability		
		1993	1994		Y[b]	D[c]	Y*D[d]
Glucose	0	5.31[e]	6.41[e]	.122	.001	.001	.001
	1	3.24[f]	3.62[f]				
	3	2.61[g]	3.41[f]				
	7	1.68[h]	2.31[g]				
	14	1.56[h]	2.09[g]				
	21	1.15[i]	1.74[h]				
	40	1.11[i]	1.52[h]				
	100	1.07[i]	1.01[i]				
Fructose	0	4.83[e]	6.30[e]	.131	.001	.001	.001
	1	1.91[f]	2.22[f]				
	3	1.38[g]	2.07[f]				
	7	0.94[h]	1.68[g]				
	14	0.76[h]	1.27[h]				
	21	0.73[h]	1.06[hi]				
	40	0.67[h]	0.87[i]				
	100	0.69[h]	0.91[i]				
Galactose	0	0.06	0.17	.002	.001	.001	.412
	1	0.06	0.24				
	3	0.15	0.31				
	7	0.10	0.29				
	14	0.09	0.25				
	21	0.10	0.27				
	40	0.08	0.24				
	100	0.06	0.23				
Xylose	0	0.07	0.06	.027	.301	.001	.826
	1	0.07	0.08				
	3	0.09	0.12				
	7	0.14	0.10				
	14	0.12	0.12				
	21	0.16	0.20				
	40	0.29	0.31				
	100	0.56	0.61				
Arabinose	0	0.07[ef]	0.16[e]	0.01	.001	.001	.001
	1	0.06[f]	0.14[efg]				
	3	0.07[ef]	0.13[fgh]				
	7	0.09[e]	0.12[ghi]				
	14	0.08[ef]	0.11[hij]				
	21	0.07[ef]	0.10[ij]				
	40	0.07[ef]	0.09[j]				
	100	0.09[e]	0.15[ef]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year * day of ensiling
[efghij] Means with unlike superscripts in the same year and carbohydrate heading differ ($P < .01$)

stages of the fermentation period when conditions were more acidic. It seems likely that the yeast, mold and lactate assimilating yeast populations utilized other substrates rather than sugars after the initial 7 d post- ensiling. Glucose and fructose contents were lower ($P<0.01$) over the entire ensiling period in forage sorghum ensiled in 1993 than in 1994. The differences in sugars content between years support the observation of a greater metabolic activity of the microbial population (e.g. enterobacteriaceae, yeasts and molds) in the 1993 silage, as evidenced by higher content of fermentation end-products (e.g. acetic acid, ethanol) with lower microbial counts. Forage sorghum ensiled in 1994 had greater ($P<0.01$) galactose content then forage ensiled in 1993. Galactose concentration was similar throughout the ensiling period. In this experiment, it would seem that galactose content did not have a major influence on the fermentation process. Xylose content of silages was similar ($P<0.01$) during both years. Over the ensiling period, xylose content increased ($P<0.01$) as length of the fermentation period increased, but a greater accumulation was observed during the last 40 d post-ensiling. This increase in xylose content may be attributed to acid hydrolysis of the hemicellulose fraction of the cell-wall. In this experiment, greater xylose content corresponded to the increase in fermentation end-products (e.g. acetic acid) throughout the fermentation period. Arabinose content was constant over time in 1993. In 1994, arabinose content remained constant during the initial 21 d of the

fermentation process, but increased ($P<0.01$) for the remainder of the ensiling period. Similar to xylose, this increase in arabinose content may result from acid hydrolysis of the hemicellulose fraction of the cell-wall. Neutral detergent fiber content tended to decrease ($P<0.06$) as the length of the ensiling period increased in 1994 (Table 2-7). In 1993, NDF content after 40 d post-ensiling was lower than the initial NDF, but similar after 100 d post-ensiling. Forage ensiled in 1993 had higher ($P<0.05$) ADF than sorghum ensiled in 1994. In both years, hemicellulose decrease ($P<0.05$) over time; which corresponded to an increase in pentose (e.g. xylose, arabinose) content. The amount of hemicellulose disappearance seems to be greater than can be accounted for by the levels of xylose and arabinose measured. Cellulose content was similar ($P<0.05$) during both years.

In both years, forage sorghum treated with silage additives had similar concentrations of organic acids as compared to untreated silage, and the silage additive by day of ensiling interaction for organic acids was not significant (data not showed). In this experiment, degradation of organic acids present in forage sorghum before ensiling were not degraded by either the silage enzyme or microbial inoculant treatments.

The interaction between year and silage additives for any of the ensiling characteristics evaluated in this experiment were non-significant; which indicates that the effect of silage additives was similar for both years. Forage sorghum treated with microbial inoculant alone or in combination

Table 2-7. Effects of year and day of ensiling on structural carbohydrate contents of forage sorghum ensiled in a temperate environment

Item, g /100 g DM	Day	Year		SEM[a]	Probability		
		1993	1994		Y[b]	D[c]	Y*D[d]
NDF	0	57.52[e]	59.88[e]	.475	.001	.060	.040
	40	56.17[f]	57.96[f]				
	100	56.76[f]	56.85[g]				
ADF	0	34.65	35.66	.570	.031	.081	.421
	40	33.92	35.69				
	100	34.92	35.19				
Hemicellulose	0	22.76[e]	24.21[e]	.430	.003	.151	.022
	40	22.24[ef]	22.27[f]				
	100	21.83[f]	21.66[g]				
Cellulose	0	29.51	28.14[G]	.471	.971	.001	.002
	40	29.53	30.83[E]				
	100	29.71	29.74[F]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year * day of ensiling
[EFG] Means with unlike superscripts in the same year and item heading differ (P<.01)
[efg] Means with unlike superscripts in the same year and item heading differ (P<.05)

with enzymes had lower ($P<0.01$) pH and higher ($P<0.01$) LAB populations than forage sorghum ensiled without microbial inoculant (Table 2-8). Lactic acid content also was increased ($P<0.01$) by inoculation when compared to control silage or silage treated only with enzymes. In this experiment, silage additives did not influence the proportion of other microbial groups or fermentation end-products. These results are in agreement with those of Bolsen et al. (1992), who reported that except for LAB, inoculation did not affect other microbial populations in alfalfa and whole-plant corn silages. Glucose content was lower ($P<0.01$), but fructose ($P<0.01$) and arabinose content were higher ($P<0.05$) in forage sorghum silage treated with the microbial inoculant. Galactose content tended ($P<.07$) to be lower in inoculated silage, but no effects of the silage additives on xylose concentrations were observed. Enzyme additive did not affect ($P<0.05$) ADF, hemicellulose, and cellulose content of forage sorghum silage. However, silages containing plant cell-wall degrading enzymes had lower ($P<0.01$) NDF content than forage sorghum ensiled without the enzyme additive.

There was a significant silage additive by day of ensiling interaction on pH and LAB population (Table 2-9). Ensiling of forage sorghum with inoculant or inoculant plus enzyme had lower ($P<0.01$) pH at 3, 7 and 14 d of fermentation, and greater ($P<0.01$) LAB population 7 d post-ensiling. Significant interactions between silage additive and day of ensiling on other

Table 2-8. **Effects of silage additives on the ensiling characteristics of forage sorghum ensiled in a temperate environment.**

Item	Silage Additive					
	No Additive	Enzyme	Inoculant	E + I	SEM[a]	Probability
pH	3.94[e]	3.95[e]	3.89[d]	3.88[d]	.007	.001
Microbial Group[b]						
Lactic acid bacteria	7.08[d]	7.02[d]	7.58[e]	7.42[e]	.043	.001
Enterobacteriaceae	3.35	3.35	3.36	3.58	.091	.185
Yeasts and molds	4.34	4.32	4.37	4.44	.070	.649
Lactate assimilating yeasts	3.03	3.10	2.90	3.03	.109	.620
Fermentation End-Products[c]						
Acetic acid	1.24	1.32	1.22	1.23	.033	.187
Lactic acid	6.29[d]	6.31[d]	7.07[e]	7.22[e]	.151	.001
Propionic acid	0.01	0.01	0.01	0.00	.001	.124
Butyric acid	0.01	0.01	0.01	0.01	.001	.492
Ethanol	0.77	0.80	0.84	0.79	.035	.463
Water Soluble Carbohydrates[c]						
Glucose	2.63[e]	2.61[e]	2.38[d]	2.35[d]	.061	.001
Fructose	1.41[d]	1.38[d]	2.15[e]	2.14[e]	.066	.001
Galactose	0.17[yz]	0.18[z]	0.15[y]	0.17[yz]	.008	.076
Xylose	0.19	0.20	0.19	0.21	.013	.888
Arabinose	0.09[D]	0.10[DE]	0.09[D]	0.11[E]	.004	.052
Structural Carbohydrates[c]						
NDF	58.83[e]	57.02[d]	57.35[de]	56.81[d]	.387	.002
ADF	32.90	34.36	34.79	34.96	.466	.135
Hemicellulose	22.92	22.66	22.56	21.85	.351	.178
Cellulose	30.16	29.74	28.89	29.51	.385	.142

[a] Standard error of the mean
[b] cfu/g of fresh material
[c] Expressed as a g/100 g of DM
[ed] Means in the same row with unlike superscripts differ (P<0.01)
[DE] Means in the same row with unlike superscripts differ (P<0.05)
[yz] Means in the same row with unlike superscripts differ (P<0.10)

Table 2-9. Effects of silage additives and day of ensiling on pH and microbial succession of forage sorghum ensiled in a temperate environment.

Item	Day	Silage Additive				SEM[a]	Probability		
		No Additive	Enzyme	Inoculant	E + I		A[b]	D[c]	A*D[d]
pH	0	5.31	5.26	5.30	5.28	.020	.001	.001	.001
	1	4.48	4.47	4.49	4.51				
	3	3.80[f]	3.83[f]	3.68[g]	3.70[g]				
	7	3.71[f]	3.70[f]	3.52[g]	3.50[g]				
	14	3.59[f]	3.60[f]	3.50[g]	3.45[g]				
	21	3.54	3.53	3.50	3.50				
	40	3.56	3.57	3.55	3.55				
	100	3.63	3.59	3.55	3.55				
Microbial Group[e]									
Lactic acid bacteria	0	3.41[g]	3.33[g]	5.22[f]	5.04[f]	.123	.001	.001	.001
	1	6.96[g]	7.25[g]	8.07[f]	8.09[f]				
	3	7.36[g]	7.66[g]	8.58[f]	8.60[f]				
	7	8.39[g]	8.41[g]	8.66[f]	8.67[f]				
	14	8.17	8.12	8.19	8.05				
	21	7.77	7.75	7.49	7.52				
	40	7.36	7.14	7.42	7.18				
	100	7.20	7.06	7.05	7.08				
Enterobacteriaceae	0	4.59	4.67	4.64	4.70	.257	.180	.001	.449
	1	5.70	5.43	5.68	5.66				
	3	4.21	4.05	3.63	3.91				
	7	2.40	2.93	2.66	2.95				
	14	2.65	2.72	2.30	2.96				
	21	2.14	2.19	2.75	3.19				
	40	2.58	2.06	2.50	2.72				
	100	2.47	2.64	2.75	2.55				
Yeasts and molds	0	3.75	4.31	4.06	4.29	.198	.649	.001	.629
	1	4.54	4.28	4.33	4.26				
	3	4.04	3.95	4.00	4.23				
	7	4.12	4.08	3.97	3.89				
	14	4.53	4.47	4.27	4.51				
	21	4.67	4.44	4.70	4.83				
	40	4.18	4.58	4.48	4.80				
	100	4.93	4.45	4.87	4.68				
Lactate assimilating yeasts	0	2.16	2.44	2.67	2.55	.309	.620	.001	.736
	1	3.06	2.90	2.92	3.18				
	3	2.92	2.69	2.57	2.92				
	7	3.03	2.71	2.86	2.77				
	14	2.65	2.65	2.50	2.16				
	21	2.91	3.48	2.26	2.89				
	40	3.49	4.27	3.88	4.15				
	100	4.02	3.70	3.56	3.59				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[e] cfu/g of fresh material
[fg] Means with unlike superscripts in the same row differ (P<.01)

microbial groups were not observed. Lactic acid content was the only fermentation end-product influenced by the silage additives during the course of ensiling (Table 2-10). Inoculation of forage sorghum resulted in higher ($P<0.05$) lactic acid concentrations after 1, 3, and 7 d post-ensiling, as compared to silage without microbial inoculant. This increase in lactic acid content corresponded with the greater LAB population and lower pH found in inoculated forage sorghum.

The effects of silage additives on glucose, galactose or xylose content (Table 2-11) were consistent at each ensiling period. Fructose content was higher ($P<0.01$) from d 1 until the end of fermentation in treatments containing bacterial inoculant as compared to forage ensiled without the microbial starter culture. It appears that the microbial culture added in this experiment had a glucose preference over fructose, as evidenced by the higher fructose and lower glucose content observed in inoculated silages. Arabinose was higher ($P<0.05$) after 14, 21, and 40 d post-ensiling in silage containing microbial inoculant plus enzymes as compared to silage without additive or silage treated only with enzymes or microbial inoculant. The sum of the 5 individual WSC in this experiment after 100 d post-ensiling, indicate that forage sorghum treated with bacterial inoculant (3.17 g/ 100 g DM) or inoculant plus enzymes (2.95 g/ 100 g DM) had greater residual WSC at the end of the fermentation process than control silage (2.33 g/100 g DM) or silage treated only with enzymes (2.35 g/ 100 g DM). In other experiments, corn silage treated with lactic acid bacterial inoculant

Table 2-10. **Effects of silage additive and day of ensiling on fermentation end-products of forage sorghum ensiled in a temperate environment**

Fermentation End-Product, g/100 g DM	Day	Silage Additive				SEM[a]	Probability		
		No additive	Enzyme	Inoculant	E+I		A[b]	D[c]	A*D[d]
Acetic acid	0	0.27	0.23	0.32	0.20	.094	.187	.001	.632
	1	0.94	0.89	0.98	0.90				
	3	1.06	1.13	1.02	1.08				
	7	1.28	1.31	1.24	1.23				
	14	1.44	1.61	1.55	1.58				
	21	1.61	1.66	1.44	1.62				
	40	1.75	1.73	1.52	1.49				
	100	1.52	1.91	1.69	1.73				
Lactic acid	0	0.67	0.70	0.46	0.51	.429	.001	.001	.048
	1	3.96[f]	3.99[f]	5.04[e]	5.41[e]				
	3	5.83[f]	5.38[f]	7.57[e]	6.70[e]				
	7	6.73[f]	6.79[f]	8.56[e]	9.65[e]				
	14	9.30	9.36	9.50	9.39				
	21	9.52	9.58	10.06	9.93				
	40	8.15	8.25	8.50	8.51				
	100	6.19	6.40	6.88	7.64				
Propionic acid	0	0.00	0.00	0.00	0.00	.001	.124	.001	.591
	1	0.00	0.00	0.00	0.00				
	3	0.01	0.01	0.01	0.01				
	7	0.01	0.00	0.00	0.01				
	14	0.01	0.00	0.00	0.01				
	21	0.01	0.01	0.01	0.01				
	40	0.01	0.01	0.01	0.01				
	100	0.01	0.01	0.01	0.01				
Butyric acid	0	0.00	0.00	0.00	0.00	0.09	.492	.001	.946
	1	0.00	0.01	0.00	0.00				
	3	0.01	0.01	0.01	0.01				
	7	0.01	0.01	0.01	0.01				
	14	0.01	0.01	0.01	0.01				
	21	0.01	0.01	0.01	0.01				
	40	0.01	0.01	0.01	0.01				
	100	0.01	0.01	0.01	0.01				
Ethanol	0	0.13	0.26	0.17	0.16	0.10	.463	.001	.924
	1	0.54	0.52	0.46	0.53				
	3	0.97	0.81	0.91	0.80				
	7	1.08	1.10	1.14	1.13				
	14	0.94	1.04	0.98	0.97				
	21	0.92	1.02	1.28	1.05				
	40	0.80	0.79	0.79	0.83				
	100	0.75	0.78	1.01	0.82				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[e,f] Means with unlike superscripts in the same row differ ($P<0.05$)

Table 2-11. Effects of silage additives and day of ensiling on WSC content of forage sorghum ensiled in a temperate environment

Carbohydrate, g/ 100 g DM	Day	Silage Additive				SEM[a]	Probability		
		No Additive	Enzyme	Inoculant	E+I		A[b]	D[c]	A*D[d]
Glucose	0	5.99	5.99	5.82	5.64	.173	.001	.001	.973
	1	3.47	3.38	3.44	3.43				
	3	3.23	3.24	2.74	2.84				
	7	2.14	2.14	1.94	1.77				
	14	2.01	2.03	1.66	1.60				
	21	1.79	1.56	1.20	1.23				
	40	1.40	1.44	1.19	1.23				
	100	1.01	1.06	1.04	1.04				
Fructose	0	5.63	5.52	5.57	5.53	.186	.001	.001	.007
	1	1.90[f]	1.84[f]	2.26[e]	2.24[e]				
	3	1.34[f]	1.25[f]	2.25[e]	2.05[e]				
	7	0.84[f]	0.84[f]	1.70[e]	1.85[e]				
	14	0.44[f]	0.56[f]	1.47[e]	1.58[e]				
	21	0.31[f]	0.34[f]	1.47[e]	1.47[e]				
	40	0.32[f]	0.25[f]	1.26[e]	1.25[e]				
	100	0.45[f]	0.42[f]	1.23[e]	1.12[e]				
Galactose	0	0.10	0.14	0.10	0.12	.023	.007	.001	.902
	1	0.14	0.17	0.16	0.14				
	3	0.21	0.25	0.22	0.25				
	7	0.21	0.20	0.18	0.21				
	14	0.17	0.21	0.15	0.17				
	21	0.22	0.20	0.17	0.16				
	40	0.15	0.18	0.14	0.18				
	100	0.15	0.15	0.14	0.14				
Xylose	0	0.07	0.06	0.07	0.05	0.39	.888	.001	.169
	1	0.08	0.07	0.08	0.07				
	3	0.09	0.12	0.11	0.11				
	7	0.12	0.12	0.12	0.12				
	14	0.13	0.11	0.13	0.13				
	21	0.13	0.14	0.13	0.33				
	40	0.33	0.32	0.25	0.31				
	100	0.60	0.61	0.62	0.51				
Arabinose	0	0.11	0.13	0.11	0.11	0.12	.052	.001	.021
	1	0.10	0.12	0.11	0.08				
	3	0.09	0.13	0.09	0.08				
	7	0.10[EF]	0.09[F]	0.10[EF]	0.12[E]				
	14	0.08[F]	0.07[F]	0.07[F]	0.13[E]				
	21	0.08[F]	0.07[F]	0.08[F]	0.11[E]				
	40	0.06[E]	0.09[EF]	0.08[F]	0.11[E]				
	100	0.12	0.11	0.12	0.14				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[ef] Means with unlike superscripts in the same row differ ($P<0.01$)
[EF] Means with unlike superscripts in the same row differ ($P<0.05$)

had higher WSC after 110 d post-ensiling than corn silage treated without microbial culture (Cleare et al., 1990). The higher residual WSC content from the current experiment and Cleare and co-workers may indicate a less extensive fermentation in the inoculated silage.

Neutral detergent fiber after 40 and 100 d post-ensiling tended to be lower ($P<0.09$) in forage sorghum containing enzymes than forage ensiled without additive or LAB inoculant (Table 2-12). The silage additive by day of ensiling interactions on other structural carbohydrates were not significant. In this experiment, enzyme mixtures decreased the NDF fraction after 100 d of fermentation as compared to silage without enzyme additive, but this did not have a signbificant effect on the fermentation process. Previous results with enzyme-treated forages have shown variable success on silage fermentation, but are difficult to compare because of the variability in enzymes complexes, different amounts of activity, and interactions with plant maturity and plant species.

Tropical Silage

The organic acid contents of fresh and ensiled forage were different ($P<0.01$) across years (Table 2-13). Malic and oxaloacetic acids acids were greater in 1994 than in 1993. Additionally, the concentration of these acids decreased over the ensiling period ($P<0.01$). For all ensiling periods, citric acid tended ($P<0.08$) to be higher in 1993 than in 1994, but succinic acid content was lower ($P<0.01$). For all ensiling periods, citric acid tended

Table 2-12. **Effects of silage additives and day of ensiling on structural carbohydrate contents of forage sorghum ensiled in a temperate environment.**

Item, g/100 g DM	Day	Additive				SEM[a]	Probability		
		No additive	Enzyme	Inoculant	E+I		A[b]	D[c]	A*D[d]
NDF	0	59.17[e]	58.95[ef]	56.97[g]	57.50[fg]	.671	.002	.060	.090
	40	58.13[e]	55.72[f]	58.00[e]	56.41[f]				
	100	59.18[e]	56.40[f]	57.00[f]	56.53[f]				
ADF	0	36.05	35.77	33.67	35.12	.807	.135	.819	.243
	40	35.76	33.13	35.22	35.10				
	100	35.89	34.18	35.48	34.66				
Hemicellulose	0	23.11	23.17	23.29	22.37	.608	.178	.152	.647
	40	22.36	22.58	22.77	21.30				
	100	23.29	22.22	21.62	21.87				
Cellulose	0	28.93	29.53	27.22	29.64	.667	.142	.019	.150
	40	30.52	29.86	30.14	30.22				
	100	31.05	29.85	29.32	28.68				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[efg] Means with inlike superscripts in the same row differ ($P<0.10$)

Table 2-13. Effects of year and day of ensiling on organic acid contents of forage sorghum ensiled in a tropical environment

Organic acid , g/ 100 g DM	Day	Year		SEM[a]	Probability		
		1993	1994		Y[b]	D[c]	Y*D[d]
Citric	0	0.64[E]	0.70[E]	.059	.001	.001	.084
	1	0.60[E]	0.44[F]				
	3	0.47[F]	0.40[F]				
	7	0.53[F]	0.46[F]				
	14	0.39[G]	0.42[F]				
	21	0.53[F]	0.31[G]				
	40	0.51[F]	0.33[G]				
	100	0.35[G]	0.16[H]				
Malic	0	4.49	4.62	.173	.001	.001	.694
	1	2.75	2.74				
	3	2.30	2.78				
	7	2.34	2.78				
	14	2.36	2.80				
	21	1.93	2.50				
	40	1.84	2.28				
	100	1.40	1.61				
Oxalacetic	0	0.84	1.22	.085	.001	.001	.177
	1	0.63	1.29				
	3	0.55	1.25				
	7	0.62	1.21				
	14	0.71	1.04				
	21	0.74	1.11				
	40	0.45	1.08				
	100	0.32	0.78				
Succinic	0	1.74[e]	3.32[e]	.159	.001	.001	.001
	1	0.99[g]	3.09[e]				
	3	1.17[fg]	2.63[f]				
	7	1.43[f]	2.45[f]				
	14	1.60[e]	2.60[f]				
	21	1.39[fg]	1.92[g]				
	40	1.13[fg]	1.80[g]				
	100	0.94[h]	1.31[h]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year by day of ensiling
[ef] Means with unlike superscripts in the same year and organic acid heading differ ($P<0.01$)
[EFG] Means with unlike superscripts in the same year and organic acid heading differ ($P<0.10$)

($P<0.08$) to be higher in 1993 than in 1994, but succinic acid content was lower ($P<0.05$). Similar to forage sorghum ensiled in a temperate environment, all organic acids were degraded in the tropical silage. Malic and succinic acids were the major organic acids found in the fresh and ensiled forage sorghum, and their concentration decreased more during the ensiling period than citric and oxaloacetic acids.

Acidity as measured by pH, of the tropical silage was influenced similarly by length of ensiling across years (Table 2-14). Over time, pH of forage sorghum silage was down-trending throughout the period of ensiling; but was variable, and reached the 4.2 value after 21 d of fermentation. In this experiment, higher and more variable pH values were found in forage sorghum ensiled in the tropical environment in comparison to the temperate location. This observation suggests that microbial ecology was changing and different substrates were being used, that would imply a very heterogenous microbial population existed in the tropical silage. Differences in pH between locations may be also associated with the higher buffering capacity of the vegetative material prior to ensiling in the tropical environment, and the higher organic acid contents throughout the fermentation process (McDonald et al., 1991).

Microbial populations were different across years as evidenced by the significant year by day of ensiling interaction. Lactic acid bacterial population was higher ($P<0.01$) the initial 7 d post-ensiling in 1994 than in 1993, but was lower ($P<0.01$) during the rest of the fermentation process.

Table 2-14. Effects of year and day of ensiling on pH and microbial succession of forage sorghum ensiled in a tropical environment

Item	Day	Year		SEM[a]	Probability		
		1993	1994		Y[b]	D[c]	Y*D[d]
pH	0	5.56	5.61	.043	.163	.001	.760
	1	4.74	4.73				
	3	4.56	4.50				
	7	4.55	4.48				
	14	4.34	4.34				
	21	4.51	4.42				
	40	4.11	4.07				
	100	4.14	4.12				
Microbial Group[e]							
Lactic acid bacteria	0	4.83^{j}	4.21^{k}	.115	.001	.001	.001
	1	5.96^{i}	8.45^{f}				
	3	7.70^{f}	8.62^{f}				
	7	7.65^{f}	7.87^{g}				
	14	7.09^{g}	7.05^{h}				
	21	6.86^{gh}	6.60^{i}				
	40	6.80^{gh}	6.45^{i}				
	100	6.73^{h}	5.79^{j}				
Enterobacteriaceae	0	7.01^{f}	5.47^{i}	.255	.001	.001	.001
	1	6.96^{f}	7.96^{f}				
	3	5.26^{g}	6.34^{g}				
	7	3.43^{kl}	5.98^{h}				
	14	3.62^{k}	3.61^{k}				
	21	2.96^{l}	4.38^{j}				
	40	3.24^{l}	3.49^{k}				
	100	2.24^{l}	3.87^{k}				
Yeasts and molds	0	6.11^{g}	3.14^{k}	.149	.001	.001	.004
	1	6.30^{g}	5.54^{i}				
	3	6.34^{g}	6.88^{g}				
	7	6.89^{f}	7.47^{f}				
	14	6.53^{f}	6.21^{h}				
	21	6.75^{f}	5.97^{h}				
	40	6.82^{f}	5.33^{i}				
	100	6.83^{f}	4.87^{j}				
Lactate assimilating yeast	0	2.55^{l}	2.95^{k}	.173	001	.001	.001
	1	3.99^{h}	5.03^{i}				
	3	4.72^{g}	6.24^{g}				
	7	6.04^{f}	7.09^{f}				
	14	5.94^{f}	6.20^{g}				
	21	6.08^{f}	5.82^{h}				
	40	5.78^{f}	5.11^{i}				
	100	6.10^{f}	4.59^{j}				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year by day of ensiling
[e] cfu/g of fresh material
[fghijkl] Means with unlike superscripts in the same year and item heading differ ($P<0.01$)

In this experiment, a greater lactic acid bacterial population found in 1994 during the first week post-ensiling did not result in lower pH values; which may indicate differences in counts or activity of other microbial groups or fermentation end-product between years. Additionally, it may suggest that what happens early in the fermentation period is of less importance than is currently thought. In 1993, enterobacteriaceae population decreased ($P<0.01$) as length of ensiling period increased, whereas, in 1994 the population increased by day 1 before showing the declining trend. Yeast and mold populations were higher ($P<0.01$) after 7 d post-ensiling in both years, but the changes were much more dramatic in 1994.

Low WSC content of tropical grasses and low homofermentative LAB population during the fermentation process have been described as the major problems associated with the ensiling of tropical forages (McDonald et al., 1991; Van Soest, 1994). However, in this experiment, LAB populations in the silage were similar between the two environments. The enterobacteriaceae, yeasts and molds, and lactate assimilating yeasts observed in forage sorghum ensiled in a tropical environment were markedly greater than microbial counts typically found in grasses ensiled in temperate environments. The higher counts of these microorganisms, described as undesirable for the fermentation process, appear to be the problem in achieving a satifactory fermentation in a tropical environments.

Similar to forage sorghum ensiled in the temperate environment, acetic acid content increased with length of the ensiling period (Table 2-15). A greater

and more variable acetic acid production was found in forage sorghum ensiled in 1994 than in 1993. This change in acetic acid content in forage ensiled in 1994, may correspond to the higher enterobacteriaceae and more yeasts and molds and lactate assimilating yeast populations found during the fermentation process. Since enterobacteriaceae population decreased after 21 d post-ensiling, it would seem that other acetic acid producing microorganisms (e.g. heterofermentative lactic acid bacteria, yeasts and molds, lactate assimilating yeast) are responsible for acetic acid production at later stages of the fermentation process. Lactic acid was the major fermentation end-product associated with the ensiling of forage sorghum in the tropical environment. A greater lactic acid production after 3 d post-ensiling was found in forage sorghum ensiled in 1994 as compared to sorghum ensiled in 1993. This higher lactic acid may indicate a more active LAB population in forage sorghum ensiled in 1994. In both years, lactic acid content increased after d 14 post-ensiling; which corresponds to changes in pH and lactic acid bacterial populations. After 14 d post-ensiling, lactic acid content decreased ($P < 0.01$) over time. It would appear from this observation that lactic acid is being used as a substrate by the microbial ecosystem late in the ensiling period.

In this experiment, forage sorghum ensiled in a tropical environment for 100 d had low acetic acid content. Levels of acetic acid indicated that few acetic acid-producing microorganisms were present in the silage microflora, that microorganisms responsible for acetic acid production had very low

Table 2-15. Effects of year and day of ensiling on fermentation end-products of forage sorghum ensiled in a tropical environment.

Fermentation End-Product , g/100 g DM	Day	Year		SEM[a]	Probability		
		1993	1994		Y[b]	D[c]	Y*D[d]
Acetic acid	0	0.04[h]	0.08[h]	.041	.001	.001	.001
	1	0.21[g]	0.25[g]				
	3	0.23[g]	0.71[e]				
	7	0.30[f]	0.74[e]				
	14	0.33[f]	0.80[e]				
	21	0.38[f]	0.69[f]				
	40	0.37[f]	0.67[f]				
	100	0.51[e]	0.72[e]				
Lactic acid	0	0.19[h]	0.10[i]	.223	.001	.001	.001
	1	1.54[g]	1.14[i]				
	3	2.19[e]	7.24[e]				
	7	3.17[e]	7.23[e]				
	14	3.55[e]	7.53[e]				
	21	2.48[f]	6.05[f]				
	40	1.81[g]	4.59[g]				
	100	1.51[g]	3.20[h]				
Propionic acid	0	0.00[g]	0.00[g]	.010	.001	.001	.007
	1	0.00[g]	0.00[g]				
	3	0.00[g]	0.00[g]				
	7	0.00[g]	0.00[g]				
	14	0.09[e]	0.04[ef]				
	21	0.07[e]	0.04[ef]				
	40	0.04[f]	0.05[e]				
	100	0.04[f]	0.02[fg]				
Butyric acid	0	0.00	0.00	.006	.899	.001	.150
	1	0.03	0.04				
	3	0.05	0.06				
	7	0.06	0.04				
	14	0.05	0.05				
	21	0.06	0.06				
	40	0.04	0.04				
	100	0.04	0.05				
Ethanol	0	0.02[j]	0.08[j]	.058	.198	.001	.001
	1	0.12[i]	0.48[h]				
	3	0.29[h]	0.65[g]				
	7	0.63[f]	0.81[f]				
	14	1.11[e]	1.10[e]				
	21	0.60[f]	0.56[g]				
	40	0.55[g]	0.40[h]				
	100	0.71[f]	0.25[i]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year by day of ensiling
[efghij] Means with unlike superscripts in the same year and fermentation end-product heading differ ($P < 0.01$)

metabolic activity, or that other microorganisms associated with the ensiling process are capable of utilizing acetic acid as a substrate. In similar experiments performed in the tropical environment of Hawaii, (Kung Jr. et al., 1982) lactic acid was also the major fermentation end-product of whole plant sugarcane (*Saccharum officinarum*) ensiled at 5 different stages of maturity for 60 d. However, results from the current experiment and the Kung Jr. study are in disagreement with other studies (Catchpoole and Willians, 1969; Catchpoole and Henzel, 1970; Panditharatne et al., 1986) which indicated that acetic acid is the major fermentation end-product associated with the ensiling of tropical grasses. The high acetate silages described in their experiments were utilizing legumes (e.i. *Phaseolus atropurpureus, Desmodium intortum, Lotononis bainessi, Setaria sphacelata*, or *Chloris gayana*) or grass species (*Panicum maximum*) with different buffering capacity and water soluble carbohydrate contents than the forage sorghum evaluated in this experiment or the sugarcane bagasse. Longer ensiling periods (200 d) were also utilized in those studies as compared to the length of ensiling utilized in the current experiment (100 d). It has been shown that increasing the length of fermentation in grass silage decreases the homofermentative LAB population and lactic acid content, and increases the pH, heterofermentative LAB and acetic acid content (Tjandraatmadja et al., 1991).

Propionic and butyric acids were also detected in both years. Butyric and propionic acid contents in the tropical silage were greater than

concentrations observed in the temperate environment. However, concentrations of these two acids were not sufficiently high to play a major role in the fermentation process.

In both years, ethanol production increased during the initial 14 d post-ensiling, being slightly higher ($P < 0.01$) in forage sorghum ensiled in 1994 than in 1993. After 14 d post-ensiling, ethanol production decreased as length of fermentation increased in 1994, but this general trend was not observed in 1993. These differences in ethanol content between years may be attributed to different metabolic activity of microorganisms associated with ethanol production. As observed with silages prepared from forages grown in a temperate climate, glucose and fructose were also the major WSC associated with the fermentation process of forage sorghum ensiled in the tropical climate (Table 2-16). In both years, glucose and fructose decreased over the entire ensiling period, with the greatest decrease occuring within the first 7 d. The greater disappearance of glucose and fructose the first week post-ensiling corresponds to the maximum LAB counts observed in this experiment. However, this decline in sugar content also corresponds to the decrease in the enterobacteriaceae population the first week of ensiling. Enterobacteriaceae and LAB must compete for the available substrates for growth. The high levels of acetic acid would suggest the fermentation was not homofermentative and that acetic acid producing bacteria utilized some of the substrate. Galactose content was greater ($P<0.01$) in 1994 than 1993, but a similar pattern across years

Table 2-16. Effects of year and day of ensiling on water soluble carbohydrate contents of forage sorghum ensiled in a tropical environment

		Year			Probability		
Carbohydrate, g/ 100 g DM	Day	1993	1994	SEM[a]	Y[b]	D[c]	Y*D[d]
Glucose	0	3.28[f]	2.93[e]	.034	.242	.001	.001
	1	1.12[f]	0.64[f]				
	3	0.51[g]	0.51[g]				
	7	0.41[h]	0.44[h]				
	14	0.14[i]	0.47[h]				
	21	0.12[i]	0.36[i]				
	40	0.08[jk]	0.30[j]				
	100	0.04[k]	0.23[j]				
Fructose	0	2.63[e]	3.25[e]	.046	.001	.001	.001
	1	0.54[f]	0.49[f]				
	3	0.34[g]	0.36[g]				
	7	0.22[h]	0.37[g]				
	14	0.23[h]	0.41[gf]				
	21	0.17[h]	0.30[gh]				
	40	0.14[h]	0.22[h]				
	100	0.06[i]	0.13[i]				
Galactose	0	0.05	0.20	.068	.001	.001	.151
	1	0.04	0.10				
	3	0.04	0.08				
	7	0.12	0.23				
	14	0.13	0.54				
	21	0.11	0.18				
	40	0.06	0.17				
	100	0.05	0.14				
Xylose	0	0.37[e]	0.67[ef]	.043	.001	.001	.001
	1	0.33[e]	0.69[ef]				
	3	0.32[e]	0.57[gh]				
	7	0.12[f]	0.65[fg]				
	14	0.15[f]	0.75[e]				
	21	0.13[f]	0.55[h]				
	40	0.11[f]	0.19[j]				
	100	0.07[f]	0.19[j]				
Arabinose	0	0.06	0.09	.012	.001	.006	.243
	1	0.07	0.08				
	3	0.07	0.07				
	7	0.06	0.08				
	14	0.07	0.13				
	21	0.07	0.10				
	40	0.05	0.10				
	100	0.11	0.12				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year by day of ensiling
[efghijk] Means with unlike superscripts in the same year and carbohydrate heading differ ($P<0.01$)

was observed. A small increase in galactose content was observed after 14 - 21 d of fermentation, however, the changes in galactose content did not correspond with any major changes in microbial population or other fermentation end-products. In contrast to forage sorghum ensiled in the temperate environment, xylose content after 100 d post-ensiling was lower ($P<0.01$) than xylose content in vegetative material prior to ensiling. Arabinose content was higher ($P<0.01$) after 100 d post-ensiling than initial arabinose concentration; which may result from cell-wall hydrolysis during the last third of the fermentation process. Neutral detergent fiber levels were reduced ($P<0.01$) as a result of fermentation (Table 2-17). Though more variable, ADF and cellulose contents increased as length of ensiling increased ($P<0.01$). This may result from a concentration of these constitutents which are more resistant to degradation. Hemicellulose decreased ($P<0.05$) as length of the ensiling period increased. This decrease in hemicellulose content did not correspond to an increase in xylose, but may explain the increase in arabinose content. There are two possible explanations for the differences observed in 4 or 5 carbon sugar appearance and hemicellulose disappearance . Firstly, it may be that forages grown in a tropical environment possess less xylose and more arabinose sugars. Another possibility may involve the rate of release versus metabolism by microorganisms. Perhaps the rate of xylose released from the cell wall is slower than its rate of utilization by the silage microflora, but the rate of arabinose hydrolysis is higher than its rate of utilization.

Table 2-17. Effects of year and day of ensiling on stuctural carbohydrate contenst of forage sorghum ensiled in a tropical environment.

Item , g/100g of DM	Day	Year		SEM[a]	Probability		
		1993	1994		Y[b]	D[c]	Y*D[d]
NDF	0	65.85	66.35	.341	.439	.001	.415
	40	63.24	62.93				
	100	63.28	63.75				
ADF	0	37.23[f]	39.70[e]	.383	.001	.001	.005
	40	37.86[f]	35.77[f]				
	100	39.23[e]	40.02[e]				
Hemicellulose	0	28.84[e]	26.43[e]	.255	.001	.001	.001
	40	25.82[f]	25.52[f]				
	100	24.41[g]	24.43[g]				
Cellulose	0	30.11[g]	32.50[e]	.471	.029	.001	.008
	40	31.51[f]	31.44[f]				
	100	32.93[e]	32.96[e]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of day of ensiling
[d] Interaction of year by day of ensiling
[efg] Means with unlike superscripts in the same year and item heading differ ($P < 0.01$)

The effects of additives were similar during both years. Similar to forage sorghum ensiled in the temperate environment, in this study silage additives did not change the plant organic acid profiles of forage sorghum ensiled in a tropical environment. Silage treated with microbial inoculant alone or in combination with enzyme had lower ($P<0.01$) pH, and greater ($P<0.01$) lactic acid bacterial population and lactic acid content than control silage or silage treated only with enzymes (Table 2-18). Similar to the results observed in the temperate environment, silage additives did not permanently influence other microbial groups or fermentation end-products in the tropical silage. Inoculated sorghum silage also had lower ($P<0.01$) glucose than forage sorghum ensiled without microbial inoculant, but higher ($P<0.01$) fructose concentration. Arabinose content in forage sorghum treated with microbial inoculant plus enzymes was higher ($P<0.01$) than control silages, but similar to forage sorghum treated with either the enzyme or microbial culture only.

There was no significant silage additive by day of ensiling interaction on pH (Table 2-19), but pH values were numerically lower most of the ensiling periods in treatments containing microbial inoculant. Forage sorghum that was inoculated before ensiling had a greater ($P<0.01$) LAB population after 1 d post-ensiling. Likewise inoculated silage tended ($P<0.07$) to have lower enterobacteriaceae and yeast and mold ($P<0.06$) populations after 3 and 7 d of fermentation, respectively, as compared to silage without a microbial inoculant. The lower pH found in silage treated with inoculant may explain

Table 2-18. Effects of silage additives on the ensiling characteristics of forage sorghum ensiled in a tropical environment.

Item	Silage Additive				SEM[a]	Probability
	No Additive	Enzyme	Inoculant	E + I		
pH	4.67[d]	4.66[d]	4.44[e]	4.42[e]	.021	.001
Microbial Group[b]						
Lactic acid bacteria	6.56[e]	6.65[e]	7.00[d]	6.95[d]	.057	.001
Enterobacteriaceae	4.83	4.75	4.71	4.66	.127	.819
Yeasts and molds	6.21	6.16	6.15	5.98	.074	.146
Lactate assimilating yeasts	5.29	5.30	5.31	5.17	.086	.625
Fermentation End-Products[c]						
Acetic acid	0.44	0.47	0.41	0.45	.020	.189
Lactic acid	3.00[e]	3.01[e]	3.69[d]	3.70[d]	.111	.001
Propionic acid	0.03	0.02	0.02	0.02	.005	.659
Butyric acid	0.04	0.05	0.05	0.04	.003	.957
Ethanol	0.51	0.53	0.52	0.54	.029	.965
Water Soluble Carbohydrates[c]						
Glucose	0.75[d]	0.76[d]	0.70[de]	0.68[e]	.017	.002
Fructose	0.56[e]	0.58[de]	0.66[d]	0.67[d]	.066	.002
Galactose	0.13	0.20	0.10	0.12	.034	.186
Xylose	0.38	0.39	0.33	0.37	.021	.210
Arabinose	0.07[e]	0.08[de]	0.07[e]	0.10[d]	.004	.005
Structural Carbohydrates[c]						
NDF	64.66	63.70	64.34	64.23	.279	.116
ADF	39.27	38.41	38.24	38.61	.313	.115
Hemicellulose	26.06	25.75	26.21	25.61	.208	.174
Cellulose	32.45	31.97	31.24	31.98	.348	.119

[a] Standard error of the mean
[b] cfu/g of fresh material
[c] Expressed as g/ 100 g of DM
[de] Means with unlike superscripts in the same row differ ($P<0.01$)

Table 2-19. Effects of silage additives and day of ensiling on pH and microbial succession of forage sorghum ensiled in a tropical environment

Item	Day	Silage Additive				SEM[a]	Probability		
		No Additive	Enzyme	Inoculant	E + I		A[b]	D[c]	A*D[d]
pH	0	5.73	5.65	5.42	5.54	.062	.001	.001	173
	1	4.93	4.80	4.59	4.63				
	3	4.69	4.78	4.37	4.30				
	7	4.72	4.64	4.37	4.34				
	14	4.42	4.43	4.26	4.24				
	21	4.54	4.63	4.35	4.33				
	40	4.18	4.17	4.07	3.95				
	100	4.16	4.19	4.13	4.04				
Microbial Group[e]									
Lactic acid bacteria	0	3.59[g]	3.81[g]	5.40[f]	5.29[f]	.163	.001	.001	.001
	1	6.65[g]	6.84[g]	7.72[f]	7.61[f]				
	3	7.80[g]	7.91[g]	8.26[f]	8.65[f]				
	7	7.66	7.88	7.96	7.84[f]				
	14	7.10	7.27	6.96	6.96				
	21	6.84	6.61	6.88	6.60				
	40	6.88	6.63	6.54	6.46				
	100	6.30	6.28	6.28	6.20				
Enterobacteriaceae	0	6.02	6.19	6.29	6.46	.361	.819	.001	.073
	1	7.70	7.78	7.48	6.88				
	3	6.05	5.83	5.72	5.60				
	7	5.78[h]	4.89[h]	3.85[i]	4.09[i]				
	14	3.36	3.97	3.94	3.19				
	21	3.63	3.73	3.50	3.82				
	40	3.00	2.88	3.96	3.62				
	100	3.09	2.74	2.94	3.44				
Yeasts and molds	0	4.74	4.60	4.88	4.28	.211	.146	.001	.060
	1	6.40[h]	6.14[h]	5.60[i]	5.55[i]				
	3	6.96	6.80	6.20	6.48				
	7	6.97	7.47	7.05	7.22				
	14	6.33	6.06	6.71	6.37				
	21	6.41	6.36	6.22	6.44				
	40	6.04	6.07	6.36	5.82				
	100	5.80	5.77	6.18	5.65				
Lactate assimilating yeasts	0	2.72	2.93	2.62	2.75	.244	.625	.001	.633
	1	4.48	4.56	4.59	4.42				
	3	5.85	5.59	5.40	5.09				
	7	6.28	6.60	6.58	6.79				
	14	6.06	5.76	6.37	6.09				
	21	6.29	6.09	6.05	5.38				
	40	5.47	5.43	5.48	5.42				
	100	5.20	5.43	5.36	5.40				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[e] cfu/g of fresh material
[fg] Means with unlike superscripts in the same row differ (P<.01)
[hi] Means with unlike superscripts in the same row differ (P<.10)

the lower enterobacteriaceae and yeast and mold populations early in the fermentation. However at d 100 post-ensiling, all silages had similar amount of enterobacteriaceae and yeast and mold populations.

Over the ensiling period, lactic acid was the only fermentation end-product influenced by silage additives (Table 2-20). Silages containing microbial inoculant had greater ($P<0.05$) lactic acid content after 3, 7 and 14 d post-ensiling as compared to forage sorghum ensiled without additives or treated only with enzymes. In contrast to sorghum ensiled in the temperate environment, the increase in lactic acid content over time due to inoculation did not correspond to periods of increased LAB counts.

Glucose was the only WSC influenced over time by silage additives (Table 2-21). Inoculated silage had lower glucose ($P<0.01$) content after 3, 7, and 14 d post-ensiling as compared to silage treated without microbial inoculant. These time periods with low glucose concentrations corresponded with the maximum lactic acid production in inoculated silages. The other sugars declined linearly ($P<0.01$) over time for all treatments. However, silage treated with microbial inoculant had greater ($P<0.01$) fructose content than treatments without the microbial culture. Neither silage additive affected any of the structural carbohydrate fractions in forage sorghum silage (Table 2-22).

The microbial inoculant utilized in this experiment, decreased the pH and glucose content, but increased lactic acid bacterial population, lactic acid content and fructose in forage sorghum, regardless of environment. The

Table 2-20. Effects of silage additives and day of ensiling on fermentation end-products of forage sorghum ensiled in a tropical environment

Fermentatin End-Product , g/ 100g DM	Day	Silage Additive				SEM[a]	Probability		
		No Additive	Enzyme	Inoculant	E+I		A[b]	D[c]	A*D[d]
Acetic acid	0	0.05	0.05	0.07	0.06	.057	.189	.001	.609
	1	0.20	0.24	0.25	0.23				
	3	0.47	0.55	0.43	0.43				
	7	0.54	0.60	0.52	0.44				
	14	0.59	0.60	0.53	0.54				
	21	0.55	0.57	0.48	0.54				
	40	0.47	0.51	0.42	0.69				
	100	0.63	0.66	0.55	0.62				
Lactic acid	0	0.12	0.13	0.18	0.14	.315	.001	.001	.024
	1	1.37	0.99	1.69	1.39				
	3	3.49[f]	3.87[f]	5.68[e]	5.80[e]				
	7	4.92[f]	4.69[f]	5.60[e]	5.60[e]				
	14	4.82[f]	5.07[f]	5.95[e]	6.38[e]				
	21	4.26	4.08	4.39	4.32				
	40	3.01	3.32	3.30	3.19				
	100	1.98	1.93	2.73	2.78				
Propionic acid	0	0.00	0.00	0.00	0.00	.014	.659	.001	.696
	1	0.00	0.00	0.00	0.00				
	3	0.00	0.00	0.00	0.00				
	7	0.00	0.00	0.00	0.00				
	14	0.11	0.06	0.05	0.05				
	21	0.05	0.05	0.04	0.03				
	40	0.03	0.03	0.06	0.05				
	100	0.04	0.04	0.03	0.03				
Butyiic acid	0	0.00	0.00	0.00	0.00	.010	.957	.001	.894
	1	0.03	0.04	0.04	0.05				
	3	0.05	0.06	0.05	0.04				
	7	0.06	0.04	0.07	0.06				
	14	0.05	0.04	0.06	0.06				
	21	0.06	0.06	0.05	0.07				
	40	0.04	0.05	0.04	0.04				
	100	0.04	0.05	0.03	0.03				
Ethanol	0	0.05	0.04	0.06	0.05	.083	.965	.001	.873
	1	0.30	0.34	0.28	0.30				
	3	0.40	0.47	0.47	0.55				
	7	0.67	0.80	0.73	0.70				
	14	1.16	0.92	1.16	1.19				
	21	0.66	0.63	0.50	0.55				
	40	0.47	0.53	0.49	0.45				
	100	0.40	0.50	0.53	0.49				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[ef] Means with unlike superscripts in the same row differ ($P<0.05$)

Table 2-21. Effects of silage additives and day of ensiling on WSC content of forage sorghum ensiled in a tropical environment

Carbohydrate , g/ 100 g DM	Day	Silage Additive				SEM[a]	Probability		
		No Additive	Enzyme	Inoculant	E+I		A[b]	D[c]	A*D[d]
Glucose	0	3.01	3.05	3.14	3.22	.049	.002	.001	.011
	1	0.84	0.89	0.90	0.89				
	3	0.61[e]	0.62[e]	0.41[f]	0.43[f]				
	7	0.54[e]	0.47[e]	0.38[f]	0.31[f]				
	14	0.34[e]	0.40[e]	0.27[f]	0.22[f]				
	21	0.29	0.28	0.21	0.19				
	40	0.23	0.24	0.19	0.12				
	100	0.16	0.17	0.13	0.10				
Fructose	0	3.05	2.91	2.89	2.93	.066	.002	.001	.745
	1	0.44	0.48	0.63	0.57				
	3	0.23	0.32	0.44	0.42				
	7	0.22	0.25	0.40	0.33				
	14	0.23	0.33	0.30	0.43				
	21	0.16	0.19	0.28	0.31				
	40	0.14	0.14	0.22	0.21				
	100	0.04	0.09	0.15	0.11				
Galactose	0	0.16	0.12	0.13	0.10	.096	.186	.003	.567
	1	0.08	0.10	0.04	0.06				
	3	0.04	0.06	0.07	0.08				
	7	0.29	0.18	0.15	0.09				
	14	0.16	0.69	0.16	0.32				
	21	0.15	0.17	0.09	0.15				
	40	0.12	0.16	0.08	0.10				
	100	0.09	0.11	0.06	0.10				
Xylose	0	0.53	0.56	0.52	0.50	.061	.210	.001	.608
	1	0.51	0.52	0.52	0.49				
	3	0.38	0.51	0.41	0.51				
	7	0.42	0.40	0.42	0.31				
	14	0.50	0.49	0.27	0.56				
	21	0.41	0.32	0.26	0.37				
	40	0.20	0.16	0.10	0.13				
	100	0.11	0.13	0.13	0.14				
Arabinose	0	0.06	0.07	0.09	0.09	.016	.005	.006	.459
	1	0.10	0.05	0.07	0.08				
	3	0.05	0.07	0.05	0.11				
	7	0.08	0.06	0.07	0.09				
	14	0.07	0.10	0.09	0.13				
	21	0.08	0.08	0.07	0.11				
	40	0.06	0.10	0.05	0.09				
	100	0.12	0.10	0.11	0.12				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[e,f] Means with unlike superscripts in the same row differ ($P<0.01$)

Table 2-22. **Effects of silage additives and day of ensiling on structural carbohydrate contents of forage sorghum ensiled in a tropical environment.**

Item, g/ 100 g DM	Day	Silage Additive				SEM[a]	Probability		
		No Additive	Enzyme	Inoculant	E+I		A[b]	D[c]	A*D[d]
NDF	0	66.17	65.65	65.81	66.79	.426	.116	.001	.343
	40	63.97	62.64	63.01	62.70				
	100	63.85	62.80	64.19	63.21				
ADF	0	38.61	37.97	37.73	39.54	.543	.115	.001	.115
	40	38.27	37.98	37.38	37.64				
	100	40.94	39.29	39.60	38.67				
Hemicellulose	0	27.79	27.43	28.07	27.25	.361	.174	.001	.423
	40	26.31	25.51	25.79	25.06				
	100	24.10	24.30	24.76	24.54				
Cellulose	0	31.57	31.30	30.12	32.23	.603	.119	.001	.505
	40	31.82	31.66	31.27	31.16				
	100	33.96	32.94	32.34	32.54				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling

lactic acid bacterial inoculant seemed to have a greater domination of the fermentation in forage sorghum ensiled in the temperate environment. The LAB inoculant applied in this study consisted of a strain of *Lactobacillus plantarum* which is a persistent species found in temperate silage, and was applied at 10^6 cfu/g of fresh material, the optimum application rate in temperate environments. Previous experiments also showed that *Lactobacillus plantarum* was the predominant lactic acid bacterial strain in forages (*Sorghum bicolor cv. Sugardrip, Panicum maximun, Digitaria decumbens, Setaria sphalaceta*) ensiled in tropical environments (Tjandraatmadja et al., 1990). However, the different chemical composition of the forage, greater amount of undesirable microorganisms present in the chopped forage prior to ensiling and during the ensiling period, or an inability for the added organisms to compete at the ambient temperature found in a tropical environment, may have affected the growth rate and metabolic activity of the microbial culture.

Similar to forage sorghum ensiled in the temperate climate, the enzyme additive did not improve the ensiling characteristics of forage sorghum ensiled in a tropical environment.

Implications

Addition of the microbial inoculant as a silage additive was effective in decreasing the pH, and increasing the lactic acid bacterial population, and lactic acid content at early stages of the fermentation process in forage

sorghum ensiled in temperate and tropical environments. Forage sorghum inoculated in the temperate area had a greater fructose content after 100 d post-ensiling in comparison with silages without the microbial inoculant. However, in the tropical environment fructose levels were not elevated 100 d post-ensiling in the inoculated treatments. More studies need to be conducted to evaluate other bacterial inoculants or specific strains of LAB that may be more suitable for use in tropical forages. Evaluation of preparations containing different enzyme (s), enzymatic activity or application rates should also be conducted.

CHAPTER 3

A TWO YEAR STUDY ON THE EFFICACY OF SILAGE ADDITIVES TO ENHANCE THE ENSILING OF FORAGE SORGHUM IN TEMPERATE AND TROPICAL ENVIRONMENTS. II. AEROBIC STABILITY

Abstract

An experiment was conducted to evaluate the effects of a commercial enzyme preparation and a microbial inoculant on the aerobic stability of forage sorghum ensiled in temperate and tropical environments. Forage sorghum was harvested in 1993 and 1994 after 90 d of growth at Michigan State University, East Lansing, and at the Lajas Agricultural Experiment Station, University of Puerto Rico, Mayaguez. In both environments, chopped forage was placed into PVC laboratory silos for 40 and 100 d, and was assigned to one of four treatments; no additive (control), enzymes (.1% of fresh material), microbial inoculant (10^6 cfu/g of fresh material), and enzymes plus microbial inoculant. Duplicate silo were prepared in both years at each location for each treatment and for each ensiling period. After removal, silage (400 g) was placed into large styrofoam containers lined

with a plastic bag and exposed to air for 0, 1, 3, 5, and 7 d. After each exposure period, silage was analyzed for pH, microbial populations (yeast and molds, and total bacteria), fermentation end-products (acetic, lactic, propionic and butyric acids, and ethanol), and water soluble carbohydrates (glucose, fructose, galactose, xylose, and arabinose). Temperature was monitored daily. The critical period of aerobic deterioration appears to occur between 0 and 3 d in the temperate silage, and between 0 and 1 d in the tropical silage. In both locations, inoculated silage spoiled faster than silage treated without the microbial inoculant. Silage exposed to air after 40 d of fermentation was less stable than silage exposed after 100 d, but a more pronounced effect due to length of fermentation was observed in the temperate silage.

Introduction

Aerobic deterioration can account for as much as 35 % of the total DM loss that occurs during the ensiling process (Honnig and Woolford, 1979; Woolford, 1990). In temperate environments, utilization of silage additives (e.g. microbial inoculant, enzymes) to improve the aerobic stability of forages has shown variable success. A microbial inoculant appeared to lower the stability of high moisture corn ensiled for 40 d (Wardynski et al. 1993). Wheat and sorghum ensiled for 45 d and treated with a bacterial inoculant spoiled faster that control silages (Weinberg et al., 1993). No

differences attributed to microbial inoculant on aerobic stability of corn ensiled for 40 and 186 d, and sorghum ensiled for 30 and 160 d was observed by Sanderson (1993). Likewise, utilization of plant cell-wall degrading enzymes also has improved or shown (Jaakkola, et al., 1991) little effect (Stokes, 1992) on aerobic deterioration. However, there is a limited information regarding the effects of silage additives on the aerobic stability of tropical forages. Because of the warmer climate one would expect aerobic stability of silages to be more challenged than in a temperate region. The objective of this experiment was to evaluate the effects of a commercial plant cell-wall degrading enzyme preparation and a homofermentative lactic acid bacteria inoculant on the aerobic stability of forage sorghum ensiled under a temperate and tropical environment. The effect of length of fermentation (40 or 100 d) on aerobic stability was also determined.

Experimental Procedure

Location of the experiment, vegetative material, harvesting and ensiling process, and experimental treatments utilized in this experiment are described in Chapter 2. In both 1993 and 1994, and at each location, duplicate silos from each treatment were emptied after 40 or 100 d of fermentation, placed into styrofoam containers lined with a plastic bag and exposed to air for 7 d. After 0, 1, 3, 5, and 7 d of aerobic exposure,

samples were analyzed for pH and yeast and mold populations as previously described (Chapter 2). Total bacteria counts were performed using selective media (Tryptic soy agar with 1.5% agar) and enumerated after 2 d of incubation. Fermentation end-products and WSC were quantified by ion exchange chromatography as described in Chapter 2. Temperature was monitored twice daily with a thermometer embedded in the surface of the exposed silage. In both years, statistical analysis was performed within environment as a completely randomized design with a 2 (years) by 2 (length of fermentation) by 4 (silage additives) by 5 (days of aerobic exposure) factorial arrangement of treatments (Steel and Torrie, 1980) using the General Linear Model Procedure of SAS (1990). The models for pH, microbial groups, fermentation end-products, and WSC were as follows:

$$Y_{ijklm} = \mu + A_i + B_j + (A*B)_{ij} + C_k + (A*C)_{ik} + (B*C)_{jk} + (A*B*C)_{ijk} + D_l + (A*D)_{il} + (B*D)_{jl} + (A*B*D)_{ijl} + (C*D)_{kl} + (A*C*D)_{ikl} + (B*C*D)_{jkl} + (A*B*C*D)_{ijkl} + E_{ijklm}$$

Where:

Y = Individual variable measured (e.g. glucose, fructose)

μ = Overall mean

A_i = Effect of year

B_j = Effect of length of fermentation

$A*B_{ij}$ = Interaction of year by length of fermentation

C_k = Effect of silage additive

$A*C_{ik}$ = Interaction of year by silage additive

$B*C_{jk}$ = Interaction of length of fermentation by silage additive

$A*B*C_{ijk}$ = Interaction of year by length of fermentation by silage additive

D_l = Effect of length of aerobic exposure

$A*D_{il}$ = Interaction of year by length of aerobic exposure

$B*D_{jl}$ = Interaction of length of fermentation by length of aerobic exposure

$A*B*D_{ijk}$ = Interaction of year by length of fermentation by length of aerobic exposure

$C*D_{kl}$ = Interaction of silage additive by length of aerobic exposure

$A*C*D_{ikl}$ = Interaction of year by silage additive by length of aerobic exposure

$B*C*D_{jkl}$ = Interaction of length of fermentation by silage additive by length of aerobic exposure.

$A*B*C*D_{ijkl}$ = Interaction of year by length of fermentation by silage additive by length of aerobic exposure

E_{ijklm} = random residual error

Mean separation was performed by Bonferroni t-test. The model for structural carbohydrate content was similar except that 8 days of aerobic exposure were utilized.

Results and Discussion

Temperate Silage

It is well documented that aerobic deterioration occurs from the metabolism of sugars and organic acids by microorganisms, and is usually detected by an increase in pH and temperature (Spoeltra et al., 1988). In this experiment, pH increased ($P<0.01$) as length of aerobic exposure increased (Table 3-1). This trend occurred during both years. By the third day, pH was significantly increased and continued in a linear fashion through d-5. During both years, the maximum temperature ($P<0.01$) was observed within 2 d of aerobic exposure. Thereafter temperature decreased slightly until the end of the aerobic exposure period. Yeast and mold populations increased ($P<0.01$) at a exponential rate after 3 and 5 days of aerobic exposure in 1993 and 1994, respectively. Simultaneous increases in pH and temperature with growth of yeasts and molds were observed in this experiment. Similar relationships have been reported in previous studies (Courtin and Spoeltra, 1990; Rust and Yokoyama, 1992) conducted in temperate environments. Total bacterial population increased ($P<0.01$) by 5 % and 12 % after 3 or 5 d of aerobic exposure in 1993 and 1994,

Table 3-1. **Effects of year and length of aerobic exposure on pH, temperature, and microbial population of forage sorghum silage exposed to air in a temperate environment**

Item	Aerobic exposure (d)	Year		SEM[a]	Probability		
		93	94		Y[b]	D[c]	Y*D[d]
pH	0	3.64	3.53	.072	.269	.001	.234
	1	3.77	3.55				
	3	4.65	4.75				
	5	5.87	5.80				
	7	6.66	6.70				
Temperature (°C)	0	20.16[i]	21.29[h]	.460	.001	.001	.001
	1	23.03[g]	26.66[f]				
	2	24.89[f]	26.75[f]				
	3	23.49[g]	22.86[g]				
	4	22.26[gh]	23.37[g]				
	5	21.95[h]	23.16[g]				
	6	22.02[h]	22.97[g]				
	7	21.33[h]	22.61[g]				
Microbial Group[e]							
Yeasts and molds	0	4.57[j]	4.61[j]	.087	.001	.001	.001
	1	5.39[h]	6.17[i]				
	3	7.08[g]	7.16[h]				
	5	7.37[f]	8.79[f]				
	7	7.65[f]	8.02[g]				
Total bacteria	0	7.15[g]	7.34[h]	.09	.001	.001	.001
	1	7.06[g]	7.77[g]				
	3	7.53[f]	7.71[g]				
	5	7.48[f]	8.35[f]				
	7	7.64[f]	8.44[f]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of length of aerobic exposure
[d] Interaction of year by length of aerobic exposure
[e] cfu/ g of fresh material
[fghij] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)

respectively. Based on proliferation of microorganisms over time, yeasts and molds appear to be the major perpetrators of deterioration during the initial stages of aerobic exposure, and total bacteria seem to be responsible for the increase in pH. This observation is supported with a previous study (Moon et al., 1983) which indicated that yeasts and molds are the most important microorganisms implicated in the initiation of aerobic deterioration. However, in maize silage, acetic acid bacteria in addition to yeasts and molds have been implicated in the initiation of aerobic deterioration (Spoeltra et al., 1988).

In both years, acetic acid content substantially decreased ($P<0.01$) after 5 d of aerobic exposure (Table 3-2). Rate of acetic acid utilization varied between years, as indicated by the decrease in acetic acid content at d 1 of aerobic exposure in the 1994 silage as compared to d 3 in 1993. Lactic acid content decreased ($P<0.05$) as length of aerobic exposure increased in both years, but a greater utilization of lactic acid was observed between 0 and 3 d of aerobic exposure. Only small concentrations of butyric and propionic acids were observed in this study, and a significant response attributed to year, additive, day of aerobic exposure or length of fermentation was not observed. Data for these two volatile fatty acids is presented in Appendix A. Ethanol content decreased ($P<0.01$) over the exposure period in both years. Changes in microbial populations and fermentation end-products observed over time in this experiment may

Table 3-2. **Effects of year and length of aerobic exposure on fermentation end-products of forage sorghum silage exposed to air in a temperate environment**

Fermentation End-Product, g /100 g DM	Aerobic Exposure (d)	Year		SEM[a]	Probability		
		93	94		Y[b]	D[c]	Y*D[d]
Acetic acid	0	1.88[e]	1.58[e]	0.08	.001	.001	.001
	1	1.84[e]	0.65[f]				
	3	1.49[f]	0.55[f]				
	5	0.29[g]	0.21[g]				
	7	0.21[g]	0.29[g]				
Lactic acid	0	7.30[E]	7.67[E]	0.27	.483	.001	.052
	1	6.23[F]	5.14[F]				
	3	2.65[G]	2.38[G]				
	5	1.20[H]	1.36[H]				
	7	0.70[I]	0.92[I]				
Ethanol	0	1.24[e]	0.54[f]	0.06	.001	.001	.001
	1	0.84[f]	0.85[e]				
	3	0.48[g]	0.45[f]				
	5	0.27[h]	0.28[g]				
	7	0.42[g]	0.28[g]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of length of aerobic exposure
[d] Interaction of year by length of aerobic exposure
[efghi] Means with unlike superscripts in the same column within an fermentation end-product heading differ ($P<0.01$)
[EFGHI] Means with unlike superscripts in the same column within an fermentation end-product heading differ ($P<0.05$)

indicate that the rate and extent of deterioration was higher in 1994 than in 1993. However, in both years the greatest amount of deterioration occurred between 0 and 3 d as evidenced by changes in microbial counts, fermentation end-products, pH and temperature.

A rapid decline in hexose and pentose content was observed within 3 d of aerobic exposure; which corresponded to the increase in pH, temperature and microbial populations (Table 3-3). Results from this experiment, demonstrated that a greater disappearance of plant organic acids and water soluble sugars occurred during the first 3 d of aerobic exposure. It appeared that differences in microbial activity or substrate availability rather than substrate preference were the major factors that influenced the variation in rate and extent of deterioration across years.

The accumulation of lactic acid and lower pH due to inoculation, has been promoted as a strategy to improve aerobic stability. Unfortunately, the low pH and high lactic acid content inhibits aerobic bacteria but does little to eliminate yeast and mold growth when air is present. Rapid accumulation of lactic acid also is recommended to improve DM recovery from the silo. However, it appears that this strategy results in less stable material, especially when epiphytic yeast and mold populations are high. The efficacy of silage additives should be based on nutrient preservation from the field to consumption.

Results from Chapter 2 indicated that forage sorghum treated with microbial

Table 3-3. **Effects of year and length of aerobic exposure on water soluble carbohydrate contents of forage sorghum silage exposed to air in a temperate environment**

Carbohydrates, g/100 g DM	Aerobic Exposure (d)	Year		SEM[a]	Probability		
		93	94		Y[b]	D[c]	Y*D[d]
Glucose	0	1.07[e]	1.37[e]	0.65	.001	.001	.001
	1	0.03[f]	1.03[f]				
	3	0.02[f]	0.64[g]				
	5	0.01[f]	0.69[g]				
	7	0.01[f]	0.62[g]				
Fructose	0	0.64[g]	0.98[e]	0.05	.001	.001	.001
	1	0.20[f]	0.77[f]				
	3	0.05[g]	0.31[g]				
	5	0.03[g]	0.25[g]				
	7	0.02[g]	0.21[g]				
Galactose	0	0.07[e]	0.25[e]	0.01	.001	.001	.001
	1	0.02[f]	0.24[e]				
	3	0.01[f]	0.09[f]				
	5	0.01[f]	0.03[g]				
	7	0.00[f]	0.03[g]				
Xylose	0	0.41[e]	0.51[e]	0.02	.001	.001	.004
	1	0.01[f]	0.18[f]				
	3	0.03[f]	0.07[g]				
	5	0.01[f]	0.03[gh]				
	7	0.01[f]	0.02[h]				
Arabinose	0	0.08[E]	0.13[E]	0.01	.001	.001	.036
	1	0.05[FG]	0.14[F]				
	3	0.03[G]	0.09[F]				
	5	0.04[G]	0.06[G]				
	7	0.02[G]	0.04[H]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of length of aerobic exposure
[d] Interaction of year by length of aerobic exposure
[efgh] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.01$)
[EFGH] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.05$)

inoculant alone or in combination with enzymes increased the acidity, lactic acid content, and residual fructose content, but did not influence other fermentation end-products. However, when the resulting silage was exposed to aerobic conditions, inoculated silage had higher ($P<0.01$) pH than forage sorghum ensiled without the microbial inoculant (Table 3-4). Temperature and yeast and mold populations were higher ($P<0.01$) in inoculated forage as compared to control silage or silage treated only with enzymes, but similar to forage treated with inoculant plus enzymes. Total bacterial population was similar regardless of silage additives. In this experiment, utilization of a microbial inoculant resulted in more unstable silage as evidenced by a greater pH, temperature, and yeast and mold populations. These results agree with Rust et al. (1989), and Wardynski et al. (1993); who reported that inoculated corn silage and high moisture corn were less stable upon exposure to air than control silage. Ethanol was the only fermentation end-product influenced by silage additives during the exposure period. Control silage had lower ($P<0.01$) ethanol content than silage treated with microbial inoculant, but similar to treatments containing the enzyme mixture. The similar counts of total bacteria among experimental treatments and higher ethanol content in inoculated silages, may indicate that the negative effect of inoculation on aerobic stability is manifested in greater yeast and mold populations. Glucose content tended ($P<0.10$) to be higher, but fructose was lower ($P<0.01$) in control silage or

Table 3-4. **Effects of silage additives on aerobic stability of forage sorghum ensiled in a temperate environment and exposed to air for 7 days**

Item	Silage Additive				SEM[a]	Probability
	No Additive	Enzyme	Inoculant	E+I		
pH	4.66[d]	4.66[d]	5.13[e]	5.12[e]	.046	.001
Temperature (°C)	22.52[e]	22.64[e]	23.71[d]	23.33[d]	.203	.001
Microbial Group[b]						
Yeasts and molds	6.40[f]	6.48[ef]	6.76[d]	6.68[de]	.055	.001
Total bacteria	7.77	7.64	7.59	7.58	.057	.088
Fermentation End-Product[c]						
Acetic acid	0.87	1.08	0.86	0.78	.168	.146
Lactic acid	3.63	3.79	3.41	3.36	.050	.221
Ethanol	0.47[f]	0.61[de]	0.64[d]	0.53[de]	.038	.009
Water Soluble Carbohydrate[c]						
Glucose	0.58[y]	0.60[y]	0.54[z]	0.47[z]	.041	.105
Fructose	0.20[f]	0.30[f]	0.44[e]	0.44[e]	.032	.001
Galactose	0.07[d]	0.10[e]	0.06[d]	0.07[d]	.008	.005
Xylose	0.12	0.14	0.12	0.11	.014	.347
Arabinose	0.05[d]	0.07[de]	0.07[de]	0.09[e]	.008	.002

[a] Standard error of the mean
[b] cfu/g fresh material
[c] g/ 100 g DM
[def] Means with unlike superscripts in the same row differ ($P<0.01$)
[yz] Means with unlike superscripts in the same row differ ($P<0.10$)

silage treated only with enzymes in comparison to treatments containing the microbial inoculant. Xylose was similar regardless of silage additive. Galactose content was higher ($P<0.01$) in silage containing the enzyme mixture, and arabinose content was higher ($P<0.01$) in silage treated with inoculant plus enzymes as compared to the other treatments.

The pH was similar for all treatments at d 0 and 1 of aerobic exposure, but silage treated with bacterial inoculant had greater ($P<0.01$) pH from d 3 to 7 than control silage or silage treated only with the enzyme mixture (Table 3-5). Initial temperature was similar for all treatments, but was higher ($P<0.01$) in silage treated with the microbial inoculant after 1 and 2 d of aerobic exposure. After 3 d of exposure, temperature for all treatments were similar. A significant silage additive by day of aerobic exposure interaction was not observed for yeast and mold populations. Even though the total bacterial population was higher ($P<0.05$) in the ensiled forage sorghum containing the microbial inoculant before exposure to air, after 1 d of aerobic exposure the bacterial population was similar among treatments. The greater pH and temperature observed in silages containing the microbial inoculant within 3 d after aerobic exposure, and the higher yeast and mold populations over the entire aerobic exposure period, indicates that inoculation increased the rate of deterioration in forage sorghum silage. Initial acetic acid levels were similar for all treatments (Table 3- 6). Silage containing microbial inoculant had lower ($P<0.01$) acetic acid content after

Table 3-5. **Effects of silage additives and length of aerobic exposure on pH, temperature, and microbial populations of forage sorghum silage exposed to air in a temperate environment**

Item	Aerobic Exposure (d)	Silage Additive				SEM[a]	Probability		
		No Additive	Enzymes	Inoculant	E+I		A[b]	D[c]	A*D[d]
pH	0	3.60	3.61	3.56	3.56	.102	.001	.001	.001
	1	3.60	3.60	3.76	3.68				
	3	4.21[g]	4.20[g]	5.14[f]	5.27[f]				
	5	5.46[g]	5.40[g]	6.31[f]	6.17[f]				
	7	6.43[g]	6.51[g]	6.86[f]	6.91[f]				
Temperature (°C)	0	20.96	20.64	20.58	20.71	.575	.001	.001	.002
	1	22.48[g]	22.68[g]	27.16[f]	26.95[f]				
	2	24.69[g]	23.37[g]	28.78[f]	26.46[f]				
	3	24.55[f]	22.94[g]	22.81[g]	22.40[g]				
	4	23.10	22.60	22.46	23.09				
	5	22.38	22.70	22.91	22.24				
	6	21.69	23.10	22.79	22.34				
	7	21.27	21.97	22.19	22.13				
Microbial Group[e]									
Yeasts and molds	0	4.46	4.54	4.72	4.65	.123	.001	.001	.770
	1	5.42	5.69	6.09	5.92				
	3	6.85	7.03	7.32	7.29				
	5	7.51	7.45	7.78	7.59				
	7	7.75	7.71	7.90	7.99				
Total bacteria	0	7.44[F]	7.29[FG]	7.20[FG]	7.04[G]	.127	.001	.088	.055
	1	7.44	7.45	7.47	7.30				
	3	7.81	7.74	7.55	7.57				
	5	8.20[F]	7.66[g]	7.79[G]	8.02[GH]				
	7	7.95	8.05	7.91	8.25				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of length of aerobic exposure
[d] Interaction of silage additive by length of aerobic exposure
[e] cfu/ g of fresh material
[fgh] Means with unlike superscripts in the same row differ ($P<0.01$)
[FGH] Means with unlike superscripts in the same row differ ($P<0.05$)

1 d of air exposure than silage without the inoculant. After 3 d of aerobic exposure, control silage had lower acetic acid content than silages containing additives, but no differences due to silage additives were observed after 5 and 7 d of aerobic exposure. Lactic acid content was similar ($P<0.01$) at d 0 and 1 of aerobic exposure regardless of silage additive. After 3 d, silages containing the microbial inoculant had lower ($P<0.01$) lactic acid content than silage without the microbial additive. The silage treated only with the microbial additive had greater ($P<0.01$) ethanol content the first day of aerobic exposure, thereafter, ethanol levels were similar among treatments at each day tested. There was a significant silage additive by day of aerobic exposure interaction on fructose ($P<0.01$) and arabinose ($P<0.05$) content (Table 3-7). Silages treated with the microbial inoculant had greater fructose content at d 0 and 1 of aerobic exposure than silages without the starter culture, but for all treatments, fructose content was similar thereafter. Differences in arabinose content among treatments were also observed the initial 3 d of aerobic exposure, but thereafter changes were small and a general trend was not observed. Changes in fermentation end-products (e.g. lactic acid) and WSC (e.g. fructose) further support the premise that inoculation increased the rate of deterioration in forage sorghum exposed to air.

It is well documented that longer storage intervals improve the stability of silages (Muck, 1988; Pitt et al., 1991a, 1991b). In this study, silages

Table 3-6. **Effects of silage additives and length of aerobic exposure on fermentation end-products of forage sorghum silage exposed to air in a temperate environment**

Fermentation End-Products, g /100 g DM	Aerobic Exposure (d)	Silage Additive				SEM[a]	Probability		
		No Additive	Enzymes	Inoculant	E+I		A[b]	D[c]	A*D[d]
Acetic acid	0	1.74	1.81	1.73	1.64	0.12	.001	.001	.004
	1	1.38[e]	1.74[e]	1.06[f]	0.81[f]				
	3	0.76	1.12	1.12	1.09				
	5	0.23	0.32	0.27	0.17				
	7	0.27	0.40	0.14	0.20				
Lactic acid	0	7.24	7.28	7.64	7.78	0.38	.221	.001	.001
	1	5.37	5.24	5.93	6.20				
	3	3.43[e]	3.24[e]	1.87[f]	1.50[f]				
	5	1.06[e]	2.41[e]	0.95[f]	0.70[f]				
	7	1.19[e]	0.81[e]	0.66[f]	0.60[f]				
Ethanol	0	0.82[f]	0.88[f]	1.03[e]	0.84[f]	0.09	.001	.001	.001
	1	0.55[g]	0.93[f]	0.80[f]	1.10[e]				
	3	0.35	0.58	0.48	0.45				
	5	0.31	0.27	0.37	0.16				
	7	0.34	0.41	0.54	0.13				

[a]Standard error of the mean
[b]Effect of silage additive
[c]Effect of length of aerobic exposure
[d]Interaction of silage additive by length of aerobic exposure
[efg]Means with unlike superscripts in the same row differ ($P<0.01$)

Table 3-7. Effects of silage additives and length of aerobic exposure on water soluble carbohydrate contents of forage sorghum silage exposed to air in a temperate environment

Carbohydrates, g /100 g DM	Aerobic Exposure (d)	Silage Additive				SEM[a]	Probability		
		No Additive	Enzymes	Inoculant	E+I		A[b]	D[c]	A*D[d]
Glucose	0	1.35	1.23	1.10	1.19	0.10	.105	.001	.695
	1	0.53	0.62	0.56	0.40				
	3	0.31	0.39	0.42	0.19				
	5	0.34	0.33	0.37	0.36				
	7	0.39	0.42	0.24	0.19				
Fructose	0	0.38[f]	0.35[f]	1.29[e]	1.21[e]	0.07	.107	.001	.001
	1	0.21[f]	0.53[e]	0.59[e]	0.61[e]				
	3	0.14	0.28	0.13	0.17				
	5	0.12	0.16	0.12	0.14				
	7	0.14	0.17	0.06	0.08				
Galactose	0	0.17	0.16	0.14	0.17	0.02	.005	.001	.267
	1	0.10	0.19	0.11	0.10				
	3	0.05	0.10	0.03	0.02				
	5	0.02	0.03	0.01	0.04				
	7	0.02	0.03	0.01	0.01				
Xylose	0	0.45	0.47	0.48	0.45	0.03	.002	.001	.983
	1	0.08	0.14	0.08	0.08				
	3	0.04	0.07	0.02	0.02				
	5	0.01	0.01	0.03					
	7	0.01	0.03	0.01	0.01				
Arabinose	0	0.11	0.09	0.11	0.13	0.02	.001	.001	.042
	1	0.05[F]	0.10[E]	0.07[EF]	0.11[F]				
	3	0.04	0.06	0.06	0.10				
	5	0.03	0.05	0.07	0.05				
	7	0.03	0.05	0.04	0.02				

[a]Standard error of the mean
[b]Effect of silage additive
[c] Effect of length of aerobic exposure
[d] Interaction of silage additive by length of aerobic exposure
[ef]Means with unlike superscripts in the same row differ ($P<0.01$)
[EF] Means with unlike superscripts in the same row differ ($P<0.05$)

exposed to air after 100 d of fermentation had lower ($P<0.01$) temperature, pH and microbial populations, but higher ($P<0.01$) pentose and fermentation end-products than silages opened after 40 d (Table 3-8). Hexose content was similar regardless of length of fermentation. Similar trends in pH elevation were observed for silages stored for both 40 and 100 d (Table 3-9), however the rate and extent of change were greater in silages stored for 40 d. Temperature reached its maximum value after 1 and 3 d of aerobic exposure for silages opened after 40 or 100 d, respectively. For both fermentation periods, yeast and mold populations increased ($P<0.01$) at a exponential rate after 5 d of aerobic exposure, but a greater increase was observed when silage was opened after 40 d. Total bacterial population increased as the time of exposure increased ($P<0.05$) regardless of fermentation period.

Acetic and lactic acid levels decreased over time with silages stored for 40 and 100 d, but the rate of decline was greater ($P<0.01$) for silages stored for the shorter time period (Table 3-10). Ethanol content decreased ($P<0.01$) over time for forage sorghum silage opened after 40 or 100 d. A significant interaction between length of storage and time of exposure was detected ($P<0.05$) but a divergent trend was not observed.

The soluble sugars decreased throughout the exposure period for silages stored for either 40 or 100 d (Table 3-11). There was an overall tendency for the 40 d silages to have lower sugar levels after 7 d of exposure, but the

Table 3-8. Effects of length of fermentation on aerobic stability of forage sorghum ensiled in a temperate environment and exposed to air for seven days

Item	Length of Fermentation (d)			
	40	100	SEM[a]	Probability
pH	5.27[e]	4.52[d]	.032	.001
Temperature (°C)	23.95[e]	22.15[d]	.014	.001
Microbial Group[b]				
Yeasts and molds	6.75[e]	6.41[d]	.038	.001
Total bacteria	7.94[e]	7.35[d]	.040	.088
Fermentation End-Products[c]				
Acetic acid	0.81[d]	0.99[e]	.118	.001
Lactic acid	2.92[d]	4.19[e]	.030	.001
Ethanol	0.51[d]	0.62[e]	.026	.001
Water Soluble Carbohydrates[c]				
Glucose	0.53	0.57	.029	.341
Fructose	0.34	0.35	.022	.730
Galactose	0.07	0.08	.006	.076
Xylose	0.08[d]	0.16[e]	.009	.001
Arabinose	0.04[d]	0.09[e]	.006	.001

[a] Standard Error of the mean
[b] cfu/g of fresh material
[c] g/ 100 g DM
[de] Means with unlike superscripts in the same row differ ($P<0.01$)

Table 3-9. **Effects of length of fermentation and length of aerobic exposure on pH, temperature, and microbial populations of forage sorghum silage exposed to air in a temperate environment**

Item	Aerobic Exposure (d)	Length of Fermentation (d)		SEM[a]	Probability		
		40	100		L[b]	D[c]	L*D[d]
pH	0	3.56[j]	3.61[i]	.072	.001	.001	.001
	1	3.72[i]	3.60[i]				
	3	5.20[h]	4.20[h]				
	5	6.48[g]	5.19[g]				
	7	7.38[f]	5.97[f]				
Temperature (°C)	0	21.66[j]	19.79[h]	.406	.001	.001	.001
	1	28.60[f]	21.10[g]				
	2	27.05[g]	24.59[f]				
	3	24.14[h]	22.21[g]				
	4	22.87[i]	22.76[g]				
	5	22.25[i]	22.86[g]				
	6	22.47[i]	22.49[g]				
	7	22.55[i]	21.38[h]				
Microbial Group[e]							
Yeasts and molds	0	4.47[f]	4.51[i]	.087	.001	.001	.001
	1	5.75[h]	5.81[h]				
	3	7.28[g]	6.96[g]				
	5	8.07[f]	7.09[g]				
	7	8.20[f]	7.47[f]				
Total bacteria	0	7.30	7.19	.090	.001	.001	.138
	1	7.77	7.06				
	3	8.06	7.17				
	5	8.18	7.66				
	7	8.41	7.67				

[a] Standard error of the mean
[b] Effect of length of fermentation
[c] Effect of length of aerobic exposure
[d] Interaction of length of fermentation by length of aerobic exposure
[e] cfu/ g of fresh material
[fghi] Means with unlike superscripts in the same column and item heading differ (P<0.01)

differences were very small and of minimal significance.

Differences in availability and utilization of substrates and microbial ecology responsible for the aerobic instability may explain this differences in rate and extent of deterioration when silage was exposed to air after 40 or 100 d of fermentation. It would appear the 40 d silage is less stable than the 100 d silage. Whatever renders stability to a silage was present in the 100 d but not in the 40 d silages. Likewise, it would appear it is some other factor besides acidity, fermentation end-products or residual sugars that leads to stable silages.

Length of fermentation also influenced the effect of silage additives on aerobic stability. Forage sorghum treated with microbial inoculant alone or in combination with enzymes and exposed to air after 40 or 100 d of fermentation had higher pH ($P<0.01$) than control silage or silage treated only with enzymes (Table 3-12). Changes in pH between inoculated and non-inoculated silages were greater when silage was opened after 40 d of fermentation. A higher ($P<0.01$) temperature was also observed in inoculated silages exposed to air after 40 d as compared to sorghum ensiled without the microbial inoculant. When forage sorghum was exposed to air after 100 d post-ensiling, temperature was higher ($P<0.05$) in silages containing the microbial inoculant plus the enzyme mixture than the other treatments.

For both periods of fermentation, yeast and mold populations were higher

Table 3-10. **Effects of length of fermentation and length of aerobic exposure on fermentation end-products of forage sorghum silage exposed to air in a temperate environment**

Fermentation End-Product, g/ 100 g DM	Aerobic Exposure (d)	Length of Fermentation (d)		SEM[a]	Probability		
		40	100		L[b]	D[c]	L*D[d]
Acetic acid	0	1.71[e]	1.76[e]	.080	.001	.001	.011
	1	0.95[f]	1.55[f]				
	3	0.99[f]	1.05[g]				
	5	0.20[g]	0.31[h]				
	7	0.21[g]	0.30[h]				
Lactic acid	0	8.21[e]	6.76[e]	.265	.001	.001	.001
	1	4.68[f]	6.69[e]				
	3	0.98[g]	4.05[f]				
	5	0.43[h]	2.13[g]				
	7	0.29[h]	1.33[h]				
Ethanol	0	0.90[e]	0.89[f]	.060	.005	.001	.001
	1	0.53[f]	1.16[e]				
	3	0.35[f]	0.59[g]				
	5	0.34[f]	0.22[g]				
	7	0.45[f]	0.26[g]				

[a] Standard error of the mean
[b] Effect of length of fermentation
[c] Effect of length of aerobic exposure
[d] Interaction of length of fermentation by length of aerobic exposure
[efgh] Means with unlike superscripts in the same column wiithin a fermentation end-product heading differ ($P < 0.01$)

Table 3-11. **Effects of length of fermentation and length of aerobic exposure on water soluble carbohydrate contents of forage sorghum silage exposed to air in a temperate environment**

Carbohydrate, g/100 g DM	Aerobic Exposure (d)	Length of Fermentation (d)		SEM[a]	Probability		
		40	100		L[b]	D[c]	L*D[d]
Glucose	0	1.38[e]	1.06[e]	0.06	.341	.001	.001
	1	0.37[f]	0.69[f]				
	3	0.36[fg]	0.29[g]				
	5	0.30[fgh]	0.40[g]				
	7	0.24[h]	0.39[g]				
Fructose	0	0.82[E]	0.80[E]	0.05	.730	.001	.028
	1	0.39[F]	0.58[F]				
	3	0.15[G]	0.21[G]				
	5	0.19[G]	0.08[G]				
	7	0.14[G]	0.08[G]				
Galactose	0	0.18[e]	0.14[e]	0.01	.073	.001	.013
	1	0.11[f]	0.14[e]				
	3	0.03[g]	0.07[f]				
	5	0.02[g]	0.03[g]				
	7	0.01[g]	0.03[g]				
Xylose	0	0.31[e]	0.61[e]	0.02	.001	.001	.001
	1	0.08[f]	0.10[f]				
	3	0.02[g]	0.05[g]				
	5	0.01[g]	0.02[g]				
	7	0.01[g]	0.02[g]				
Arabinose	0	0.08[e]	0.13[e]	0.01	.001	.001	.004
	1	0.07[e]	0.13[e]				
	3	0.02[f]	0.11[e]				
	5	0.04[f]	0.05[f]				
	7	0.02[f]	0.04[f]				

[a] Standard error of the mean
[b] Effect of length of fermentation
[c] Effect of length of aerobic exposure
[d] Interaction of length of fermentation by length of aerobic exposure
[efgh] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.01$)
[EF] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.05$)

Table 3-12. Effects of silages additive and length of ensiling on pH, temperature, and microbial population of forage sorghum silage exposed to air in a temperate environment

Item	Silage Additive	Length of Fermentation (d) 40	100	SEM[a]	Probability A[b]	L[c]	A*L[d]
pH	No additive	4.87[g]	4.45[g]	.065	.001	.001	.001
	Enzyme	5.00[g]	4.32[g]				
	Inoculant	5.58[f]	4.67[f]				
	E +I	5.62[f]	4.62[f]				
Temperature (°C)	No additive	22.80[g]	22.47[g]	.287	.001	.001	.001
	Enzyme	23.09[g]	21.94[g]				
	Inoculant	25.28[f]	22.15[g]				
	E + I	24.63[f]	23.02[f]				
Microbial Group[e]							
Yeasts and molds	No additive	6.39[h]	6.40[g]	.077	.001	.001	.001
	Enzyme	6.66[g]	6.30[g]				
	Inoculant	7.07[f]	6.45[f]				
	E + I	6.89[f]	6.48[f]				
Total bacteria	No additive	8.14	7.40	.080	.080	.001	.182
	Enzyme	7.99	7.29				
	Inoculant	7.82	7.34				
	E + I	7.82	7.37				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of length of ensiling
[d] Interaction of silage additive by length of ensiling
[e] cfu/ g of fresh material
[fgh] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)

($P<0.01$) in forage sorghum treated with the microbial inoculant as compared to the other treatments. Similar to changes in pH and temperature, differences in yeast and mold counts between inoculated and non-inoculated silages were greater in silages stored for 40 d than 100 d. Total bacterial populations were similar for all silage additives regardless of length on fermentation; which supports the observation that yeasts and molds are the initiators of deterioration rather than bacteria.

The patterns of change in fermentation end-products were similar ($P<0.05$) for the four treatments in silages stored for 40 and 100 d (Table 3-13). There was a significant interaction detected for acetic acid, but a divergent trend was not evident.

Water soluble carbohydrate levels for the silage treatments were similar for both the 40 and 100 d silages (Table 3-14).

Results from this experiment indicated that inoculated forage sorghum silage spoiled faster than non-inoculated silages, regardless of length of fermentation. However, the negative effect on aerobic stability due to inoculation was more pronounced when silage was exposed to air after 40 than 100 d of fermentation.

Tropical Silage

Silage exposed to air in 1993 had a higher ($P<0.01$) pH than in 1994 (Table 3-15). The pH increased ($P<0.01$) as length of aerobic exposure increased

Table 3-13. Effects of silage additives and length of ensiling on fermentation end-products of forage sorghum silage exposed to air in a temperate environment

Fermentation End-Product, g/ 100 g DM	Silage Additive	Length of Fermentation (d) 40	100	SEM[a]	Probability A[b]	L[c]	A*L[d]
Acetic acid	No additive	0.89[e]	0.86[f]	.072	.001	.001	.056
	Enzyme	0.90[e]	1.27[e]				
	Inoculant	0.79[f]	0.95[f]				
	E +I	0.67[f]	0.90[f]				
Lactic acid	No additive	2.99	4.33	.237	.221	.001	.326
	Enzyme	2.95	4.63				
	Inoculant	2.77	4.06				
	E + I	2.96	3.76				
Ethanol	No additive	0.40	0.56	.053	.001	.001	.876
	Enzyme	0.58	0.65				
	Inoculant	0.60	0.69				
	E + I	0.48	0.59				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of length of ensiling
[d] Interaction of silage additive by length of ensiling
[ef] Means with unlike superscripts in the same column within an item heading differ ($P<0.05$)

Table 3-14. Effects of silages additive and length of ensiling on water soluble carbohydrate contents of forage sorghum silage exposed to air in a temperate environment

Carbohydrate , g/100 g DM	Silage Additive	Length of Fermentation (d)		SEM[a]	Probability		
		40	100		A[b]	L[c]	A*L[d]
Glucose	No additive	0.57	0.60	0.05	.105	.341	.721
	Enzyme	0.58	0.63				
	Inoculant	0.49	0.59				
	E +I	0.48	0.45				
Fructose	No additive	0.21	0.20	0.04	.001	.730	.916
	Enzyme	0.30	0.30				
	Inoculant	0.41	0.47				
	E + I	0.44	0.45				
Galactose	No additive	0.06	0.08	0.01	.005	.076	.608
	Enzyme	0.08	0.12				
	Inoculant	0.05	0.06				
	E + I	0.07	0.07				
Xylose	No additive	0.08	0.15	0.01	.374	.001	.967
	Enzyme	0.10	0.19				
	Inoculant	0.07	0.16				
	E + I	0.08	0.15				
Arabinose	No additive	0.03	0.06	0.01	.002	.001	.240
	Enzyme	0.05	0.08				
	Inoculant	0.04	0.09				
	E + I	0.06	0.13				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of length of ensiling
[d] Interaction of silage additive by length of ensiling

in the 1993 silage. In 1994, acidity was similar at d 0 and 1, but then decreased as length of aerobic exposure increased. Overall, similar trends occurred over time in both years. Temperature reached a maximum value after 1 d of aerobic exposure in 1993 and 1994, then slightly decreased until the end of the aerobic exposure period. These silages were unstable based on criteria of an exponential increase ($P<0.01$) in yeasts and molds and total bacterial populations within the first 24 h in 1993. In 1994, microorganisms enumerated increased over time, but at a slower rate ($P<0.01$). In contrast to forage sorghum exposed to air in the temperate environment, it appears that greater deterioration in tropical silage occurred before 1 d of aerobic exposure, as evidenced by changes in pH, temperature and microbial populations. The higher pH, ambient temperature and yeast and mold populations when the tropical silage is exposed to air may be responsible for the higher rate of deterioration. It has been shown in models developed by different author's (Courtin and Spoeltra, 1990; Woolford, 1990: Pitt et al., 1991; Muck and O'Keily, 1992) that initial counts of yeasts and molds, high pH and high temperatures negatively influence the stability of silages.

Acetic acid content increased ($P<0.01$) the initial 3 d of aerobic exposure in the 1993 silage, then decreased thereafter (Table 3-16). In 1994, acetic acid decreased ($P<0.01$) during the first 3 d of exposure and, then remained constant. In both years, lactic acid content decreased ($P<0.01$)

Table 3-15. Effects of year and length of aerobic exposure on pH, temperature, and microbial population of forage sorghum silage exposed to air in a tropical environment

Item	Aerobic Exposure (d)	Year		SEM[a]	Probability		
		93	94		Y[b]	D[c]	Y*D[d]
pH	0	4.13[j]	4.11[i]	.101	.001	.001	.001
	1	4.68[i]	4.22[i]				
	3	6.23[h]	4.62[h]				
	5	6.89[g]	5.07[g]				
	7	7.17[f]	5.38[f]				
Temperature (°C)	0	29.50[I]	30.07[I]	.325	.005	.001	.024
	1	36.29[E]	35.96[E]				
	2	34.28[G]	34.60[G]				
	3	33.54[G]	33.62[H]				
	4	32.27[H]	32.33[I]				
	5	31.59[H]	31.49[J]				
	6	30.47[I]	31.98[J]				
	7	29.47[I]	30.99[J]				
Microbial Group[e]							
Yeasts and molds	0	5.91[j]	5.20[j]	.101	.001	.001	.001
	1	7.12[i]	5.51[i]				
	3	7.29[h]	5.57[h]				
	5	7.38[g]	5.63[g]				
	7	7.65[f]	6.03[f]				
Total bacteria	0	5.92[i]	6.16[h]	.067	.001	.001	.001
	1	7.14[h]	6.34[g]				
	3	7.63[g]	6.42[f]				
	5	7.69[fg]	6.35[fg]				
	7	7.77[f]	6.35[fg]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of length of aerobic exposure
[d] Interaction of year by length of aerobic exposure
[e] cfu/ g of fresh material
[fghij] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)
[FGHIJ] Means with unlike superscripts in the same column within an item heading differ ($P<0.05$)

as length of aerobic exposure increased. Levels were significantly higher in 1994 as compared to 1993. Ethanol content was higher ($P<0.01$) after 1 d, but lower thereafter. Since yeasts are known to produce ethanol, this observation would support the premise that yeasts are the early initiators of the deterioration process. Similar to silage exposed to air in the temperate climate, microbial ecology and rate of deterioration differ between years in the tropical silage, but the major changes occurred within the initial 24 h of aerobic exposure.

Sugars levels were low in all silages and tended to decrease as length of exposure increased (Table 3-17). Sugars levels were higher ($P<0.01$) in 1994 than in 1993. In contrast to what happened during aerobic exposure of temperate silage, microorganisms in tropical silage appeared to utilize organic acids to greater extent than residual sugars. The changes in microbial populations, fermentation end-products and residual WSC observed in this experiment, suggest that lactate assimilating (*Candida krusei*) rather than sugars utilizing (*Saccharomyces cerevisiae*) are the predominant yeasts associated with the instability of forage sorghum, and that acetic acid bacteria which oxidize ethanol to acetic acid and; acetic and lactic acids to CO_2 and H_2O (Fenton et al., 1995) may also play a major role in the deterioration.

In contrast to the relatively large negative effect of inoculation on the aerobic stability of forage sorghum ensiled the temperate environment, in

Table 3-16. **Effects of year and length of aerobic exposure on fermentation end-products of forage sorghum silage exposed to air in a tropical environment**

Fermentation End-Product, g /100 g DM	Aerobic Exposure (d)	Year		SEM[a]	Probability		
		93	94		Y[b]	D[c]	Y*D[d]
Acetic acid	0	0.49[g]	0.77[e]	.056	.006	.001	.001
	1	0.71[f]	0.54[f]				
	3	0.83[e]	0.40[g]				
	5	0.48[g]	0.30[g]				
	7	0.37[h]	0.36[g]				
Lactic acid	0	1.57[e]	3.99[e]	.124	.001	.001	.001
	1	1.05[f]	2.72[f]				
	3	0.93[g]	2.25[g]				
	5	0.56[h]	2.05[h]				
	7	0.14[i]	1.30[j]				
Ethanol	0	0.60[f]	0.35[fg]	.060	.231	.001	.001
	1	1.02[e]	0.70[e]				
	3	0.37[g]	0.46[fg]				
	5	0.12[h]	0.39[g]				
	7	0.08[i]	0.52[f]				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of length of aerobic exposure
[d] Interaction of year by length of aerobic exposure
[efghi] Means with unlike superscripts in the same column within a fermentation end-product heading differ ($P<0.01$)

Table 3-17. **Effects of year and length of aerobic exposure on water soluble carbohydrate contents of forage sorghum silage exposed to air in a tropical environment**

Carbohydrates, g/100 g DM	Aerobic Exposure (d)	Year		SEM[a]	Probability		
		93	94		Y[b]	D[c]	Y*D[d]
Glucose	0	0.07[e]	0.33[e]	.027	.001	.160	.020
	1	0.02[f]	0.37[e]				
	3	0.05[e]	0.34[e]				
	5	0.07[e]	0.23[f]				
	7	0.02[f]	0.28[f]				
Fructose	0	0.10	0.20	.023	.001	.001	.337
	1	0.15	0.18				
	3	0.07	0.16				
	5	0.02	0.11				
	7	0.01	0.12				
Galactose	0	0.06	0.15	.024	.001	.185	.655
	1	0.06	0.21				
	3	0.02	0.16				
	5	0.03	0.17				
	7	0.02	0.13				
Xylose	0	0.10	0.18	.017	.001	.001	.500
	1	0.05	0.19				
	3	0.01	0.16				
	5	0.02	0.13				
	7	0.02	0.12				
Arabinose	0	0.09	0.10	.015	.001	.001	.061
	1	0.10	0.14				
	3	0.05	0.12				
	5	0.01	0.08				
	7	0.01	0.11				

[a] Standard error of the mean
[b] Effect of year
[c] Effect of length of aerobic exposure
[d] Interaction of year by length of aerobic exposure
[e,f] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.05$)

the tropical climate utilization of silage additives had only a small effect on aerobic stability. On d 1, temperature was higher ($P<0.01$) for silages treated with the microbial inoculant, but thereafter temperatures were similar (Table 3-18). Other measures of aerobic stability such as pH, microbial populations, fermentation end-products, and water soluble carbohydrates were similar in forage sorghum upon exposure to air regardless of additive treatment (Tables 3-18, 3-19, 3-20). Results from chapter 2 indicated that the ensiling characteristics of forage sorghum differ when ensiled in temperate and tropical environments, and the utilization of a microbial inoculant improved the fermentation process in both locations. However, based on microbiological (e.g. yeasts and molds populations) and biochemical (e.g. residual sugar content) changes observed over time in both environments, the inoculant reduced the stability of the silages. The faster pH decline and accumulation of lactic acid during fermentation due to the inoculant mostly likely inhibited production of the conditions that render stability to silages. It would appear that other factors besides pH and lactic acid are also important in stability of silages.

In the temperate environment, forage sorghum silage exposed to air after 40 d of fermentation was less stable than silage opened after 100 d. In contrast, length of fermentation had smaller effects in silages from the tropical environment. There was a tendency for silage fermented for 40 d to have a greater temperature increase during exposure than silage

Table 3-18. **Effects of silage additive and length of aerobic exposure on pH, temperature, and microbial populations of forage sorghum silage exposed to air in a tropical environment**

Item	Aerobic exposure (d)	Silage Additive				SEM[a]	Probability		
		No additive	Enzymes	Inoculant	E+I		A[b]	D[c]	A*D[d]
pH	0	4.16	4.19	4.12	4.02	.142	.718	.001	.593
	1	4.62	4.45	4.30	4.42				
	3	5.35	5.47	5.19	5.68				
	5	5.98	5.95	6.03	5.97				
	7	6.20	6.17	6.34	6.39				
Temperature (°C)	0	28.88	29.62	29.82	29.81	.460	.451	.001	.001
	1	35.11[g]	34.72[g]	38.09[f]	36.58[f]				
	2	33.69	34.99	34.13	34.94				
	3	32.49	34.51	33.71	33.60				
	4	32.74	32.27	31.73	32.45				
	5	31.56	31.63	31.15	31.83				
	6	31.57	31.06	31.41	30.85				
	7	30.40	30.59	30.05	29.87				
Microbial Group[e]									
Yeasts and molds	0	5.61	5.50	5.48	5.65	.143	.354	.001	.697
	1	6.30	6.16	6.31	6.51				
	3	6.45	6.51	6.32	6.44				
	5	6.77	6.51	6.32	6.40				
	7	6.96	6.86	6.85	6.69				
Total bacteria	0	6.16	6.05	6.10	5.85	.093	.939	.001	.060
	1	6.74	6.92	6.55	6.74				
	3	6.98	6.89	7.09	7.15				
	5	6.97	6.97	7.08	7.06				
	7	7.04	6.96	7.08	7.16				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of length of aerobic exposure
[d] Interaction of silage additive by length of aerobic exposure
[e] cfu/ g of fresh material
[fg] Means with unlike superscripts in the same row differ ($P<0.01$)

Table 3-19. **Effects of silage additives and length of aerobic exposure on fermentation end-products of forage sorghum silage exposed to air in a tropical environment**

Fermentation End-Products, g/ 100 g DM	Aerobic Exposure (d)	Silage Additive				SEM[a]	Probability		
		No Additive	Enzymes	Inoculant	E+I		A[b]	D[c]	A*D[d]
Acetic acid	0	0.61	0.52	0.67	0.72	0.08	.992	.001	.730
	1	0.63	0.59	0.59	0.69				
	3	0.64	0.62	0.63	0.56				
	5	0.35	0.52	0.34	0.34				
	7	0.39	0.34	0.37	0.37				
Lactic acid	0	2.69	2.75	3.06	2.70	0.17	.072	.001	.836
	1	1.69	2.05	2.03	1.78				
	3	1.57	1.78	1.56	1.51				
	5	1.26	1.54	1.43	0.98				
	7	0.66	0.72	0.70	0.80				
Ethanol	0	0.42	0.53	0.47	0.46	0.09	.108	.001	.296
	1	0.78	0.82	0.75	1.11				
	3	0.40	0.45	0.39	0.42				
	5	0.31	0.20	0.26	0.25				
	7	0.36	0.23	0.16	0.44				

[a]Standard error of the mean
[b] Effect of silage additive
[c] Effect of length of aerobic exposure
[d] Interaction of silage additive by length of aerobic exposure

Table 3-20. **Effects of silage additives and length of aerobic exposure on water soluble carbohydrate contents of forage sorghum silage exposed to air in a tropical environment**

Carbohydrates, g/ 100 g DM	Aerobic Exposure (d)	Silage Additive				SEM[a]	Probability		
		No Additive	Enzymes	Inoculant	E+I		A[b]	D[c]	A*D[d]
Glucose	0	0.25	0.21	0.21	0.13	0.03	.631	.163	.494
	1	0.19	0.20	0.18	0.20				
	3	0.16	0.21	0.21	0.18				
	5	0.21	0.15	0.13	0.11				
	7	0.17	0.09	0.15	0.19				
Fructose	0	0.11	0.12	0.19	0.17	0.02	.168	.001	.738
	1	0.16	0.16	0.14	0.19				
	3	0.10	0.09	0.13	0.12				
	5	0.06	0.03	0.11	0.06				
	7	0.04	0.09	0.07	0.06				
Galactose	0	0.10	0.13	0.08	0.12	0.03	.192	.185	.706
	1	0.11	0.19	0.09	0.14				
	3	0.10	0.08	0.09	0.08				
	5	0.08	0.08	0.07	0.17				
	7	0.08	0.12	0.05	0.05				
Xylose	0	0.15	0.14	0.12	0.16	0.02	.070	.001	.734
	1	0.10	0.18	0.08	0.13				
	3	0.09	0.07	0.08	0.10				
	5	0.07	0.09	0.07	0.09				
	7	0.07	0.11	0.04	0.05				
Arabinose	0	0.08	0.11	0.08	0.11	0.02	.160	.001	.185
	1	0.09	0.15	0.08	0.16				
	3	0.07	0.06	0.11	0.09				
	5	0.04	0.02	0.08	0.03				
	7	0.04	0.06	0.05	0.08				

[a]Standard error of the mean
[b]Effect of silage additive
[c]Effect of length of aerobic exposure
[d]Interaction of silage additive by length of aerobic exposure

fermented for 100 d, however, these differences are small and may be of little practical significance (Table 3-21). Length of fermentation did not influence the rate of pH decline. Collectively, the pattern of changes observed in all criteria to measure stability were similar. Yeast and mold and total bacteria populations were greater ($P<0.01$) in the 40 d as compared to 100 d silages. Acetic acids levels were greater ($P<0.01$) whereas lactic acid and ethanol levels were less in the 100 d silages (Table 3-22). Glucose and fructose levels were higher ($P<0.01$) in the 40 d silage (Table 3-23), but galactose and xylose were lower ($P<0.05$). There were few significant interactions between length of fermentation and silage additives detected in this study (Tables 3-24, 3-25, 3-26). Parameters where a significant interaction was detected included yeasts and molds ($P<0.06$), ethanol ($P<0.01$), and arabinose ($P<0.06$) content. In total, the results from this experiment indicated that the effect of silage additives on the aerobic stability of forage sorghum were essentially the same regardless of length of fermentation in tropical silages.

Implications

Utilization of microbial inoculant did not prevent the aerobic deterioration of forage sorghum ensiled in temperate or tropical environments. In contrast, inoculation increased the rate of deterioration of forage sorghum ensiled in the temperate location, and a more pronounced negative effect was noticed

Table 3-21. **Effects of length of fermentation and length of aerobic exposure on pH, temperature, and microbial population of forage sorghum silage exposed to air in a tropical environment**

Item	Aerobic Exposure (d)	Length of Fermentation (d)		SEM[a]	Probability		
		40	100		L[b]	D[c]	L*D[d]
pH	0	4.11[j]	4.13[j]	.100	.975	.001	.001
	1	4.40[i]	4.50[h]				
	3	5.45[h]	5.40[g]				
	5	5.78[g]	6.18[f]				
	7	6.51[f]	6.04[f]				
Temperature (°C)	0	30.07[j]	29.50[k]	.325	.001	.001	.001
	1	37.15[f]	35.10[f]				
	2	32.52[h]	33.35[g]				
	3	34.55[g]	32.61[h]				
	4	33.01[gh]	31.58i				
	5	31.72[i]	31.36[i]				
	6	31.47[i]	30.97[j]				
	7	30.28[j]	30.18[k]				
Microbial Group[e]							
Yeasts and molds	0	5.84	5.28	.101	.001	.001	.287
	1	6.74	5.90				
	3	6.82	6.04				
	5	6.98	6.02				
	7	7.16	6.52				
Total bacteria	0	6.27[h]	5.18[h]	.067	.001	.001	.016
	1	7.09[g]	6.40[g]				
	3	7.18[g]	6.87[f]				
	5	7.28[g]	6.76[f]				
	7	7.42[f]	6.70[f]				

[a] Standard error of the mean
[b] Effect of length of fermentation
[c] Effect of length of aerobic exposure
[d] Interaction of length of fermentation by length of aerobic exposure
[e] cfu/ g of fresh material
[fg] Means with unlike superscripts in the same column within an item heading differ (P<0.01)

Table 3-22. **Effect of length of fermentation and length of aerobic exposure on fermentation end-products of forage sorghum silage exposed to air in a tropical environment**

Fermentation End-Product, g/ 100 g DM	Aerobic Exposure (d)	Length of Fermentation (d)		SEM[a]	Probability		
		40	100		L[b]	D[c]	L*D[d]
Acetic acid	0	0.60[e]	0.66[f]	.056	.001	.001	.001
	1	0.33[f]	0.93[e]				
	3	0.33[f]	0.89[e]				
	5	0.19[g]	0.59[f]				
	7	0.25[f]	0.48[e]				
Lactic acid	0	3.10	2.47	.124	.001	.001	.382
	1	2.05	1.72				
	3	1.69	1.50				
	5	1.48	1.13				
	7	0.81	0.63				
Ethanol	0	0.48[F]	0.47[F]	.058	.001	.001	.045
	1	0.96[E]	0.77[E]				
	3	0.55[F]	0.29[G]				
	5	0.37[G]	0.14[H]				
	7	0.49[F]	0.11[H]				

[a] Standard error of the mean
[b] Effect of length of fermentation
[c] Effect of length of aerobic exposure
[d] Interaction of length of fermentation by length of aerobic exposure
[ef] Means with unlike superscripts in the same column within a fermentation end-product heading differ ($P<0.01$)
[FG] Means with unlike superscripts in the same column within a fermentation end-product heading differ ($P<0.05$)

Table 3-23. Effect of length of fermentation and length of aerobic exposure on water soluble carbohydrate contents of forage sorghum silage exposed to air in a tropical environment

Carbohydrate, g/ 100 g DM	Aerobic Exposure (d)	Length of Fermentation (d)		SEM[a]	Probability		
		40	100		L[b]	D[c]	L*D[d]
Glucose	0	0.24	0.17	0.027	.001	.163	.076
	1	0.25	0.14				
	3	0.23	0.15				
	5	0.13	0.13				
	7	0.20	0.11				
Fructose	0	0.19[e]	0.11[e]	0.05	.007	.001	.001
	1	0.12[f]	0.08[f]				
	3	0.13[f]	0.09[f]				
	5	0.10[f]	0.03[g]				
	7	0.09[f]	0.03[g]				
Galactose	0	0.12	0.09	0.024	.025	.185	.274
	1	0.10	0.17				
	3	0.07	0.10				
	5	0.06	0.14				
	7	0.07	0.09				
Xylose	0	0.15	0.13	0.017	.026	.001	.229
	1	0.09	0.15				
	3	0.06	0.11				
	5	0.06	0.09				
	7	0.06	0.07				
Arabinose	0	0.08[ef]	0.11[f]	0.015	.805	.001	.001
	1	0.08[ef]	0.16[e]				
	3	0.09[e]	0.08[g]				
	5	0.07[f]	0.02[h]				
	7	0.09[e]	0.03[h]				

[a] Standard error of the mean
[b] Effect of length of fermentation
[c] Effect of length of aerobic exposure
[d] Interaction of length of fermentation by day of aerobic exposure
[efgh] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.01$)

Table 3-24. Effects of silage additives and length of fermentation on pH, temperature, and microbial population of forage sorghum silage exposed to air in a tropical environment

Item	Silage Additive	Length of Fermentation (d) 40	100	SEM[a]	Probability A[b]	L[c]	A*L[d]
pH	No additive	5.35	5.17	.091	.718	.975	.172
	Enzyme	5.24	5.20				
	Inoculant	5.14	5.25				
	E +I	5.21	5.38				
Temperature (°C)	No additive	32.69	31.67	.023	.451	.001	.886
	Enzyme	33.04	31.81				
	Inoculant	33.01	32.01				
	E + I	33.14	31.84				
Microbial Group[e]							
Yeasts and molds	No additive	6.92	5.92	.090	.354	.001	.062
	Enzyme	6.73	5.88				
	Inoculant	6.55	5.96				
	E + I	6.63	6.04				
Total bacteria	No additive	7.06	6.50	.059	.939	.001	.236
	Enzyme	7.09	6.43				
	Inoculant	6.99	6.57				
	E + I	7.06	6.53				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of length of fermentation
[d] Interaction of silage additive by length of fermentation
[e] cfu/ g of fresh material

Table 3-25. Effects of silage additives and length of fermentation on fermentation end-products of forage sorghum silage exposed to air in a tropical environment

Fermentation End-Product, g/ 100 g DM	Silage Additive	Length of Fermentation (d) 40	100	SEM[a]	Probability A[b]	L[c]	A*L[d]
Acetic acid	No additive	0.33	0.72	.051	.992	.001	.336
	Enzyme	0.29	0.75				
	Inoculant	0.34	0.70				
	E +I	0.39	0.67				
Lactic acid	No additive	1.75	1.35	.111	.070	.001	.495
	Enzyme	1.99	1.55				
	Inoculant	1.95	1.56				
	E + I	1.62	1.49				
Ethanol	No additive	0.08[e]	0.06	.006	.001	.108	.006
	Enzyme	0.07[e]	0.06				
	Inoculant	0.05[f]	0.07				
	E + I	0.06[f]	0.07				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of length of fermentation
[d] Interaction of silage additive by length of fermentation
[e,f] Means with unlike superscripts in the same column within a fermentation end-product heading differ (P<0.01)

Table 3-26. Effect of silage additives and length of fermentation on water soluble carbohydrate contents of forage sorghum silage exposed to air in a tropical environment

Carbohydrate, g/ 100 g DM	Silage Additive	Length of Fermentation (d) 40	100	SEM[a]	Probability A[b]	L[c]	A*L[d]
Glucose	No additive	0.21	0.18	0.02	.631	.006	.146
	Enzyme	0.20	0.15				
	Inoculant	0.20	0.16				
	E +I	0.23	0.10				
Fructose	No additive	0.10	0.09	0.01	.168	.007	.871
	Enzyme	0.12	0.07				
	Inoculant	0.15	0.11				
	E + I	0.14	0.10				
Galactose	No additive	0.07	0.12	0.02	.192	.025	.695
	Enzyme	0.11	0.13				
	Inoculant	0.07	0.08				
	E + I	0.09	0.14				
Xylose	No additive	0.07	0.12	0.01	.074	.021	.619
	Enzyme	0.12	0.12				
	Inoculant	0.07	0.09				
	E + I	0.09	0.12				
Arabinose	No additive	0.05	0.08	0.01	.164	.805	.060
	Enzyme	0.09	0.07				
	Inoculant	0.08	0.08				
	E + I	0.11	0.08				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of length of fermentation
[d] Interaction of silage additive by length of fermentation

after a short storage period.

CHAPTER 4

MICROBIAL INOCULANT AND ENZYMES IN FORAGE SORGHUM ENSILED AT TWO STAGES OF MATURITY IN A TROPICAL ENVIRONMENT.

Abstract

An experiment was conducted to evaluate the beneficial effects of silage additives (enzymes and inoculant) on the ensiling characteristics and aerobic stability of forage sorghum ensiled at two stages of maturity in a tropical environment. Forage sorghum was harvested after 90 (22.75% DM) and 110 d (32.73% DM) of growth in the Lajas Agricultural Experiment Station, University of Puerto Rico, and was mechanically chopped into 2.5 cm pieces. Chopped forage at each stage of maturity was assigned to one of four treatments; no additive (control), enzymes (.1% of fresh material), inoculant (10^6 cfu/g of fresh material) and enzymes plus inoculant, and placed into PVC silos. Three silos per treatment at each stage of maturity were opened after 6 ensiling periods (0, 1, 3, 7, 21, and 100 d) and analyzed for plant organic acids (citric, malic, oxaloacetic, and succinic acids), pH, microbial succession (enterobacteriaceae, lactic acid bacteria, yeasts and molds, and lactate assimilating yeast) silage fermentation end-

products (organic acids and ethanol) and water soluble carbohydrates (glucose, fructose, galactose, xylose, and arabinose). Structural carbohydrates (NDF, ADF, hemicellulose, and cellulose) were determined at d 0 and 100 post-ensiling. For aerobic stability studies, duplicate silos for each treatment at each stage of maturity were opened after 100 d post-ensiling and exposed to air for 7 d. Acidity, fermentation end-products, and water soluble carbohydrates were determined after 0, 3, and 7 d. Temperature was monitored daily. Fermentation patterns of forage sorghum silage varied with stage of maturity. Addition of lactic acid bacterial inoculant alone or in combination with enzymes improved the ensiling characteristics of forage sorghum, but a more beneficial response was observed in the more immature silage. A greater deterioration of forage sorghum silage ensiled at 90 or 110 d occurred within the first 3 d of aerobic exposure. Silage additives did not prevent the aerobic deterioration of forage sorghum silage, regardless of stage of maturity.

Introduction

As plant mature, numerous changes occur in plant composition (Van Soest, 1994), and epiphytic microflora (Woolford, 1984) which may influence the fermentation process and the effectiveness of silage additives (Satter et al., 1991). In temperate environments, ensiling of forages at different stages of maturity with or without silage additives has been the subject of many

studies. In one study, a different fermentation pattern was observed when forage sorghum was ensiled with silage additives at various stages of maturity (Black et al, 1980). Bolsen et al. (1992), reported that final pH in alfalfa silage decreased as stage of maturity advanced.

Likewise, the effect of maturity (Stokes, 1992) and silage additives (Rust et al., 1989) on aerobic deterioration of forages ensiled in temperate environments have shown variable results. Forage sorghum ensiled at the end of flowering stage was more stable than sorghum ensiled at milk stage (Weinberg et al., 1993). Cleare et al (1990) found that inoculated corn silage had lower pH after 48 and 72 h of aerobic exposure than untreated corn silage. Moon et al. (1983), reported than inoculation of wheat silage with bacteria or fungi accelerated aerobic deterioration after 4 d of aerobic exposure.

In tropical environments, silage fermentation is different than typically observed in a temperate climate (McDonald, 1991), and a different response to aerobic exposure would be expected. Additionally, forages mature at a faster rate in tropical environments (Van Soest et al., 1994). In previous chapters (3 and 4) we demonstrated that the application of a lactic acid bacterial inoculant alone or in combination with enzymes decreased the pH, and increased lactic acid bacteria and lactic acid content in forage sorghum ensiled at 90 d of growth, but did not prevent aerobic deterioration. However, the diversity of microorganisms on the fodder plant at different

stages of maturity prior to ensiling and their subsequent growth during silage fermentation in tropical climates in unknown. In addition, the effects of silage additives on the ensiling characteristics and aerobic stability of tropical forage ensiled at different stages of maturity is not well documented. The objective of this experiment was to determine the effect of a homofermentative lactic acid bacterial inoculant and a commercial enzyme preparation on the ensiling characteristics and aerobic stability of forage sorghum ensiled at two stages of maturity in a tropical environment.

Experimental Procedure

Forage Harvesting and Ensiling

Forage sorghum (Hi Energy Hybrid, Agri-pro Seed, Hereford, TX) was harvested at 90 (23.75% DM) and 110 (32.73% DM) d of growth at the Lajas Agriculture Experiment Station, University of Puerto Rico (67°00' west longitude, 18°00' north latitude), located at the southwest end of the island 27 m above the sea. Harvested forage sorghum at both stages of maturity was chopped mechanically into 2.5 cm pieces with a forage harvester. Chopped forage was analyzed for DM (55°C for 72 h), ash (550°C for 12 h), total-N (AOAC, 1990), buffering capacity (Playne and McDonald, 1966), organic acid content, water soluble and structural carbohydrates (Van Soest et al., 1991, Method A), and epiphytic microbial populations. Vegetative material prior to ensiling at each stage of maturity

was treated with one of four treatments: no additive (control), enzymes (Viscozyme™L, Novo Nordisk Bioindustrials, Inc. Farhmam, Survey, UK), lactic acid bacterial inoculant (Ecosyl™, Zeneca Bioproducts, Farnham, Survey, UK), and enzymes plus inoculant. The enzyme additive consisted of a multi-enzyme preparation containing arabinase, cellulase, ß-glucanase, hemicellulase and xylanase and was applied at .1% of fresh material. The microbial inoculant consisted of *Lactobacillus plantarum* and was applied at 10^6 cfu/g of fresh material. Treatments were applied to weighed portions (1.6 kg) of forage, manually mixed, and packed into laboratory silos fitted with release valves to allow gas escape. Laboratory silos were maintained at room temperature (27-30°C) until opened. The control treatment received a similar amount of water as forage treated with the additives.

Sample collection and analysis

Triplicate silos for each treatment at each stage of maturity were analyzed for plant organic acid content, pH, microbial succession, silage fermentation end-products, and water soluble carbohydrates after 0, 1, 3, 7, 21, and 100 d post-ensiling. Fifty g of forage from each silo were placed into 450 ml of distilled water and homogenized for 5 minutes with a stomacher apparatus (Tekman 3500, Cincinnati, OH). Homogenates were strained through 8 layers of cheesecloth and analyzed for pH with a pH meter fitted with a combination electrode (Fisher Scientific, Pittsburgh, PA). For microbial

succession determinations, tenfold dilutions of the clarified homogenate were prepared for each sample in sterile peptone solution (.1%) and enumerated for four distinct groups of microorganisms. Plates for all groups of microbes were poured with selective media and enumerated after specific incubation periods. Microbial groups included: lactic acid bacteria (Rogosa SL agar, Difco Laboratories, Detroit, MI) enumerated after 2 d of incubation, Enterobacteriaceae (Violet red agar, Difco, Laboratories, Detroit, MI, modified with 5% glucose) enumerated after 1 d of incubation, yeasts and molds (Rose bengal agar supplemented with chloramphenicol, Difco Laboratories, Detroit, MI) enumerated after 7 d, and lactate assimilating yeast (Yeast nitrogen base supplemented with .01% lactic acid and 1.5 % agar; Difco Laboratories, Detroit, MI) enumerated after 3 d of incubation.

Plant organic acids (citric, malic, oxaloacetic, and succinic acids), and silage fermentation end-products (lactic, acetic, propionic and butyric acids, and ethanol) were determined by ion exchange-exclusion high performance liquid chromatography (BIORAD aminex HPX-87H) following the general procedure of Canale et al., (1984). Mobile phase consisted of .005 N H_2SO_4 at a flow rate of .9 ml/min. Column was maintained at 65°C by an external column heater (Waters Millipore). Three ml of homogenate from each treatment, maturity, and ensiling period were filtered through 2 μm ion chromatography syringes filters (Gelman Acrodisk, 25 mm, Ann Arbor, MI) into 4 ml HPLC sample vials (National Scientific, Atlanta, GA). Filtered samples were stored

at -20°C until analysis. Fifteen μl of the filterate were injected by an autoinjector (Water WISP 712) and analytes was detected by refractive index (Waters 410 refractive index detector). Peak heights were quantified by a commercial HPLC software program (Turbochem 3, PE Nelson) and compared to commercial standards. Water soluble carbohydrates were also determined by ion exchange chromatography (Biorad aminex HPX - 87P), except that 20 μl of clarified extract were injected. Millipore water at a flow rate of .6 ml/min was used as the mobile phase, and column temperature was maintained at 85°C. Structural carbohydrates; NDF, ADF, hemicellulose (calculated as the difference between NDF and ADF), and cellulose (calculated as the difference between (ADF and lignin), were determined after 0 and 100 d post-ensiling (Goering and Van Soest, 1970; Van Soest et al., 1991 Method A).

Aerobic Stability

Duplicate silos from each treatment at each stage of maturity were opened after 100 d of ensiling. After emptying, 400 g from each silo were placed into styrofoam containers lined with plastic, and exposed to air for 7 d. After 0, 3, and 7 d of aerobic exposure; pH, fermentation end-products, and WSC content were determined as previously described. Temperature was monitored twice daily with thermometers embedded in the surface of the exposed silage.

Statistical Analyses

Results from the ensiling process; pH, microbial succession, fermentation-end products, and water soluble carbohydrate contents; were analyzed as a completely randomized design with a 2 (stage of maturity) by 4 (silage additives) by 6 (day of ensiling) factorial arrangement of treatments using the General Linear Model subroutine of SAS (1990) with the following model:

$$Y_{ijkl} = \mu + A_i + B_j + (A*B)_{ij} + C_k + (A*C)_{ik} + (B*C)_{jk} + (A*B*C)_{ijk} + E_{ijkl}$$

Where:

Y_{ijkl} = Individual response variable measured (e.g. pH, fermentation end-product)

μ = Overall mean

A_i = Effect of stage of maturity

B_j = Effect of silage additive

$A*B_{ij}$ = Interaction of stage of maturity by silage additive

C_k = Effect of day of ensiling

$A*C_{ik}$ = Interaction of stage of maturity by day of ensiling

$B*C_{jk}$ = Interaction of silage additive by day of ensiling

$A*B*C_{ijk}$ = Interaction of stage of maturity by silage additive by day of ensiling

E_{ijkl} = residual error

Bonferroni-t tests were used for mean separation. The model for structural carbohydrate content was similar except that two days of ensiling (0 and 100 d) were utilized. Aerobic stability data was analyzed as a completely randomized design with a 2 (stage of maturity) by 4 (silage additives) by 3 (length of aerobic exposure) factorial arrangement of treatments using the General Linear Model Subroutine of SAS (1990). Themodel for aerobic stability was similar to the model for fermentation characteristics except that day of ensiling was substituted by the days of aerobic exposure (e.g. temperature, 8 d; pH, 3 d).

Results and Discussion

Forage Composition

Initial DM, total-N content, NDF, and hemicellulose were higher in forage sorghum harvested at 110 d of growth than in sorghum harvested at 90 d, but pH, and buffering capacity were lower (Table 4-1). Organic matter, cellulose, ADF and lignin contents were similar regardless of stage of maturity. Initial glucose content was higher in forage harvested at 110 d, but galactose and pentose content were lower. Fructose was similar regardless of stage of maturity. Malic and succinic acids were the major organic acids present in forage sorghum prior to ensiling, and were found at similar concentrations for both stages of maturity. Citric acid content was

Table 4-1. Characteristics of forage sorghum harvested at two stages of maturity in a tropical environment

Item	Maturity (days)			
	90	SD	110	SD[a]
Chemical Composition				
DM, %	23.75	0.54	32.73	1.41
OM[b]	93.08	1.02	94.05	0.87
NDF[b]	66.50	2.53	68.02	2.20
ADF[b]	39.99	2.42	39.12	2.41
Hemicellulose[b]	26.30	0.81	28.90	1.06
Cellulose[b]	32.50	2.43	32.92	2.39
Lignin[b]	5.54	0.78	5.39	0.77
Glucose[b]	2.93	0.17	7.25	0.79
Fructose[b]	3.25	0.30	3.61	1.01
Galactose[b]	0.20	0.05	0.09	0.04
Xylose[b]	0.67	0.06	0.05	0.03
Arabinose[b]	0.09	0.02	ND	ND
Total-N[b]	1.04	0.09	0.74	0.01
pH	5.61	0.29	5.44	0.02
Buffering capacity[c]	24.27	2.75	19.65	1.57
Organic Acids				
Citric[b]	0.70	0.20	1.47	0.42
Malic[b]	4.62	0.40	4.88	0.13
Oxaloacetic[b]	1.19	0.17	0.14	0.02
Succinic[b]	3.21	0.65	3.70	0.89
Epiphytic Microflora[d]				
Enterobacteriaceae	5.47	0.96	3.39	0.30
Lactic acid bacteria	3.30	0.70	6.53	0.13
Yeasts and molds	3.14	0.44	4.47	0.45
Lactate assimilating yeasts	2.75	0.41	3.35	0.10

[a] Standard deviation
[b] g/ 100 g DM
[c] meq/ 100 g DM
[d] cfu/g of fresh material
ND = No detectable

lower in the more immature forage, but oxaloacetic acid was higher. All four epiphytic microorganisms enumerated in chopped forage prior to ensiling were higher in sorghum harvested at 110 d as compared to 90 d of growth. As expected, forage sorghum harvested at different stages of maturity in a tropical environment differ in their initial chemical composition and epiphytic microflora. These changes in chemical composition and epiphytic microorganisms in the plant material due to stage of maturity have been extensively reviewed (McDonald et al. 1991; Van Soest, 1994).

Ensiling Characteristics

Significant interactions between maturity and day of ensiling were evident for malic, oxaloacetic and succinic acids (Table 4-2). Over the entire ensiling period, citric acid content was lower ($P<0.01$) in forage sorghum ensiled at 90 d of maturity than in sorghum ensiled at 110 d. For both stages of maturity, citric acid content decreased ($P<0.01$) as length of ensiling increased. Fermentation of malic acid occurred over time in both stages of maturity, but a faster decrease was observed in forage ensiled at 90 d than at 110 d. Oxaloacetic acid decreased ($P<0.01$) over time in sorghum ensiled at 90 d, but remained constant in sorghum ensiled at 110 d. Additionally, oxaloacetic acid levels were greater in the 90 d maturity forage as compared to the 110 d. For both stages of maturity, succinic acid content decreased ($P<0.05$) as length of ensiling increased. In this

Table 4-2. Effects of stage of maturity and day of ensiling on plant organic acid content of forage sorghum ensiled in a tropical environment

Organic Acid, g/ 100 g DM	Day of Ensiling	Maturity (d)		SEM[a]	Probability		
		90	110		M[b]	D[c]	M*L[d]
Citric	0	0.70	1.47	.074	.001	.001	.111
	1	0.45	1.34				
	3	0.40	1.15				
	7	0.46	1.08				
	21	0.31	0.80				
	100	0.16	0.79				
Malic	0	4.62[e]	4.88[e]	.160	.001	.001	.001
	1	2.74[f]	4.79[e]				
	3	2.78[f]	4.76[e]				
	7	2.78[f]	4.39[f]				
	21	2.25[g]	4.09[f]				
	100	1.61[h]	3.40[f]				
Oxaloacetic	0	1.22[e]	0.14[f]	.061	.001	.001	.001
	1	1.29[e]	0.16[ef]				
	3	1.25[e]	0.25[ef]				
	7	1.21[e]	0.26[e]				
	21	1.11[f]	0.27[e]				
	100	0.78[g]	0.17[ef]				
Succinic	0	3.32[e]	3.70[e]	.178	.001	.001	.001
	1	3.09[e]	3.20[f]				
	3	2.63[f]	1.42[g]				
	7	2.45[f]	1.41[g]				
	21	1.92[g]	1.41[g]				
	100	1.31[h]	1.13[h]				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[efgh] Means with unlike superscripts in the same column within an organic acid heading differ ($P < 0.01$)

experiment, all plant organic acids of forage sorghum silage were degraded, regardless of stage of maturity.

Over the ensiling period, a similar trend in pH was observed for both stages of maturity (Table 4-3). Acidity increased ($P<0.01$) as length of ensiling increased in forage ensiled at 90 or 110 d, but a greater decrease in pH values from d 3 to 21 were observed in sorghum ensiled at 110 d of maturity than at 90 d. Significant stage of maturity by day of ensiling interactions for all microbial groups enumerated were observed. For both stages of maturity, lactic acid bacterial populations reached its maximum counts after 3 d post-ensiling, then decreased as length of fermentation increased. A greater increase in lactic acid bacterial populations after 3, 7, and 21 d post-ensiling were observed in forage ensiled at 110 d. Enterobacteriaceae population increased during the first 24 h and then decreased ($P<0.01$) throughout the reminder of the ensiling period. Yeast and mold populations increased ($P<0.01$) at a exponential rate 1 and 3 d post-ensiling in forage sorghum ensiled at 90 and 110 d, respectively, then decreased for the remainder of the ensiling period. For both stages of maturity, growth curve of lactate assimilating yeast followed the same trend as exhibit by yeast and mold populations. However, greater yeast, mold, and lactate assimilating yeast populations were observed in sorghum ensiled at 110 d of maturity.

A different trend in acetic acid content over time was observed between

Table 4-3. Effects of stage of maturity and day of ensiling on pH and microbial succession of forage sorghum ensiled in a tropical environment

Item	Day of Ensiling	Maturity (d)		SEM[a]	Probability		
		90	110		M[b]	D[c]	M*L[d]
pH	0	5.61[f]	5.55[f]	0.03	.001	.001	.001
	1	4.73[g]	4.71[g]				
	3	4.50[h]	4.19[h]				
	7	4.48[hi]	4.16[h]				
	21	4.42[i]	4.20[h]				
	100	4.12[j]	4.12[i]				
Microbial Group[e]							
Lactic acid bacteria	0	4.21[k]	4.22[k]	.101	.001	.001	.001
	1	8.45[g]	8.67[g]				
	3	8.62[f]	9.23[f]				
	7	7.87[h]	8.30[h]				
	21	6.60[i]	6.99[i]				
	100	5.79[j]	5.50[j]				
Enterobacteriaceae	0	5.47[h]	6.54[g]	.234	.001	.001	.001
	1	7.96[f]	7.15[f]				
	3	6.34[g]	4.95[h]				
	7	5.98[g]	5.37[h]				
	21	4.38[i]	3.72[i]				
	100	3.87[j]	3.38[j]				
Yeasts and molds	0	3.14[k]	4.54[i]	.100	.001	.001	.001
	1	5.54[i]	6.34[g]				
	3	6.88[g]	7.08[f]				
	7	7.47[f]	6.96[f]				
	21	5.97[h]	6.17[g]				
	100	4.87[j]	5.80[h]				
Lactate assimilating yeast	0	2.95[k]	3.36[h]	.111	.001	.001	.001
	1	5.03[i]	6.14[g]				
	3	6.24[g]	6.78[f]				
	7	7.09[f]	6.94[f]				
	21	5.82[h]	6.05[g]				
	100	4.59[j]	5.63[h]				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[e] cfu/g of fresh material
[fghijk] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)

stages of maturity (Table 4-4). Acetic acid levels reached maximum values 3 d post-ensiling, and then remained constant or decreased in the 90 or 110 d silage, respectively. Acetic acid content was greater in the 90 d silage as compared to the 110 d silage. Lactic acid was the major fermentation end-product associated with the ensiling of forage sorghum. For both stages of maturity, lactic acid reached its maximum value after 3 d post-ensiling, then decreased thereafter. Lactic acid levels measured between d 1 - 21 were greater for the 90 d maturity forage as compared to 110 d silage. In this study the highest lactic acid levels were not associated with the greatest population of LAB. Small levels of butyric acid were observed after 1 d post-ensiling and remained relatively constant thereafter. Propionic acid levels were small and detected only 21 d post-ensiling. Similar to results obtained in Chapter 2, concentrations of butyric and propionic acids were not of major significance in the fermentation process. For both stages of maturity, ethanol content increased ($P<0.01$) through 7 d post-ensiling, then decreased slightly. The greatest ethanol content was observed on d-7 in the 110 d maturity silage.

Glucose and fructose were the major water soluble carbohydrates present in the fresh forage at both maturities (Table 4-5). Both of these sugars were metabolized rapidly during the ensiling process. The majority of the glucose ($P<0.01$) and fructose ($P<0.05$) was metabolized during the first 24 h. Glucose levels continued to decline in the immature forage whereas the

Table 4-4. Effects of stage of maturity and day of ensiling on fermentation end-products of forage sorghum ensiled in a tropical environment

Fermentation End-Product , g/ 100 g DM	Day of Ensiling	Maturity (d)		SEM[a]	Probability		
		90	110		M[b]	D[c]	M*L[d]
Acetic acid	0	0.08[g]	0.04[h]	.032	.001	.001	.001
	1	0.25[f]	0.25[g]				
	3	0.71[e]	0.50[e]				
	7	0.74[e]	0.31[f]				
	21	0.69[e]	0.28[fg]				
	100	0.72[e]	0.24[g]				
Lactic acid	0	0.09[j]	0.10[j]	.180	.001	.001	.001
	1	1.44[h]	1.15[i]				
	3	7.24[e]	5.73[f]				
	7	7.23[e]	4.52[g]				
	21	6.05[f]	4.29[g]				
	100	3.20[g]	3.35[h]				
Propionic acid	0	0.00[f]	0.00[f]	.010	.548	.001	.003
	1	0.00[f]	0.00[f]				
	3	0.00[f]	0.00[f]				
	7	0.00[f]	0.00[f]				
	21	0.03[e]	0.01[e]				
	100	0.02[e]	0.03[e]				
Butyric acid	0	0.00	0.00	.010	.899	.001	.150
	1	0.04	0.04				
	3	0.04	0.06				
	7	0.05	0.05				
	21	0.06	0.06				
	100	0.03	0.04				
Ethanol	0	0.08[h]	0.06[h]	.060	.198	.001	.001
	1	0.47[g]	0.55[g]				
	3	0.66[f]	1.41[f]				
	7	0.81[e]	1.85[e]				
	21	0.57[f]	1.80[e]				
	100	0.45[g]	0.62[g]				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[efgh] Means with unlike superscripts in the same column within a fermentation end-product heading differ ($P<0.01$)

levels were unchanged after 3 d in the 110 d maturity forage. With fructose, the levels declined throughout the fermentation period regardless of maturity. Galactose levels in the 90 d silage were similar througout the fermentation period, whereas levels increased over time in the mature forage. As a result of these trends, the final concentration of galactose was higher in the mature silage. It also appears that galactose was not metabolized by the microbial ecosystem. Xylose content decreased ($P<0.01$) over time in the 90 d silage, but increased slightly in the 110 d material. This would suggest some hemicellulose degradation was occurring in the silage prepared from the 90 d maturity forage sorghum. Arabinose levels tended to increase ($P<0.01$) during the fermentation period with both degrees of maturity, however, the levels were small. Neutral detergent fiber, hemicellulose and cellulose levels were greater ($P<0.01$) in silage made from the more mature forage as compared to the 90 d forage (Table 4-6). The NDF content decreased ($P<0.01$) during the ensiling process with both maturities. This loss in NDF content was the result of hemicellulose hydrolysis which could result from microbial activity or the acid conditions. Hemicellulose content was also lower ($P<0.01$) in the 90 d fresh forage than 110 d. Acid detergent fiber levels were similar across maturities and length of ensiling.

Results from this experiment indicate that ensiling characteristics of forage sorghum ensiled in a tropical environment varied with stage of maturity.

Table 4-5. Effects of stage of maturity and day of ensiling on water soluble carbohydrate contents of forage sorghum ensiled in a tropical environment

Carbohydrate, g/ 100 g DM	Day of Ensiling	Maturity (d)		SEM[a]	Probability		
		90	110		M[b]	D[c]	M*L[d]
Glucose	0	2.93[e]	7.25[f]	.098	.001	.001	.001
	1	0.64[f]	0.94[f]				
	3	0.51[fg]	0.47[g]				
	7	0.44[gh]	0.56[g]				
	21	0.36[H]	0.65[g]				
	100	0.23[I]	0.67[g]				
Fructose	0	3.25[E]	3.61[E]	.121	.001	.001	.030
	1	0.49[F]	1.50[F]				
	3	0.36[F]	1.03[G]				
	7	0.38[F]	0.84[G]				
	21	0.30[F]	0.66[H]				
	100	0.13[G]	0.39[I]				
Galactose	0	0.20	0.09[h]	.112	.001	.001	.001
	1	0.10	0.56[f]				
	3	0.08	0.21[g]				
	7	0.23	0.64[f]				
	21	0.18	1.21[e]				
	100	0.14	1.44[e]				
Xylose	0	0.67[e]	0.05[f]	.039	.001	.001	.001
	1	0.69[e]	0.10[f]				
	3	0.57[e]	0.10[f]				
	7	0.65[e]	0.12[f]				
	21	0.55[e]	0.13[f]				
	100	0.19[f]	0.25[e]				
Arabinose	0	0.09[fg]	0.00[f]	.009	.001	.001	.001
	1	0.08[gh]	0.00[f]				
	3	0.07[h]	0.01[f]				
	7	0.08[gh]	0.04[f]				
	21	0.10[ef]	0.06[f]				
	100	0.12[e]	0.15[e]				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[efgh] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.01$)
[EFGHI] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.05$)

Table 4-6. Effects of stage of maturity and day of ensiling on structural carbohydrate contents of forage sorghum ensiled in a tropical environment

Carbohydrate, g/ 100 g DM	Day of Ensiling	Maturity (d)		SEM[a]	Probability		
		90	110		M[b]	D[c]	M*L[d]
NDF	0	65.84	68.02	.551	.012	.001	.204
	100	63.04	63.79				
ADF	0	39.62	39.13	.564	.207	.885	.677
	100	39.94	39.97				
Hemicellulose	0	26.26[e]	28.90[e]	.337	.001	.001	.005
	100	24.18[f]	24.82[f]				
Cellulose	0	32.25	38.73	.770	.001	.938	.403
	100	32.96	38.14				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of day of ensiling
[d] Interaction of silage additive by day of ensiling
[ef]Means with unlike superscripts in the same column within a carbohydrate heading differ (P<0.01)

Based on changes in fermentation end-products throughout the ensiling period, it appears that the microbial ecology and substrate utilization differ when forage sorghum was ensiled at different stages of maturity. The smaller enterobacteriaceae population and lower acetic acid content found in the 110 d silage at early stages of the fermentation is likely a result of the lower pH observed. Additionally, forage sorghum ensiled at 90 d had greater oxaloacetic acid content than forage ensiled at 110 d. Oxaloacetic acid has a stronger buffering capacity and has been shown that influence the pH of silages (Playne and McDonald, 1966). The greater increase in lactic acid bacterial populations in the 110 d silage did not result in higher lactic acid content, which may indicate that a more active lactic acid bacterial population may be present in forage ensiled at 90 d or substrate was limiting. Yeast and mold populations seem to play a predominant role in the fermentation process of forage sorghum ensiled at 110 d, as evidenced by the higher ethanol content during the initial one third of the fermentation process.

In forage sorghum ensiled at 90 d, the decrease in lactic acid, and increase in acetic acid production at later stages of the fermentation process may indicate a shift in lactic acid bacteria from homofermentative to heterofermentative. Substrate availability and utilization was variable in forage ensiled at 90 d as evidenced by changes in water soluble (e.g. xylose, arabinose) and structural carbohydrate (e.g. hemicellulose) contents.

In forage sorghum ensiled at 110 d, it appears that a limited microbial activity occurs after the first 21 d post-ensiling as evidenced by changes in fermentation end-products, water soluble and structural carbohydrates. Hemicellulose hydrolysis was observed in sorghum ensiled at both stages of maturity. However, this decrease in hemicellulose content did not result in higher xylose content in forage sorghum ensiled at 90 d; which may indicate a greater rate of xylose utilization than xylose degradation. In forage sorghum ensiled at 110 d, the decreased hemicellulose after 100 d post-ensiling may correspond to the increase in galactose and pentose content. These changes in structural carbohydrates and water soluble sugars in the more mature silage may indicate a greater hemicellulose hydrolysis than utilization, and support the observation of a limited microbial activity in the more mature silage after the initial third of the fermentation period. Even though fermentation patterns seem to differ over time in forage sorghum ensiled at 90 and 110 d, final pH, and fermentation end-products (except acetic acid) were similar. However, the residual WSC were higher in the silage from the more mature forage. These results support the observations of Tjandraatmadja et al. (1994) who found that final pH and residual WSC increased with increasing maturity in forages ensiled in tropical environments. Panditharatne et al. (1988), also reported different fermentation patterns in guinea grass ensiled in a tropical environment after two regrowth periods.

Silage additives did not influence the organic acid hydrolysis in forage sorghum regardless of stage of maturity (Data not showed). Forage sorghum ensiled at 90 or 110 d and treated with microbial inoculant alone or in combination with enzymes had lower pH ($P<0.01$) than control silage or silage treated only with the enzyme mixture (Table 4-7). The microbial inoculant treatments had greater ($P<0.05$) lactic acid bacterial population than non-inoculated silages regardless of maturity stage of the forage. Silage additives did not influence other populations of microbial groups and did not alter the levels of acetic, propionic, butyric acids or ethanol (Table 4-8). Lactic acid levels were significantly greater ($P<0.01$) for silages treated with the microbial inoculant. Previous results (Chapter 2) indicated that the microbial inoculant decreased glucose, but increased fructose content in forage sorghum ensiled after 90 d. In this experiment, small increases in galactose and arabinose contents in forage sorghum treated the enzyme was observed (Table 4-9; $P<0.01$). The other sugars were a similar levels for the four treatments. Even though WSC contents were not significantly influenced by silage additives, a numerical trend for lower glucose and greater fructose content due to inoculation was observed, which support the results from Chapter 2. Structural carbohydrate contents were similar for all treatments (Table 4-10). Results from this experiment indicated that the beneficial changes due to inoculation (pH, lactic acid bacterial population, and fermentation end-products) were greater in forage sorghum

Table 4-7. Effects of silage additives and stage of maturity on pH and microbial succession of forage sorghum ensiled in a tropical environment

Item	Silage Additive	Maturity (d) 90	Maturity (d) 110	SEM[a]	Probability A[b]	Probability M[c]	Probability A*M[d]
pH	No additive	4.78[e]	4.57[e]	.03	.001	.001	.001
	Enzyme	4.80[e]	4.53[e]				
	Inoculant	4.51[f]	4.41[f]				
	E+I	4.50[f]	4.38[f]				
Microbial Group[e]							
Lactic acid bacteria	No additive	6.53	7.01	.08	.001	.001	.675
	Enzyme	6.75	7.17				
	Inoculant	7.28	7.57				
	E+I	7.14	7.51				
Enterobacteriaceae	No additive	5.99	5.39	.20	.133	.001	.663
	Enzyme	5.51	5.31				
	Inoculant	5.56	5.09				
	E+I	5.59	4.96				
Yeasts and molds	No additive	5.72	6.09	.08	.981	.001	.300
	Enzyme	5.71	6.05				
	Inoculant	5.65	6.16				
	E+I	5.50	6.21				
Lactate assimilating yeast	No additive	5.25	5.79	.09	.829	.001	.191
	Enzyme	5.38	5.68				
	Inoculant	5.24	5.88				
	E+I	5.27	5.92				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of stage of maturity
[d] Interaction of silage additive by stage of maturity
[e] cfu/g of fresh material
[fg] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)

Table 4-8. Effects of silage additives and stage of maturity on fermentation end-products of forage sorghum ensiled in a tropical environment

Fermentation End-Product, g/ 100 g DM	Silage Additive	Maturity (d)		SEM[b]	Probability		
		90	110		A[c]	M[d]	A*M[e]
Acetic acid	No additive	0.53	0.29	.026	.816	.001	.085
	Enzyme	0.58	0.24				
	Inoculant	0.51	0.27				
	E+I	0.50	0.29				
Lactic acid	No additive	3.73[f]	3.05[f]	.147	.001	.001	.012
	Enzyme	3.77[f]	3.08[f]				
	Inoculant	4.73[g]	3.31[g]				
	E+I	4.60[g]	3.43[g]				
Propionic acid	No additive	0.01	0.01	.002	.896	.548	.528
	Enzyme	0.01	0.01				
	Inoculant	0.01	0.01				
	E+I	0.01	0.01				
Butyric acid	No additive	0.04	0.04	.001	.702	.820	.215
	Enzyme	0.04	0.04				
	Inoculant	0.05	0.03				
	E+I	0.04	0.05				
Ethanol	No additive	0.45	0.93	.071	.329	.001	.425
	Enzyme	0.53	1.09				
	Inoculant	0.47	1.02				
	E+I	0.44	1.15				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of stage of maturity
[d] Interaction of stage additive by stage of maturity
[f,g] Means with unlike superscripts in the same column within a fermentation end-product heading differ ($P<0.01$)

Table 4-9. Effects of silage additives and stage of maturity on water soluble carbohydrate contents of forage sorghum ensiled in a tropical environment

Carbohydrate, g/ 100 g DM	Silage Additive	Maturity (d)		SEM[a]	Probability		
		90	110		A[b]	M[c]	M*A[d]
Glucose	No additive	0.88	1.88	.080	.401	.001	.765
	Enzyme	0.87	1.76				
	Inoculant	0.83	1.66				
	E+I	0.83	1.73				
Fructose	No additive	0.80	1.21	.099	.208	.001	.619
	Enzyme	0.78	1.25				
	Inoculant	0.84	1.36				
	E+I	0.86	1.53				
Galactose	No additive	0.18	0.53	.092	.092	.001	.139
	Enzyme	0.17	0.94				
	Inoculant	0.12	0.59				
	E+I	0.14	0.71				
Xylose	No additive	0.57	0.15	.032	.485	.001	.781
	Enzyme	0.58	0.12				
	Inoculant	0.52	0.13				
	E+I	0.55	0.09				
Arabinose	No additive	0.08	0.03	.007	.001	.001	.569
	Enzyme	0.08	0.04				
	Inoculant	0.08	0.04				
	E+I	0.12	0.06				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of stage of maturity
[d] Interaction of silage additive by stage of maturity

Table 4-10. Effects of silage additives and stage of maturity on structural carbohydrate contents of forage sorghum ensiled in a tropical environment

Carbohydrate, g/ 100 g DM	Silage Additive	Maturity (d)		SEM[a]	Probability		
		90	110		A[b]	M[c]	M*A[d]
NDF	No additive	64.16	66.15	.780	.878	.012	.850
	Enzyme	63.91	65.83				
	Inoculant	64.64	65.68				
	E+I	65.05	65.97				
ADF	No additive	41.31	39.31	.798	.301	.201	.171
	Enzyme	38.93	39.85				
	Inoculant	39.00	39.17				
	E+I	39.87	37.86				
Hemicellulose	No additive	23.10	26.84	.474	.135	.001	.142
	Enzyme	24.98	25.98				
	Inoculant	25.64	26.50				
	E+I	25.18	28.10				
Cellulose	No additive	33.38	38.53	1.08	.568	.001	.435
	Enzyme	31.95	39.85				
	Inoculant	31.67	37.51				
	E+I	33.42	37.86				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of stage of maturity
[d] Interaction of silage additive by stage of maturity

ensiled at 90 d of maturity than in forage ensiled at 110 d. Similar to results from Chapter 2, the enzyme mixture did not improve the ensiling characteristics of forage sorghum regardless of stage of maturity.

Aerobic Stability

Acidity of forage sorghum silage decreased ($P<0.01$) as length of exposure increased with both maturities(Table 4-11). A much larger increase ($P<0.05$) in pH occurred in the 110 d maturity forage than the more immature forage. Maximum temperature during air exposure was attained within 1 d and 2 d for the 90 and 110 d maturity silages, respectively. For both stages of maturity, acetic acid was lower ($P<0.01$) after 3 d of aerobic exposure than d 0, but unchanged thereafter (Table 4-12). Lactic acid levels were decreased ($P<0.01$) by 75 and 63% during the exposure period for the 90 and 110 d maturity forages. Clearly, the lactic acid was metabolized by the spoilage organisms. Ethanol content was higher ($P<0.01$) after 3 d of aerobic exposure than d 0 in the 90 d forage. An opposite trend was observed in the 110 d maturity forage. Ethanol content was lower ($P<0.05$) after 3 and 7 d
of air exposure as compared to d 0. In the immature forage, ethanol was produced during the exposure period whereas it disappeared with the more mature forage. A net disappearance of glucose during the exposure period was not observed in this study (Table 4-13). However, the residual glucose

Table 4-11. Effects of stage of maturity and length of aerobic exposure on pH and temperature of forage sorghum silage exposed to air in a tropical environment

Item	Aerobic Exposure (d)	Maturity (d)		SEM[a]	Probability		
		90	110		M[b]	D[c]	M*D[d]
pH	0	4.07[e]	4.12[e]	0.04	.001	.001	.001
	3	4.25[f]	5.06[f]				
	7	4.28[f]	5.23[g]				
Temperature (°C)	0	30.75[h]	29.30[h]	0.62	.001	.001	.003
	1	35.90[e]	30.55[e]				
	2	35.53[e]	32.63[f]				
	3	35.85[e]	32.98[f]				
	4	33.71[f]	31.52[g]				
	5	31.78[g]	30.76[g]				
	6	33.15[f]	29.79[h]				
	7	31.43[g]	29.51[h]				

[a] Standard error of the mean
[b] Effect of stage of maturity
[c] Effect of length of aerobic exposure
[d] Interaction of stage of maturity by length of aerobic exposure
[efgh] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)

Table 4-12. Effects of stage of maturity and length of aerobic exposure on fermentation end-products of forage sorghum silage exposed to air in a tropical environment

Fermentation End-Product, g/ 100 g DM	Aerobic Exposure (d)	Maturity (d)		SEM[a]	Probability		
		90	110		M[b]	D[c]	M*D[d]
Acetic acid	0	0.71[e]	0.23[e]	0.03	.001	.001	.001
	3	0.39[f]	0.12[f]				
	7	0.33[f]	0.09[f]				
Lactic acid	0	3.37	3.29	0.28	.585	.001	.702
	3	1.41	1.49				
	7	0.84	1.22				
Ethanol	0	0.30[f]	0.54[e]	0.05	.009	.115	.001
	3	0.43[e]	0.17[f]				
	7	0.52[e]	0.16[f]				

[a] Standard error of the mean
[b] Effect of stage of maturity
[c] Effect of days of aerobic exposure
[d] Interaction of stage of maturity by length of aerobic exposure
[ef] Means with unlike superscripts in the same column within a fermentation end-product heading differ ($P<0.01$)

Table 4-13. Effects of stage of maturity and length of aerobic exposure on water soluble carbohydrate contents of forage sorghum exposed to air in a tropical environment

Carbohydrate, g/100 g DM	Aerobic Exposure (d)	Maturity (d)		SEM[a]	Probability		
		90	110		M[b]	D[c]	M*D[d]
Glucose	0	0.29	0.67	0.07	.001	.986	.351
	3	0.41	0.57				
	7	0.35	0.62				
Fructose	0	0.15[e]	0.38[e]	0.03	.001	.001	.001
	3	0.13[e]	0.14[f]				
	7	0.07[f]	0.09[g]				
Galactose	0	0.14	1.48[e]	0.05	.001	.001	.001
	3	0.18	0.03[f]				
	7	0.14	0.02[f]				
Xylose	0	0.19[e]	0.25[e]	0.02	.020	.023	.014
	3	0.19[e]	0.03[f]				
	7	0.13[f]	0.02[f]				
Arabinose	0	0.11	0.16	0.02	.086	.001	.063
	3	0.09	0.07				
	7	0.05	0.09				

[a] Standard error of the mean
[b] Effect of stage of maturity
[c] Effect of day of aerobic exposure
[d] Interaction of stage of maturity by length of aerobic exposure
[e,f] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P < 0.01$)

levels from d 0 were greater for the mature forage (110 d) as compared to the less mature (90 d). Fructose ($P<0.01$), xylose ($P<0.01$), and arabinose ($P<0.06$) content decreased over the period of aerobic exposure with both forage maturities. Galactose levels decreased ($P<0.01$) during the exposure period with the mature forage with little change in the immature forage. Results from this experiment indicate that for both stages of maturity, the majority of the deterioration occurred within the first 3 d of aerobic exposure. However, based on differences in utilization of fermentation end-products and residual water soluble carbohydrates of the silages during exposure to air, it appears that the microbial ecology associated with the deterioration (e.g. yeasts and molds; sugar utilizing or lactate assimilating yeasts) was different between stages of maturity.

All treatments added to the immature forage sorghum and ensiled had similar pH values upon exposure to air (Table 4-14). However, with forage sorghum ensiled at 110 d, silage treated with bacterial inoculant alone or in combination with enzymes had higher ($P<0.01$) pH than sorghum ensiled without an inoculant. Temperature tended to be greater ($P<0.01$) for the immature forage as compared to the mature forage, and the inoculated silages also had greater ($P<0.01$) temperatures.

A significant interaction between silage additive and stage of maturity for acetic and lactic acids was not observed (Table 4-15). Ethanol content was higher ($P<0.05$) in forage ensiled at 90 d of growth and containing the

Table 4-14. Effects of silage additives and stage of maturity on pH and temperature of forage sorghum silage exposed to air in a tropical environment

Item	Silage Additive	Maturity (d)		SEM[a]	Probability		
		90	110		A[b]	M[c]	A*M[d]
pH	No additive	4.25	4.28[f]	0.05	.001	.001	.001
	Enzyme	4.24	4.30[f]				
	Inoculant	4.19	5.36[e]				
	E+I	4.15	5.28[e]				
Temperature (°C)	No additive	32.54	29.47	0.44	.001	.001	.130
	Enzyme	33.50	29.89				
	Inoculant	33.56	31.73				
	E+I	34.42	32.43				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of stage of maturity
[d] Interaction of silage additive by stage of maturity
[e,f] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)

Table 4-15. Effects of silage additives and stage of maturity on fermentation end-products of forage sorghum silage exposed to air in a tropical environment

Fermentation End-Product, g/100 g DM	Silage Additive	Maturity (d) 90	Maturity (d) 110	SEM[a]	Probability A[b]	Probability M[c]	Probability A*M[d]
Acetic acid	No additive	0.43	0.14	0.04	.141	.001	.163
	Enzyme	0.59	0.15				
	Inoculant	0.39	0.16				
	E+I	0.50	0.15				
Lactic acid	No additive	1.85	2.34	0.32	.310	.585	.748
	Enzyme	2.09	2.32				
	Inoculant	1.72	1.58				
	E+I	1.84	1.75				
Ethanol	No additive	0.32^{G}	0.39^{E}	0.06	.408	.009	.047
	Enzyme	0.48^{EF}	0.34^{EF}				
	Inoculant	0.37^{FG}	0.23^{FG}				
	E +I	0.50^{E}	0.18^{G}				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of stage of maturity
[d] Interaction of silage additive by stage of maturity
[efg] Means with unlike superscripts in the same column within a fermentation end-product heading differ ($P<0.05$)

enzyme mixture than in control silage or silage treated with microbial inoculant only. In forage sorghum ensiled at 110 d of growth, inoculated silages had lower ($P<0.05$) ethanol content than non-inoculated silages. A significant interaction between silage additive and stage of maturity was observed ($P<0.01$) for fructose, xylose, and arabinose (Table 4-16). Most of the changes in these sugars occurred in the more mature forages. Fructose levels were lowest in the inoculated treatments, with enzyme only treatment being intermediate. Glucose and galactose were lowest ($P<0.01$) for the inoculated as compared to the control treatments. The enzyme treatments increased the levels of xylose and arabinose in the mature forage during exposure period with smaller effects in the immature forages. Results from this experiment further support the previous conclusion from Chapter 2, that silage additives do not improve the aerobic stability of forage sorghum ensiled in a tropical environment. Rather, for both stages of maturity, addition of microbial inoculant alone or in combination with enzymes appears to increased the rate of deterioration.

Implications

Utilization of silage additives in the form of a microbial inoculant showed little improvement on the fermentation characteristics, and did not prevent the aerobic deterioration of forage sorghum ensiled after 90 or 110 d of growth in a tropical environment. In fact the bacterial inoculant utilized in

Table 4-16. Effects of silage additives and stage of maturity on water soluble carbohydrate contents of forage sorghum silage exposed to air in a tropical environment

Carbohydrate, g/ 100 g DM	Silage Additive	Maturity (d) 90	Maturity (d) 110	SEM[a]	Probability A[b]	Probability M[c]	Probability A*M[d]
Glucose	No additive	0.58	0.70	0.08	.014	.001	.398
	Enzyme	0.34	0.76				
	Inoculant	0.23	0.52				
	E+I	0.25	0.50				
Fructose	No additive	0.12[fg]	0.45[e]	0.04	.001	.001	.001
	Enzyme	0.06[g]	0.20[fg]				
	Inoculant	0.18[ef]	0.12[gh]				
	E+I	0.10[fg]	0.06[h]				
Galactose	No additive	0.22	0.13	0.03	.001	.034	.586
	Enzyme	0.19	0.14				
	Inoculant	0.12	0.07				
	E +I	0.17	0.06				
Xylose	No additive	0.09[ef]	0.12[fg]	0.02	.251	.086	.019
	Enzyme	0.06[g]	0.16[ef]				
	Inoculant	0.10[ef]	0.06[h]				
	E+I	0.08[fg]	0.08[gh]				
Arabinose	No additive	0.21	0.15[g]	0.06	.001	.001	.001
	Enzyme	0.18	0.71[e]				
	Inoculant	0.12	0.31[f]				
	E +I	0.11	0.42[f]				

[a] Standard error of the mean
[b] Effect of silage additive
[c] Effect of stage of maturity
[d] Interaction of silage additive by stage of maturity
[ef gh] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.01$)

this experiment increased the rate of deterioration (e.g. temperature, undesirable microorganisms, substrate availability). Therefore, evaluation of selected bacterial strains more suitable for tropical forages should be conducted to improve the quality of ensiled feedstuffs in tropical areas.

STUDIES ON THE EFFICACY OF A HOMOFERMENTATIVE LACTIC ACID-PRODUCING BACTERIAL INOCULANT AND COMMERCIAL, PLANT CELL-WALL-DEGRADING ENZYME MIXTURES TO ENHANCE THE FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF FORAGES ENSILED IN TEMPERATE AND TROPICAL ENVIRONMENTS

By

Abner Antonio Rodríguez-Carías

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CHAPTER 5

FERMENTATION CHARACTERISTICS OF JOHNSON GRASS ENSILED AT TWO REGROWTH PERIODS WITH SILAGE ADDITIVES

Abstract

An experiment was conducted to evaluate the effects of silage additives (microbial inoculant plus enzymes) on the fermentation characteristics of Johnson grass (*Sorghum halapense)* ensiled after two regrowth periods. Johnson grass was harvested at 45 (22.64 DM%) and 110 d of regrowth (43.84 DM%) at the Lajas Agriculture Experiment Station, University of Puerto Rico. Forage at each vegetative stage was placed into PVC laboratory silos and assigned to two treatments: no additive (control) and enzymes plus inoculant. Enzyme was applied at .1% of fresh material and the microbial inoculant at 10^6 cfu/g of fresh material. Three silos per treatment at each regrowth period were opened after 7 ensiling periods (0,1,3,7,14,21 and 100 d) and analyzed for pH, lactic acid-producing bacterial populations (LAB), fermentation end-products (acetic, lactic, and butyric acids, end ethanol), and water soluble carbohydrates (glucose,

fructose, xylose, and arabinose). Structural carbohydrate content was determined at d 0 and 100 post-ensiling. Fermentation characteristics in Johnson grass silage varied due to regrowth period. For both stages of regrowth, Johnson grass treated with microbial inoculant plus enzymes had lower pH and higher lactic acid bacterial populations and lactic acid contents than control silages. Silage additives also decreased butyric acid content in Johnson grass ensiled at 45 d, and ethanol content in the more mature forage.

Introduction

In tropical environments, the introduction of silage as a major feed component into ruminant diets to avoid the fluctuation in forage quantity and quality during the dry season represents an urgent need. Johnson grass (*Sorghum halapense*) is a perennial plant that grows wild in southwest Puerto Rico. Because of its aggressiveness, abundance, and high yield, production of silage could represent a tool to improve its utilization. However, in tropical climates, forages are generally low in water soluble carbohydrates and at ensiling, result in fermentations that fail to achieve acceptable populations of lactic acid producing bacteria (McDonald, 1991). Results from Chapters 2 and 4 indicated that addition of a microbial inoculant alone or in combination with enzymes improved the fermentation characteristics of forage sorghum silage ensiled at 90 or 110 d of growth.

There is limited information regarding the effects of silage additives on the ensiling characteristics of Johnson grass harvested in a tropical environment. The objective of this experiment was to evaluate the effects of an enzyme mixture and bacterial inoculant on the fermentation characteristics of Johnson grass ensiled at two regrowth periods in the tropical environment of Puerto Rico.

Experimental Procedure

The study was conducted at the Lajas Agriculture Experiment Station, University of Puerto Rico. Johnson grass (*Sorghum halapense)* was harvested manually at 45 (22.64 DM%) and 110 d (43.84 DM%) of regrowth and chopped into 2.5 cm pieces. Chopped forage from each vegetative stage was assigned to two treatments; no additive (Control) and enzymes (Viscozyme™L, Novo Nordisk Bioindustrials, Inc. Farnham, Survey, UK) plus microbial inoculant (Ecosyl™, Zeneca Bioproducts, Farnham, Survey, UK), and packed into PVC silos. Laboratory silos were fitted with release valves for provide gas escape and maintained at room temperature. The enzyme additive consisted of an enzyme mixture containing arabinase, cellulase, ß-glucanase, hemicellulase and xylanase and was applied at 0.1% of fresh material. The microbial inoculant consisted of *Lactobacillus plantarum* and was applied to the forage at a rate of 10^6 cfu/g of fresh material. Triplicate samples of each treatment at each vegetative state were

opened at 0,1,3,7,14,21, and 100 d post-ensiling. After silos were opened, fifty g of forage were placed into 450 ml of distilled water and homogenized for 5 minutes in a Stomacher apparatus (Tekmar 3500, Tekmar, Cincinnati, OH). Homogenates were strained through 8 layers of cheesecloth and analyzed for pH with a pH meter fitted with a combination electrode (Fisher Scientific, Pittsburgh, PA). For LAB enumeration, ten-fold serial dilutions were prepared for each sample at each ensiling day in sterile peptone solution (0.01%). Plates were poured with a Rogosa SL agar (Difco Laboratories, Detroit, MI), incubated for 48 h at 40°C and manually enumerated with a digital colony counter. Fermentation end-products were determined by ion exchange-exclusion HPLC (BIORAD aminex HPX-87H) following the general procedures of Canale et al. (1984). Mobile phase consisted of .005 N H_2SO_4 at a flow rate of .9 ml/min. Column temperature was maintained at 65°C by an external column heater (Waters Millipore). Three ml of homogenate from each treatment were filtered through .2 μm ion chromatography syringe filters (Gelman Acrodisk, 25 mm, Ann Arbor, MI) into 3 ml HPLC sample vials (National Scientific, Atlanta, GA). Filtered samples were stored at -20°C until analysis. Fifteen μl of the filtered samples were injected by an autoinjector (Water WISP 712) and analytes were detected by refractive index (Waters 410 refractive index detector). Peak heights were quantified by a commercial HPLC software package (Turbochrom 3, PE Nelson) and compared to standards for the selected

analytes (Supelco).

Water soluble carbohydrates (glucose, fructose, xylose, and arabinose) were also determined by ion exchange-exclusion HPLC (Biorad aminex HPX-87P) except that 20 μl of the filtered sample were injected. Millipore water was used for the mobile phase at a flow rate of .6 ml/min and the column temperature was maintained at 85°C. Structural carbohydrates; NDF, ADF, hemicellulose (calculated as the difference between NDF and ADF), and cellulose (calculated as the difference between ADF and lignin), were determined at 0 and 100 d post-ensiling (Goering and Van Soest, 1970; Van Soest et al., 1991 Method A). Statistical analysis was performed as a completely randomized design with 2 (regrowth periods) by 2 (silage additives) by 6 (ensiling periods) factorial arrangement of treatments (Steel and Torrie, 1978) using the General Linear Model Procedure of SAS (1990). The models for pH, lactic acid-producing bacterial populations, fermentation end-products, and water soluble carbohydrates were as follows:

$$Y_{ijkl} = \mu + A_i + B_j + (A*B)_{ij} + C_k + (A*C)_{ik} + (B*C)_{jk} + (A*B*C)_{ijk} + E_{ijkl}$$

Where:

Y_{ijkl} = Individual response variable measured (e.g. pH, fermentation end-products)

μ = Overall mean

A_i	=	Effect of regrowth period
B_i	=	Effect of silage additive
$A*B_{ij}$	=	Interaction of regrowth period by silage additive
C_k	=	Effect of day of ensiling
$A*C_{ik}$	=	Interaction of regrowth period by day of ensiling
$B*C_{jk}$	=	Interaction of silage additive by day of ensiling
$A*B*C_{ijk}$	=	Interaction of regrowth period by silage additive by day of ensiling
E_{ijkl}	=	random residual error

Bonferroni-t test was used for mean separation (SAS, 1990). The model for structural carbohydrate contents was similar except that only 2 (0 and 100 d) ensiling periods were utilized.

Results and Discussion

The pH of forage sorghum ensiled at 45 d of regrowth was reduced ($P<0.01$) after d 1 post-ensiling and remained constant thereafter (Table 5-1). However, in Johnson grass ensiled at 110 d of regrowth, pH decreased more slowly ($P<0.01$) and after the first 21 d post-ensiling increased to 4.94 by d-100. This would suggest that a less efficient fermentation occurred in the mature forage. Additionally, the final pH was lower in silage made from the immature as compared to mature forage. For both regrowth

Table 5-1. Effect of regrowth period and days of ensiling on pH and lactic acid bacterial population of Johson grass silage

Item	Day of Ensiling	Regrowth Period (d)		SEM[a]	Probability		
		45	110		R[b]	D[c]	R*D[d]
pH	0	5.69[f]	5.85[f]	.081	.001	.001	.001
	1	4.71[g]	4.98[gh]				
	3	4.70[g]	5.14[g]				
	7	4.64[g]	4.93[gh]				
	14	4.77[g]	4.83[h]				
	21	4.67[g]	4.55[i]				
	100	4.64[g]	4.94[gh]				
Lactic acid bacteria[e]	0	4.74[i]	4.66[i]	.116	.001	.001	.001
	1	8.08[f]	7.80[f]				
	3	8.02[f]	7.97[f]				
	7	7.24[g]	7.80[f]				
	14	7.06[g]	6.63[g]				
	21	7.26[g]	6.82[g]				
	100	6.81[h]	6.38[h]				

[a] Standard error of the mean
[b] Effect of regrowth period
[c] Effect of day of ensiling
[d] Interaction regrowth period by day of ensiling
[e] cfu/g of fresh material
[fghi] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)

periods, lactic acid bacterial population reached its maximum counts within 1 d post-ensiling, then decreased throughout the ensiling period. Greater ($P<0.01$) lactic acid bacterial population numbers were found in the more immature silage; which correspond to the higher acidity observed throughout the fermentation process.

Acetic acid levels increased ($P<0.01$) thoughout the fermentation period in the immature forage whereas levels were essentially the same over the fermentation period in the mature forage (Table 5-2). Lactic acid content reached a maximum ($P<0.01$) value by d-3 with forage from both regrowth periods. However, the extent and rate of decline till opening was greater in the silage from the immature forage. Lactic acid levels were very low in all silages in this study. For both regrowth periods, butyric acid was detected throughout the fermentation period. In Johnson grass ensiled at 110 d, concentrations of butyric acid were below the minimum value (.1% DM) to influence the fermentation process (McDonald et al., 1991), but in the more immature silage, butyric acid reached levels higher than .1% DM after 7, 14, and 21 d of fermentation. The greater butyric acid levels found in Johnson grass ensiled after 45 d of regrowth, may result from the high water content of the forage prior to ensiling; which may have promoted the growth of microorganisms associated with butyric acid production (e.g. clostridia, yeasts and molds). Propionic acid was not detected throughout the fermentation period in either regrowth period (Data not shown).

Table 5-2. Effect of regrowth period and days of ensiling on fermentation end-products of Johnson grass silage

Fermentation End-Product, g/100 g DM	Day of Ensiling	Regrowth Period (d) 45	110	SEM[a]	Probability R[b]	D[c]	R*D[d]
Acetic acid	0	0.01[i]	0.02[f]	.051	.001	.001	.001
	1	0.11[h]	0.13[e]				
	3	0.15[h]	0.10[e]				
	7	0.27[g]	0.08[ef]				
	14	0.47[f]	0.12[e]				
	21	0.47[f]	0.05[f]				
	100	0.56[e]	0.03[f]				
Lactic acid	0	0.00[j]	0.07[h]	.074	.032	.001	.001
	1	0.53[f]	0.63[ef]				
	3	0.90[e]	0.72[e]				
	7	0.46[f]	0.55[f]				
	14	0.48[fg]	0.49[fg]				
	21	0.36[g]	0.41[g]				
	100	0.16[h]	0.41[g]				
Butyric acid	0	0.00[g]	0.00[f]	.039	.001	.001	.001
	1	0.02[g]	0.01[f]				
	3	0.02[g]	0.06[ef]				
	7	0.13[f]	0.02[f]				
	14	0.35[e]	0.09[e]				
	21	0.15[f]	0.01[f]				
	100	0.05[g]	0.01[f]				
Ethanol	0	0.00[G]	0.00[G]	.017	.536	.001	.054
	1	0.03[F]	0.05[F]				
	3	0.04[F]	0.10[E]				
	7	0.05[F]	0.07[E]				
	14	0.05[F]	0.07[E]				
	21	0.10[E]	0.05[F]				
	100	0.04[F]	0.03[F]				

[a] Standard error of the mean
[b] Effect of regrowth period
[c] Effect of day of ensiling
[d] Interaction of regrowth period by day of ensiling
[efghi] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)

Ethanol content increased ($P<0.05$) after 21 d post-ensiling in Johnson grass ensiled at 45 and after 3 d in the 110 d silage, then for both regrowth periods, ethanol decreased until the end of the fermentation period.

In Johnson grass ensiled at 45 d of regrowth, glucose content declined rapidly (Table 5-3), which corresponds with changes observed in acidity and lactic acid bacterial populations. After 1 d post-ensiling, glucose content continued to decrease but at a slower rate. In the 110 d regrowth silage, glucose levels were lower and declined till d-100. Fructose content was variable and no consistent trends emerged even though a significant interaction was detected. Xylose decreased ($P<0.01$) throughout the ensiling period with the immature forage, but did not change with the more mature forage . Arabinose remained similar throughout the fermentation. Concentrations of WSC evaluated in this experiment were lower in the more mature silage than in the less mature forage; which corresponded to the higher lactic acid bacterial population and lactic acid content observed in Johnson grass ensiled after 45 d of regrowth.

Hemicellulose content decreased ($P<0.01$) over time for silages from both regrowth periods (Table 5-4). Cellulose, NDF, and ADF contents were greater in the more mature forage.

The silages in this experiment did not reach a pH of 4.2, or a lactic acid concentration greater than 1.5% DM; which are the recommended levels for a stable silage (McCullough, 1978). The low concentrations of WSC found

Table 5-3. Effect of regrowth period and days of ensiling on water soluble carbohydrate contents of Johnson grass silage

Carbohydrate, g/100 g DM	Day of Ensiling	Regrowth Period (d)		SEM[a]	Probability		
		45	110		R[b]	D[c]	R*D[d]
Glucose	0	0.72[e]	0.14[e]	.031	.001	.001	.001
	1	0.12[fgh]	0.08[ef]				
	3	0.09[g]	0.06[fg]				
	7	0.16[f]	0.05[fg]				
	14	0.18[f]	0.06[fg]				
	21	0.11[fgh]	0.05[fg]				
	100	0.06[h]	0.03[g]				
Fructose	0	0.04[fg]	0.11[e]	.015	.016	.678	.007
	1	0.04[fg]	0.09[e]				
	3	0.07[e]	0.06[f]				
	7	0.05[ef]	0.04[f]				
	14	0.07[e]	0.06[f]				
	21	0.06[ef]	0.06[f]				
	100	0.03[g]	0.05[f]				
Xylose	0	0.84[e]	0.07	.027	.001	.001	.001
	1	0.29[f]	0.07				
	3	0.08[h]	0.09				
	7	0.06[h]	0.06				
	14	0.15[g]	0.08				
	21	0.07[h]	0.10				
	100	0.06[h]	0.10				
Arabinose	0	0.01	0.01	.012	.550	.001	.711
	1	0.01	0.01				
	3	0.01	0.01				
	7	0.02	0.00				
	14	0.01	0.00				
	21	0.03	0.01				
	100	0.05	0.02				

[a] Standard error of the mean
[b] Effect of regrowth period
[c] Effect of day of ensiling
[d] Interaction of regrowth period by day of ensiling
[efgh] Means with unlike superscripts in the same column within a carbohydrate heading differ ($P<0.01$)

Table 5-4. Effect of regrowth period and days of ensiling on structural carbohydrate contents of Johson grass silage

Carbohydrates, g/100 g DM	Day of Ensiling	Regrowth Period (d)		SEM[a]	Probability		
		45	110		R[b]	D[c]	R*D[d]
NDF	0	68.15	70.23	1.11	.010	.475	.553
	100	66.51	70.25				
ADF	0	39.92	42.02	0.73	.002	.110	.913
	100	39.54	42.80				
Hemicellulose	0	28.02	28.80	0.57	.700	.001	.223
	100	27.17	26.85				
Cellulose	0	33.87	34.11	1.02	.001	.172	.176
	100	33.17	34.48				

[a] Standard eror of the mean
[b] Effect of regrowth period
[c] Effect of day of ensiling
[d] Interaction of regrowth period by day of ensiling

in Johnson grass at 45 or 110 d of regrowth may be the major limiting factor to the use of ensiling to improve the utilization of Johnson grass. However, other factors such as the presence of undesirable microorganisms (e.g. enterobacteriaceae, yeasts and molds) not enumerated in this experiment may also have influenced the fermentation process.

For both regrowth periods, Johnson grass treated with the microbial inoculant plus enzymes had lower ($P<0.01$) pH and higher ($P<0.01$) lactic acid bacterial populations than control silages (Table 5-5). Acetic acid levels were similar for the treated and untreated silages (Table 5-6). However, silages from the shorter regrowth period had more acetic acid than the 110 d regrowth. Lactic acid levels were increased ($P<0.01$) by the use of the inoculant and enzyme mixture. The control silage from the 110 d regrowth period tended to have more lactic acid than the 45 d regrowth forage. Utilization of the microbial inoculant plus the enzyme mixture decreased ($P<0.01$) butyric acid content in Johnson grass ensiled at 45 d and ethanol in Johnson grass ensiled at 110 d of regrowth. No effect of silage additive by regrowth period interaction on carbohydrate contents was observed (Table 5-7). For both regrowth periods, hemicellulose was lower ($P<0.05$) in silages containing the inoculant plus the enzyme, but no effects on NDF, ADF, and cellulose were observed. These results indicate that addition of microbial inoculant plus enzyme to Johnson grass ensiled at 45 or 110 d of regrowth showed a small

Table 5-5. Effect of silage additives and regrowth period on pH and lactic acid bacterial populations of Johnson grass silage

Item	Silage Additive	Regrowth Period (d)		SEM[a]	Probability		
		45	110		R[b]	A[c]	R*A[d]
pH	Control	4.97[f]	5.36[f]	.041	.001	.001	.001
	E + I	4.70[g]	4.72[g]				
Lactic acid bacteria[e]	Control	6.77[g]	6.66[g]	.041	.001	.001	.018
	E + I	7.29[f]	6.88[f]				

[a] Standard error of the mean
[b] Effect of regrowth period
[c] Effect of silage additive
[d] Interaction of regrowth period by silage additive
[e] cfu/g of fresh material
[fg] Means with unlike superscripts in the same column within an item heading differ ($P<0.01$)

Table 5-6. Effect of silage additives and regrowth period on fermentation end-products of Johson grass silage

Fermentation End-Product, g/100 g DM	Silage Additive	Regrowth Period (d) 45	110	SEM[a]	Probability R[b]	A[c]	R*A[d]
Acetic acid	Control	0.30	0.08	.027	.001	.543	.740
	E + I	0.28	0.07				
Lactic acid	Control	0.17	0.33	.041	.030	.001	.110
	E + I	0.65	0.68				
Butyric acid	Control	0.18[e]	0.04	.021	.001	.001	.001
	E + I	0.02[f]	0.02				
Ethanol	Control	0.04	0.07[E]	.001	.536	.475	.021
	E + I	0.06	0.04[F]				

[a] Standard error of the mean
[b] Effect of regrowth period
[c] Effect of silage additive
[d] Interaction of regrowth period by silage additive
[ef] Means with unlike superscripts in the same column within a fermentation end-product heading differ ($P<0.01$)
[EF] Means with unlike superscripts in the same column within a fermentation end-product heading differ ($P<0.05$)

Table 5-7. Effect of silage additives and regrowth period on water soluble and structural carbohydrate contents of Johnson grass silage

Carbohydrate, g/100 g DM	Silage Additive	Regrowth Period (d) 45	110	SEM[a]	Probability R[b]	A[c]	R*A[d]
Water Soluble							
Glucose	Control	0.23	0.06	.022	.001	.894	.162
	E + I	0.19	0.04				
Fructose	Control	0.04	0.07	.008	.016	.090	.309
	E + I	0.06	0.08				
Xylose	Control	0.21	0.07	.010	.001	.249	.958
	E + I	0.23	0.09				
Arabinose	Control	0.02	0.02	.006	.550	.113	.741
	E + I	0.01	0.01				
Structural							
NDF	Control	67.95	70.83	1.11	.018	.245	.975
	E + I	66.71	69.66				
ADF	Control	39.92	42.02	.738	.002	.794	.443
	E + I	39.54	42.80				
Hemicelullose	Control	28.02	28.80	.594	.709	.030	.372
	E + I	27.17	26.85				
Cellulose	Control	33.87	34.91	1.02	.460	.873	.608
	E + I	33.17	34.48				

[a] Standard error of the mean
[b] Effect of regrowth period
[c] Effect of silage additive
[d] Interaction of regrowth period by silage additive

improvement in the fermentation characteristics , as evidenced by lower pH, higher lactic acid bacterial populations and lactic acid content. The silage additive also decreased the butyric acid content in the less mature forage and ethanol in Johnson grass ensiled after 110 d of regrowth. However, quality of the resulting silage did not reach the criteria to be considered as a good quality silage. Research to evaluate other sources of additives and appropiate application rates for the inoculant or the enzyme need to be conducted for tropical forages.

Implications

Treatment of Johnson grass with silage additives (microbial inoculant plus enzymes) improved the ensiling characteristics, regardless of stage of regrowth. However, more research needs to be conducted to evaluate the potential of Johnson grass as a forage source in tropical climates.

CHAPTER 6

CELL-WALL DISAPPEARANCE IN FORAGE SORGHUM AND JOHNSON GRASS AFTER TREATMENT WITH A COMMERCIAL MULTI-ENZYME PREPARATION

Abstract

The effects of a commercial enzyme preparation on cell wall disappearance from two tropical forages were determined. Johnson grass (*Sorghum halapense*) at 45 (22.64 DM%) and 110 (43.84 DM%) d of regrowth, and forage sorghum (High energy hybrid, Agripro-seed, Hereford, TX; 26.04 DM%) at 90 d of growth were harvested and triplicate samples were incubated in sodium acetate buffer (pH = 4.5) at 40°C for 7 d with a commercial multi-enzyme preparation containing arabinase, cellulase, ß-glucanase, hemicellulase and xylanase. The enzyme was applied at 0, 1, 4, and 8 X the application rate recommended by the manufacturer. Recommended application rate of the enzyme was .1% of fresh material. Cell-wall disappearance was calculated by the difference between the initial (d 0) and final (d 7) NDF, ADF, hemicellulose, and cellulose content from

each sample. Neutral detergent fiber disappearance was higher ($P<0.05$) for Johnson grass harvested at 45 d than for Johnson grass harvested at 110 d or for forage sorghum. For both forages evaluated, application of the enzyme mixture at 8 X the recommended rate increased NDF, hemicellulose and cellulose disappearance in comparison to the 0, 1 or 4 X levels. Results from this experiment indicated that cell-wall fraction disappearance differs among forage evaluated when treated with a commercial enzyme preparation. Rates of application greater than recommended appeared to improved cell-wall disappearance from Johnson grass and forage sorghum .

Introduction

Because of seasonal rainfall, production of high quality conserved forage for use as a feedstuff during dry periods is a critical need in tropical climates. Preservation of forage by ensiling requires adequate amounts of water soluble carbohydrates (WSC) to ensure a fermentation dominated by lactic acid producing bacteria (LAB). However, tropical grasses are generally low in WSC (Van Soest, 1994) thereby, limiting the fermentation process. In temperate climates, utilization of commercial enzyme preparations to accelerate the fermentation by providing higher amounts of WSC produced from the hydrolysis of starch, cellulose and hemicellulose to oligosacharides and simple sugars has been studied with variable results (Jaster and Moore, 1988; Van Vuuren et al., 1989; Chen et al., 1994). Reports have shown

that the efficacy of an enzyme preparation can be influenced by forage species, environmental conditions and application rates (Henderson et al., 1982; Spoeltra and Van Wikselaar, 1992). There is limited information regarding the utilization of enzyme preparations to improve silage fermentation in tropical climates. We previously showed (Rodriguez et al., 1994) that a multi-enzyme preparation applied at the recommended rate did not improve the ensiling characteristics of forage sorghum. The objective of this study was to determine the effects of this multi-enzyme preparation applied at different rates on the in vitro cell-wall disappearance from Johnson grass and forage sorghum grown in Puerto Rico.

Experimental Procedure

Johnson grass (*Sorghum halapense)* at 45 (22.64 DM%) and 110 (43.84 DM%) d of regrowth and forage sorghum (Hi Energy Hybrid, Agripro seed, Hereford, TX) at 90 d of growth were harvested by hand at the Lajas Agriculture Experimental Station, University of Puerto Rico. Samples from each forage were oven-dried at 60°C for 72 h, ground in a Udy mill (Arthur Thomas CO. Philadelphia; 1 mm screen), and stored at room temperature (27 - 30 °C) until analysis. For cell-wall disappearance determination, triplicate samples from each forage (.5 g) were treated with four levels of a liquid, multi-enzyme preparation (Viscozyme™ 120L, Novo Nordisk Bioindustrials, Danbury, CT) containing arabinase, cellulase, ß-glucanase,

hemicellulase and xylanase (Table 6-1). The enzyme preparation was applied on a dry weight basis at 0, 1, 4 and 8 X the rate suggested by the manufacturer (.1% of fresh material). Forage samples were incubated at 40°C for 7 d in 75 ml test tubes containing the respective enzyme application rate and sufficient sodium acetate buffer (.1 M; pH = 4.5) to provide a total volume of 50 ml. Plant cell-wall fractions; NDF, ADF, hemicellulose (calculated as the difference between NDF and ADF), and cellulose (calculated as the difference between ADF and lignin) were determined at the beginning and end of the incubation period (Goering and Vansoest, 1970; Van Soest et al., 1991, method A). Disappearances of plant cell-wall fractions were calculated as the differences between the initial and final proportions of each fraction in the forage residue. Statistical analyses were performed as a completely randomized design by analysis of variance (Steel and Torrie, 1980) utilizing the General Linear Model Procedure of SAS (1990) using the following model:

$$Y_{ijk} = u + A_i + B_j + (A*B)_{ij} + E_{ijk}$$

Where:

Y = Plant cell-wall fraction disappearance (i.e NDF, ADF)

u = Overall mean

A = Effect of forage species

B = Effect of enzyme application rate

A*B = Interaction of forage species by enzyme application rate

Table 6-1. Description of the commercial multi-enzyme preparation evaluated

Trade Name	Viscozyme™ 120L
Supplier	Novo Nordisk Bio-Industrials Danbury, CT
Source	*Aspergillus niger*
Active Enzymes	arabinase cellulase ß-glucanase hemicellulase xylanase
Declared Activity	120 FBG/ml[1]
Recommended Application Rate	.1% of fresh material
Optimum pH conditions	3.3 - 5.5
Optimum temperature conditions	40 - 50 °C
g protein/ 100 ml enzyme	8

[1] FBG = Fungal beta-glucanase; where 1 FBG is the amount of enzyme which under standard conditions liberates glucose or other reducing carbohydrate with a reducing power corresponding to 1 umol glucose per minute.

E = Random residual error

Mean separation was performed by a Bonferroni-t test.

Results and Discussion

Initial structural carbohydrate contents of the forages evaluated (Table 6-2) are similar to values previously reported for tropical grasses (Minson and McLeod, 1970; Vicente-Chandler et al., 1983). As expected, Johnson grass harvested at 110 d of regrowth had higher cell-wall material than Johnson grass harvested at 45 d. Forage sorghum had a lower cell-wall content than Johnson grass, but higher lignin content. Neutral detergent fiber disappearance was higher ($P<.001$) in Johnson grass harvested at 45 d of regrowth than in Johnson grass harvested at 110 d of regrowth, or forage sorghum harvested at 90 d of growth (Figure 6-1).

For both forage species evaluated, adding the enzyme preparation at 4 X the recommended rate increased NDF disappearance as compared to untreated forage, but NDF disappearance was similar to the untreated for the recommended application rate (Figure 6-2). Increasing the enzyme preparation to 8 X the recommended application rate provided greater ($P<0.01$) NDF disappearance than 0, 1 and 4 X the suggested application rate. This result differs from previously reported experiments evaluating forage sorghum in temperate climates (Rodriguez et al., 1995), where increased application rates of the same enzyme did not improve NDF disappearance

Table 6-2. Composition of initial plant cell-wall fractions of Johnson grass and forage sorghum

Cell-wall fraction	Forage Species					
	Johnson - 45 d	SD[1]	Johnson - 110 d	SD	Sorghum - 90 d	SD
NDF	67.38	1.35	69.87	0.73	64.00	1.34
ADF	36.05	0.09	38.53	1.09	35.18	1.10
Hemicellulose	31.33	1.26	31.34	1.75	28.82	0.24
Cellulose	32.87	0.26	33.78	1.06	29.36	0.80
Lignin	3.17	0.16	4.74	0.22	5.81	0.42

[1] Standard Deviation

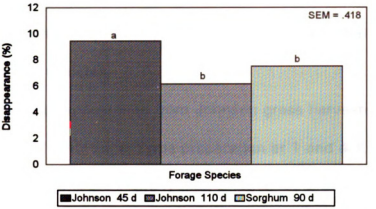

Figure 6-1. NDF disappearance from Johnson grass and forage sorghum after treatment with a commercial enzyme mixture

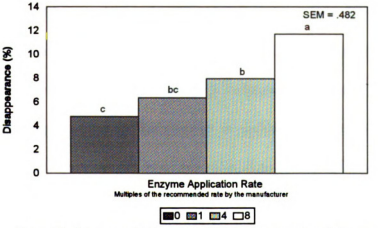

Figure 6-2. Enzyme application rate on NDF disappearance from Johnson grass and forage sorghum

from forage sorghum harvested at 90 d of growth. In the latter study, NDF disappearance was similar for application rates up to 8 times the manufacturer recommendation. It would appear that the suggested application rate for this enzyme preparation may be inappropriate for forages harvested in tropical climates.

Acid detergent fiber disappearance from Johnson grass harvested at 45 d of regrowth and treated with the enzyme preparation at 1 and 4 X the suggested rate was higher ($P < 0.01$) than untreated samples, but lower than 8 X the recommended application rate (Figure 6-3). None of the enzyme application rates improved ADF disappearance from Johnson grass harvested at 110 d of regrowth as compared to the untreated forage. In forage sorghum, ADF disappearance increased as application rate of the enzyme preparation increased. The lower response to the enzyme preparation observed in the ligno-cellulolytic fraction of Johnson grass harvested at 110 d of regrowth may be explained by a greater thickness of the cell-wall as compared to more immature forages (Van Soest, 1994). As plants mature, changes occur in the degree of polymerization within and between glucose polymers within the cell-wall, crytallinity of the cellulose fraction is increased, and physical incrustation of cellulose with lignin is greater. These factors collectively render the plant material less susceptible to enzyme degradation.

Hemicellulose disappearance in Johnson grass harvested at 45 d of

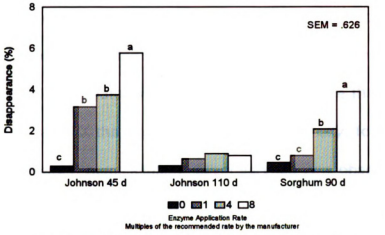

Figure 6-3. Interaction between enzyme application rate and forage species on ADF disappearance from Johnson grass and forage sorghum

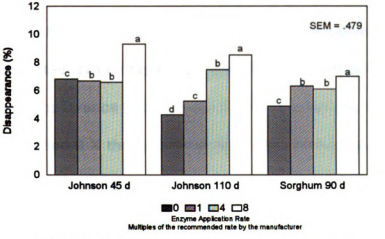

Figure 6-4. Interaction between enzyme application rate and forage species on hemicellulose disappearance from Johnson grass and forage sorghum

regrowth was only improved when enzyme application rate was 8 X the recommended rate (Figure 6-4). In Johnson grass harvested at 110 d of regrowth, hemicellulose disappearance increased as enzyme application rate was increased. Application rates of 1 and 4 X the recommended rate in sorghum forage yielded greater hemicellulose disappearance than untreated forage, but lower than 8 X the suggested rate. In this study, for both forage species evaluated, hemicellulose disappearance was observed in the control samples, which may have resulted from microbial activity or solubility of the hemicellulose. It appears that solubility of hemicellulose is greater in more immature forages, but it is less susceptible to enzyme attack. This concept is supported by the observation of increased hemicellulose disappearance as enzyme level increased.

Cellulose disappearance from Johnson grass harvested at 45 d of regrowth, and forage sorghum followed a similar pattern to ADF disappearance when treated with the multi-enzyme preparation (Figure 6-5). With both forages, higher cellulose disappearance values were obtained when the enzyme preparation was applied 8 X the recommended rate. With Johnson grass harvested at 110 d, cellulose disappearance was also improved when the enzyme complex was applied 8 X the recommended rate as compared to 0, 1, and 4 X treatments, but at a lower order of magnitude than observed with the immature forage.

The results from this experiment support the premise that forages harvested

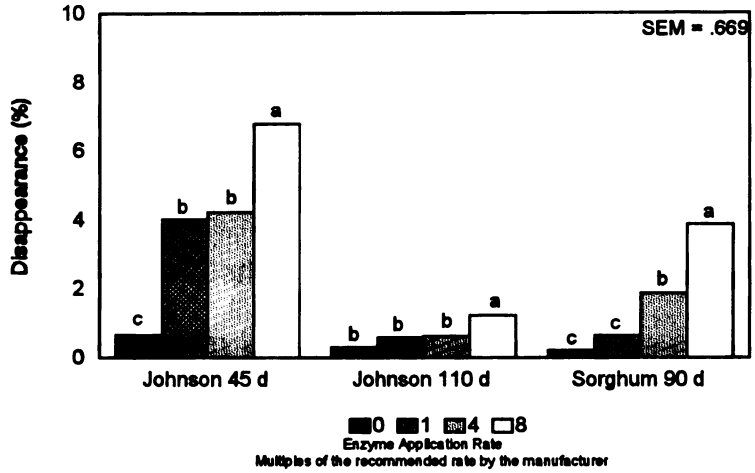

Figure 6-5. Interaction between enzyme application rate and forage species on cellulose disappearance from Johnson grass and forage sorghum

at an early stage of maturity are more susceptible to enzyme degradation than forage harvested at later stage of growth. This variability in response to the enzyme preparation among forage species evaluated may be due to differences in concentration of individual carbohydrates, covalent bonding of phenolic acids to cell-wall, or crystalline structure of the cellulose in the plant cell wall . In similar experiments conducted in temperate environments, Sheperd and Kung, Jr. (1994) showed than an enzyme additive was more effective in altering the fiber content of corn at milk stage than black layer stage of maturity.

The enzyme preparation evaluated in this experiment consisted of a multi-enzyme preparation containing five different enzyms. Individual concentration of each enzyme were not provided, and disappearance of simple sugars (arabinose, xylose), or specific sugars in the hemicellulose (xylan) fraction were not measured. However, its appears that an enzyme preparation containing cellulase and hemicellulase is needed in younger forages, whereas only a hemicellulase is needed in more mature forages. Similar results were observed with forages grown under temperate conditions (Hoffman et al., 1995); where a commercial enzyme preparation reduced cell wall content in alfalfa harvested at late-bloom by reducing the hemicellulose and pectin fractions, but had a little effect on the ADF and cellulose fractions.

Implications

Cell wall disappearance differed among forage type in this experiment when treated with a commercial multi-enzyme preparation. It appears application rates greater than recommended by the manufacturer are needed to ensure an improvement in cell-wall disappearance from Johnson grass and forage sorghum harvested in a tropical climate.

CHAPTER 7

NEUTRAL DETERGENT FIBER DISAPPEARANCE FROM FORAGE SORGHUM TREATED WITH COMMERCIAL ENZYME MIXTURES

Abstract

Five experiments were conducted to determine the effects of commercial enzyme mixtures on NDF disappearance from forage sorghum. At 90 d of growth, forage sorghum (19.06 % DM) was harvested, stored frozen (-20°C) or oven-dried (60°C), and treated with 5 enzyme preparations. Incubations in all experiments were done in sodium acetate buffer (pH = 4.5) for 7 d at 40°C. In experiment 1, frozen-thawed and dried forages were incubated with a multi-enzyme mixture (E1) containing arabinase, cellulase, ß-glucanase, hemicellulase and xylanase. Enzyme mixture was applied at 0, .5, 1 and 4 times the rate suggested by the manufacturer. Disappearance of NDF in dried sorghum decreased when application of E1 was lower than the recommended rate, but NDF disappearance did not increase when E1 was applied 4 times the suggested rate. In frozen-thawed sorghum, disappearance of NDF was similar for all application rates of E1.

In experiment 2, dried forage sorghum was incubated at 3 pH levels (3.5, 4.5, and 5.5) with E1 applied at 0, 1, 2, 4 and 8 times the rate suggested by the manufacturer. Activity of E1 was lower at pH 3.5 than at pH 4.5 and 5.5 when applied at the rate recommended, but was similar across pH values at the higher application rates. At pH values typically seen early in the fermentation process, an increased application rate of E1 above the recommended level did not improve NDF disappearance from forage sorghum. In experiment 3, dried forage sorghum was incubated with a combinations of cellulase (E2) and a mixture of xylanase, cellulase and ß-glucanase (E3) enzymes applied at E2:E3 ratios of 2:1, 4:1 and 6:1 (wt/wt). Each ratio was applied at 2, 5, and 10 g/ton of DM. Neutral detergent fiber disappearance was similar for all enzyme mixtures or application rates for E2:E3. In experiment 4, xylanase (E4) was applied to dried forage at .08 and .16 ml/kg and in experiment 5, an enzyme mixture containing cellulase and hemicellulase (E5) was applied to dried sorghum at .25 and .50 ml\kg of fresh forage. The different application rates of E4 and E5 yielded similar NDF disappearance. Comparison of enzyme preparations across experiments showed that forage sorghum treated with E5 had greater NDF disappearance than the other enzyme mixtures evaluated. Commercial enzyme mixtures degraded some NDF of forage sorghum, but application rates greater than recommended did not improve disappearance values. Activities of enzyme preparations were affected by pH. Commercial enzyme

mixtures differ in their ability to degrade the NDF fraction of forage sorghum.

Introduction

Utilization of enzyme preparations to improve silage fermentation has been the subject of several studies (Jaster and Moore, 1988; Van Vuuren et al., 1989; Chen et al., 1994; Sheperd et al., 1995;). Enzymes are presumed to accelerate fermentation by providing microorganisms with higher concentrations of water soluble carbohydrates (WSC) resulting from the hydrolysis of starch, cellulose and hemicellulose to oligosaccharides and simple sugars.

Reports on the efficacy of enzyme preparations to improved silage fermentation have been variable. Enzyme application did not consistently increase the WSC in wilted alfalfa (Kung Jr. et al., 1991), but in coastal bermudagrass treated with cellulase, silage characteristics and fiber digestibility were improved (McFan, 1986). The efficacy of enzyme preparations to improve fermentation could be affected by forage specie, environmental conditions and application rates of the enzymes. In one study, marked cellulose hydrolysis occurred when .4% cellulase was added to grass and legume silage (Henderson et al., 1982). Degradation of plant cell walls from whole crop maize significantly increased when a multi-enzyme mixture containing cellulolytic, hemicellulolytic, and amylolytic

activity was applied at higher rates than recommended (Spoeltra and van Wikselaar, 1992). Results from Chapter 2 indicated that a multi-enzyme mixture applied at .1% of fresh material however did not improved the fermentation characteristics of forage sorghum ensiled in a temperate environment. The objectives of the following series of experiments were to determine the effects of commercial enzyme preparations applied at different rates on NDF disappearance from forage sorghum.

Experimental Procedure

Forage sorghum (HI Energy II, Agripro Seed, Hereford, TX) was harvested at 90 d of growth (19.06% DM) at Michigan State University, East Lansing. Forage was chopped mechanically with a forage harvester (2.5 cm) and either stored frozen (-20°C) or oven dried (60°C) for 72 h. The dried sample was ground in a Udy mill (Arthur H. Thomas Co., Philadelphia; 1 mm screen) and stored at room temperature until analyzed. Forage samples were treated with five commercial enzyme preparations (Table 7-1). In experiment (Exp) 1, triplicate samples of dry (.5 g) and thawed (2.5 g) forage sorghum, were treated with four levels of a liquid, multi-enzyme preparation (E1) containing arabinase, cellulase, ß-glucanase, hemicellulase and xylanase. The multi-enzyme preparation was applied at 0, .5, 1 and 4 (ml\kg) times the rate suggested by the manufacturer. Incubations were performed in 75 ml test tubes containing the forage samples, respective

Table 7-1. Description of commercial enzyme mixtures evaluated

Enzyme Preparation	Supplier	Source	Active enzymes	Declared Activity
E1 - Viscoenzyme 120 L	Novo Nordisk Bioindustrials Danbury, CT	*Aspergillus spp.*	arabinase cellulase ß - glucanase hemicellulase xylanase	120 FBG/ml[a]
E2 - Biocellulase A	Quest International Inc. Sarasota, FL	*Aspergillus niger*	cellulase	3000-4000 CMC u/gm[b]
E3 - Bioxylanase A	Quest International Inc. Sarasota, FL	*Trichoderma reesie*	xylanase cellulase ß - glucanase	3000-80000 u/gm[c]
E4 - Ecogram	Zeneca Bio-Products Cleveland, England	*Neocallismastix*	xylanase	62,000 IU/ml[d]
E5 - Cellulose G	Zeneca Bio-Products Cleveland, England	*Trichoderma reesie*	cellulase hemicellulase	145 CMC u/gm[e]

[a] FBG = Fungal beta-glucanase; where 1 FBG is the amount of enzyme which under standard conditions liberates glucose or other reducing carbohydrates with a reduction power corresponding to 1 umol glucose per minute

[b] CMC = Carboxymethylcellulose; where 1 unit is the amount of enzyme that will produce 1 mg of reducing sugar (expressed as glucose per hour at 37°C and pH 4.6)

[c] One unit is the amount of enzyme which will produce 1 micromole of reducing sugar (as xylose) per minute under standard conditions

[d] International Units

[e] CMC = Carboxymethylcellulose; where 1 unit releases 1 micro-mole of glucose from CMC in 1 min at pH 4.6 and 40°C

enzyme preparations and sufficient sodium acetate buffer (.1 M; pH = 4.5) to provide a total volume of 50 ml. Enzyme application rates were 0, 1.25, 2.50, and 10 µl per tube for the 0, .5, 1 and 4 times treatments, respectively. Samples were incubated at 40°C for 7 d. In Exp 2, dried forage sorghum (.5 g) was incubated in triplicate with E1 at three pH levels (3.5, 4.5, and 5.5). Incubations were performed at 40°C for 7 d in 75 ml test tubes containing 0, 2.5, 5, 10, and 20 µl of enzyme preparation and sodium acetate buffer (.1 M) to provide a total volume of 50 ml. Enzyme was applied at 0, 1, 2, 4, and 8 times the rate suggested by the manufacturer. In Exp 3, the effects of blends of a concentrated cellulase (E2) and an enzyme preparation containing xylanase, cellulase and ß-glucanase (E3) on NDF disappearance from forage sorghum were evaluated. Enzyme preparations were blended (wt/wt) to E2:E3 ratios of 2:1, 4:1 (recommended blend), and 6:1. Each enzyme E2:E3 ratio was diluted with distilled water (1:1000), and applied to dry forage at rates of 2, 5, and 10 g of dry enzyme preparation per ton of dry matter. The E2:E3 mixtures were added to test tubes that were prepared as previously described, at rates of 5.5, 13.7 and 27.5 µl per test tube for the 2, 5, and 10 g/ton of DM treatments, respectively. Dry forage sorghum (.5 g) was added to each tube before the enzyme was added. Sodium acetate buffer (.1 M; pH = 4.5) was added to provide a final volume of 50 ml. Incubations were done at 40°C for 7 d. In Exp 4, a commercial liquid preparation of xylanase (E4),

and in Exp 5, a commercial liquid preparation containing cellulase and hemicellulase (E5) were applied in triplicate to dry forage sorghum at two different concentrations. Two enzyme concentrations were obtained for each enzyme by mixing 4 and 8 μl of E4, and 12.5 and 25 μl of E5 with a sodium acetate buffer (.1 M; pH = 4.5) to a final volume of 1 L. Fifty ml from each enzyme solution was placed in triplicate in 75 ml test tubes containing .5 g of dried sorghum. Tubes were incubated at 40°C for 7 d. These solutions were equivalent to .08 and .16 ml/kg for E4 and .25 and .50 ml/kg for E5. The recommended application rates for E4 and E5 are .08 and .25 ml/kg of fresh forage, respectively. In the five experiments, NDF (Van Soest et al., 1991, Method A) was determined at d 0 and after 7 d of incubation. Disappearance of NDF was calculated by difference between the initial and final weight of NDF in each tube. Statistical analyses were performed using the General Linear Models Procedure of SAS (1990). The model for each experiment included: enzyme application rate within forage preparation (dry or frozen-thawed), Exp 1; pH, enzyme application rate, and their interaction Exp 2; and enzyme ratio, application rate, and their interaction Exp 3. In Exp 4 and Exp 5, application level was utilized as a class variable within each enzyme preparation. Mean separation within experiment was performed by a Bonferroni t-test. Comparison of enzyme mixtures across experiments was calculated using the following model:

$$Y_{ij} = \mu + A_i + E_{ij},$$

Where

μ = Overall mean

A = Commercial enzyme (calculated as the difference between activity of each enzyme using the recommended application rate and the control group for each experiment)

E = Random residual error

RESULTS AND DISCUSSION

Neutral detergent fiber disappearance from dried forage sorghum decreased ($P < .05$) when the application of E1 was lower than the recommended rate, however application rates 4 times the recommended level did not increase NDF disappearance (Table 7-2). In frozen-thawed sorghum NDF, disappearance was similar for all application rates of E1. In similar experiments, Hunt et al. (1995) observed a decrease in DM and NDF content of fresh, dried, mid-bloom alfalfa, and late-milk tall fescue incubated for 48 h with two fibrolytic-enzyme preparations applied at different rates. In this study, a small disappearance of NDF from dried and frozen-thawed sorghum was observed in the control samples and may have resulted from microbial activity or solubilization of the hemicellulose fraction of the cell wall. The lack of response to the enzyme preparation observed in frozen-thawed forage sorghum could be explained by an increase in NDF

Table 7-2. Effect of enzyme (E1) application rate on NDF disappearance from dried and frozen-thawed forage sorghum (Exp. 1)

Forage Preparation	Application Rate, Multiples of rate Suggested by Manufacturer				SEM[a]	Probability
	0	.5	1	4		
	NDF Disappearance (%)					
Dried	6.24[b]	7.74[b]	10.11[c]	11.00[c]	.47	.001
Frozen-thawed	3.50	4.31	4.72	5.90	.71	.191

[a] Standard error of the mean

[bc] Means with unlike superscripts in the same row differ ($P < 0.05$)

Number of observation per mean = 3

content of fresh forage due to freezing and thawing sequence. In previous experiments (Nelson and Bozich, 1995), a large increase in NDF content was observed in fresh alfalfa after storage at -20°C. O'Neil and Allen (1992) reported an increase in NDF content after a freeze-thaw exposure, which was suggested as being due to both the concentration of fiber components through microbial respiration of water soluble carbohydrates and to an increase in the amount of NDF. Kohn and Allen (1992) attributed the increased NDF after a freeze-thaw sequence in fresh bromegrass and alfalfa to increased acid detergent insoluble protein, hemicellulose and lignin. In Exp 2, NDF disappearance was less ($P < .05$) at pH 3.5 than at either pH 4.5 or 5.5 when the enzyme was applied at 0 or the recommended application rate (Table 7-3). However, at greater enzyme application rates the effect of pH disappeared. This would suggest that the effects of low pH on enzyme activity can be overcome by increasing the enzyme concentration. These results are in agreement with Sheperd and Kung Jr. (1994) who reported that activity of a enzyme complex containing cellulase and hemicellulase applied at the recommended rate was reduced by 50 % when pH was decreased from 4.5 to 3.5.

In experiment 3, modifying the ratio of E2:E3 did not affect NDF disappearance of forage sorghum (Table 7-4). Forage sorghum receiving any rate of E2:E3 had greater ($P < .01$) NDF disappearance than when no E2:E3 was added.

Table 7-3. Interaction between pH and enzyme application (E1) rate on neutral detergent fiber disappearance from forage sorghum. (EXP. 2)

	Application rate, Multiples of rate Suggested by Manufacturer						Probability		
pH	0	1	2	4	8	SEM[a]	P[b]	A[c]	A*P[d]
	NDF Disappearance (%)								
3.5	3.60^{ey}	9.08^{fy}	10.32^{fy}	10.45^{fy}	11.47^{fy}	.437	.01	.01	.01
4.5	6.85^{ez}	11.68^{fz}	11.73^{fy}	9.05^{fy}	10.93^{fy}				
5.5	6.41^{ez}	11.55^{fz}	11.18^{fy}	11.56^{fy}	11.19^{fy}				

[a] Standard error of the mean

[b] pH

[c] Application rate

[d] pH*application rate interaction

[ef] Means with unlike superscripts in the same row differ ($P < .05$)

[yz] Means with unlike superscripts in the same column differ ($P < .05$)

Number of observation per mean = 3

Table 7-4. Interaction between E2 to E3 ratio and application rate on NDF disappearance from forage sorghum (EXP 3)

	Application rate, g/ton/of DM					Probability		
E2:E3 Ratio	0	2	5	10	SEM[a]	R[b]	A[c]	R*A[d]
	NDF Disappearance (%)							
2:1	4.74	6.71	7.56	6.58	.57	.56	.01	.61
4:1	4.40	6.35	6.47	6.67				
6:1	4.67	6.28	7.04	7.51				

[a] Standard error of the mean
[b] Enzyme ratio
[c] Application ratio
[d] Enzyme ratio by application rate interaction
Number of observations per mean = 3

No differences in NDF disappearance between the control samples and the sorghum treated with E4 were observed (Table 7-5). Neutral detergent fiber disappearance in forage sorghum was substantially higher ($P<.05$) when treated with E5 as compared to forage without enzyme application. However, an application level of E5 greater than recommended by the manufacturer did not increase NDF disappearance of forage sorghum.

In this study, when all commercial enzymes were applied at recommended rates and incubated for 7 d, the NDF fraction from forage sorghum was degraded, but increasing the recommended application rate by 2, 4 or 8 times did not improve NDF disappearance. These results indicate that under in vitro conditions, using enzyme preparations at rates that are recommended by the manufacturer, and using incubation periods representative of the length of a typical aerobic phase during the ensiling process did not increased NDF disappearance in forage sorghum. Previous experiments have also shown that 50 d of incubation and 500 times the recommended rate from a cellulase derived from *Trichoderma reesie* was needed to solubilize alfalfa NDF and cellulose (Kung Jr. et al., 1991). Hopking and Bass (1987) demonstrated that hydrolysis of cell wall in vitro required amounts of cell wall degrading enzymes that are to expensive for commercial application. Pitt (1991) also reported that application rates higher than recommended, and long storage times would be needed to increase enzyme activity in silage.

Table 7-5. Neutral detergent fiber disappearance from forage sorghum treated with enzyme preparations applied at different rates (EXP. 4 and EXP. 5)

Enzyme	Application Rate (ml/kg)[d]	NDF Disappearance (%)	SEM[a]	Probability
E4	0	6.25	.26	.512
	.08	6.36		
	.16	6.67		
E5	0	6.25[b]	.36	.001
	.25	13.40[c]		
	.50	12.89[c]		

[a] Standard error of the mean

[bc] Means with unlike superscripts within enzyme heading differ ($P < .05$)

[d] Application rate on a dry matter basis

Number of observations per mean = 3

Table 7-6. NDF disappearance from forage sorghum treated with commercial enzyme preparations expressed as a difference above control

Enzyme mixture	NDF disappearance (%)	SEM[a]	Probability
E1	4.4[c]	.19	.001
E2/E3	1.9[d]	.21	
E4	0.4[e]	.48	
E5	7.0[b]	.48	

[a] Standard error of least square mean
[bcde] Means with unlike superscripts differ ($P < .05$)

Forage sorghum treated with E5 had greater ($P < .05$) NDF disappearance than forage sorghum treated with other enzyme preparations (Table 7-6) when compared at rates recommended by the manufacturer. Cell wall material from forage sorghum appears to be more susceptible to degradation when treated with an enzyme preparation containing cellulase and hemicellulase (E5) than when arabinase, ß-glucanase or xylanase were present in the preparation. It also appears that enzyme preparations containing hemicellulase (E1 and E5) have a greater activity on the NDF fraction of forage sorghum than enzyme preparations without hemicellulase, and the use of enzyme preparations containing xylanase (E2/E3 and E4) had marginal effects on NDF disappearance from forage sorghum.

Reports of declared activity, percentages of individual enzymes, source, and recommended application rates of the enzyme preparations utilized in this study differ among manufacturers. Also, grams of protein per ml of enzyme preparation and suggested application level based on g of enzyme preparation per ton of forage differed (Table 7-7). Previous studies (Sheperd et al., 1995) have shown that enzyme preparations containing different cellulase and amylase activities differ in their ability to degrade cell wall components in alfalfa silage. Different fermentation patterns were observed in a timothy, meadow fescue and red clover silage when two enzymes prepararations were applied at the same cellulase activity, but derived from different microorganisms (Selmer-Olsen, 1994). Bertin and co-workers.

Table 7-7. Suggested application rate and protein content of the enzyme preparations evaluated

Enzyme Mixture	Suggested Application Rate	Protein Content (g/100 ml enzyme)	Enzyme Added (g/ton)	Enzyme Activity Added (Units/ton)[b]
E1	.1% of fresh forage	8	72	108,960
E2 / E3	5 g\ton[a]	100	5	277,500
E4	.08 ml\kg	6	54	4,503,680
E5	.25 ml\kg	12	110	32,915

[a] Application ratio suggested at 4:1
[b] Units of enzyme activity not constant across mixtures

(1985) found that enzymes derived from *Aspergillus niger* had more hemicellulolytic activity than *Trichoderma viride* enzymes, but Henderson and McDonald (1977) reported that enzymes derived from *Trichoderma viride* were more active than enzymes derived from *Aspergillus niger*. Therefore, a more appropiate comparison of enzyme mixtures should be based on equivalent concentrations of enzyme activity, equivalent amounts of protein (g/ton of forage), and enzymes from common sources.

Implications

Enzyme preparations applied at recommended rates degraded the NDF portion in forage sorghum, but application rates greater than recommended did not improve NDF disappearance. Activity of enzyme preparations could be affected by pH. Commercial enzyme preparations differ in their ability to degrade the NDF fraction of forage sorghum.

CHAPTER 8

FERMENTATION CHARACTERISTICS OF FORAGE SORGHUM ENSILED WITH COMMERCIAL ENZYME MIXTURES

Abstract

The effects of enzyme preparations on the fermentation characteristics of forage sorghum ensiled in a temperate environment were evaluated. Forage sorghum was harvested at 90 d of growth at Michigan State University, East Lansing and chopped into 2.5 cm pieces. Chopped forage prior to ensiling was treated with enzyme preparations applied at 1 or 2 X the manufacturers recommended rate. Experimental treatments included; no enzyme (control), Viscozyme (.1 % fresh material), Ecogram (.08 and .16 ml/kg of fresh material), and Cellulose G (.25 and .50 ml/kg of fresh material). Three silos per treatment were opened after 3 ensiling periods (0, 40 and 100 d), and analyzed for pH, fermentation end-products, and water soluble and structural carbohydrate contents. The pH of forage sorghum silage was similar regardless of enzyme treatment. Acetic acid and ethanol contents differed among treatments and across time, but a consistent trend

due to enzyme additive was not observed. Glucose content was higher in forage sorghum ensiled with 1 or 2 X the application rate of cellulase G as compared to control silages, but xylose was lower. Structural carbohydrate content was similar regardless of enzyme treatment.

Introduction

Variable responses to enzyme addition to enhance the fermentation of plant material have been shown (Kung Jr. et al., 1991; Sheperd et al., 1995). Enzyme preparations differ in declared activity, percentages of individual enzymes, source, and recommended application rates. Results from Chapter 2 indicated that a enzyme preparation containing 5 different enzymes and applied at the recommended rate (.1% of fresh material) did not improve the fermentation characteristics of forage sorghum. Previous experiments (Chapter 7) also showed that under controlled conditions (e.g. pH, temperature), application rates greater than recommended did not improved NDF disappearance in forage sorghum, and that commercial enzyme preparations differ in their ability to degrade the NDF fraction of forage sorghum. We demonstrated that an enzyme mixture containing hemicellulase and cellulase increased the NDF disappearance in forage sorghum in comparison with a preparation containing 5 different enzymes or a preparation containing only xylanase. The objective of this experiment was to evaluate the effect of three commercial enzyme preparations on the

fermentation characteristics of forage sorghum.

Experimental Procedure

Forage sorghum (Hi Energy Hybrid, Agri-pro Seed, Hereford, TX) was harvested at 90 of growth (20.04% DM) at Michigan State University, East Lansing. Harvested forage sorghum was chopped mechanically into 2.5 cm pieces with a forage harvester. Chopped forage, prior to ensiling, was treated with 3 commercial enzyme preparations assigned to one of six treatments (Table 8-1). Treatments were applied to weighed portions (1.6 kg) of forage, manually mixed, and packed into laboratory silos fitted with release valves for provide gas escape. Laboratory silos were maintained at room temperature (27-30°C) until opened. Triplicate silos from each treatment were analyzed for pH, fermentation end-products, and water soluble and structural carbohydrate contents at 0, 40 and 100 d post-ensiling using the methodology discussed in previous Chapters. Data was analyzed as a completely randomized design with a 6 (enzyme preparations) by 3 (ensiling periods) factorial arrangement of treatments using the General Linear Model Procedure of SAS (1990) with the following model:

$$Y_{ijk} = u + A_i + B_j + (A*B)_{ij} + E_k$$

Where:

Y_{ijk} = Individual response variable measured (e.i. pH, fermentation end-product)

Table 8-1. Description of treatments and commercial enzyme mixtures evaluated

Treatment	Enzyme Preparation	Supplier	Active Enzymes	Application Rate
1	No enzyme (Control)	----------	----------	----------
2	Viscoenzyme 120 L	Novo Nordisk Bioindustrials Danbury, CT	arabinase cellulase ß - glucanase hemicellulase xylanase	.1 % of fresh material[a]
3	Ecogram	Zeneca Bio-Products Cleveland, England	xylanase	.08 ml\kg[a]
4	Ecogram	Zeneca Bio-Products Cleveland, England	xylanase	.16 ml\kg
5	Cellulose G	Zeneca Bio-Products Cleveland, England	cellulase hemicellulase	.25 ml\kg[a]
6	Cellulose G	Zeneca Bio-Products Cleveland, England	cellulase hemicellulase	.50 ml\kg

[a] Suggested application rate

u = Overall mean

A_i = Effect of enzyme mixture

B_j = Effect of day of ensiling

$A*B_{ij}$ = Interaction of enzyme mixture by day of ensiling

E_{ijk} = Residual error

Bonferroni-t test was used for mean separation.

Results and Discussion

No significant interactions between enzyme treatment by day of ensiling for pH or fermentation end-products were observed (Table 8-2). Over the entire ensiling period, pH tended to be higher ($P<0.09$) in forage sorghum treated with Viscozyme in comparison to forage treated with the other enzyme treatments, but was similar to control silage. Forage sorghum treated with Ecogram (T 3 and T4) or Cellulase G (T5 and T6) at 1 or 2X the suggested application rate had lower ($P<0.05$) acetic acid content than control silage or silage treated with Viscozyme (T2). However, ethanol content was higher ($P<0.05$) in silages containing Viscozyme and the 2 X application rate of cellulase G as compared to the other enzyme treatments. For all enzyme treatments, lactic acid content was similar over the entire ensiling period.

Glucose content was higher ($P<0.05$) after 40 d post-ensiling in silages containing 1 or 2 X the recommended application rate of Ecogram and

Table 8-2. Effect of enzyme treatment and day of ensiling on pH and fermentation end-products of forage sorghum silage.

Item	Day of Ensiling	Treatment						SEM[a]	Probability		
		1	2	3	4	5	6		E[b]	D[c]	E*D[d]
pH	0	5.23	5.36	5.23	5.30	5.16	5.23	.043	.094	.001	.127
	40	3.53	3.56	3.53	3.53	3.55	3.56				
	100	3.68	3.66	3.55	3.48	3.58	3.56				
Acetic acid*	0	0.25	0.21	0.13	0.10	0.19	0.16	.101	.047	.001	.300
	40	1.60	1.68	1.60	1.58	1.59	1.51				
	100	1.68	1.77	1.41	1.33	1.21	1.52				
Lactic acid*	0	0.77	0.60	0.55	0.52	0.70	0.53	.632	.126	.001	.257
	40	8.26	7.84	6.70	9.54	8.33	6.99				
	100	6.28	6.00	7.82	7.59	7.35	5.80				
Ethanol*	0	0.13	0.23	0.06	0.43	0.06	0.07	.166	.001	.001	.963
	40	0.73	1.20	0.77	1.06	0.56	0.84				
	100	0.61	0.98	0.57	0.96	0.69	0.60				

[a] Standard error of the mean
[b] Effect of enzyme
[c] Effect of day of ensiling
[d] Interaction of enzyme by day of ensiling
* g/ 100 g DM

Cellulase G as compared to control silage of forage treated with Viscozyme (Table 8-3). After 100 d post-ensiling, silages containing Cellulase G had greater ($P<0.05$) glucose content than other enzyme treatments. In contrast to glucose content, xylose concentration was higher ($P<0.05$) after 40 and 100 d post-ensiling in silages without enzyme additive or forage treated with Viscozyme. Over the entire ensiling period, concentrations of fructose, galactose and arabinose were similar regardless of enzyme treatment. Over the entire ensiling period, except for a tendency ($P<0.09$) in control silage to have greater ADF content as compared to silage containing enzyme additives, NDF, hemicellulose and cellulose content were not influenced by enzyme preparation (Tbale 8-4). However, for all cell-wall fractions, a small numerical decrease was observed in silages containing enzyme mixtures as compared to control silage.

Results from this experiment indicated that forage sorghum treated with Ecogram or Cellulase G increased the residual glucose content after 100 d of fermentation. However, neither enzyme preparation increased the acidity or lactic acid content of the resulting silage, and a decrease in xylose content was observed. Application of the enzyme mixtures did not significantly decrease cell-wall components of forage sorghum silage. Even though the higher residual glucose content due to enzyme addition may improve the digestibility of the resulting silage, it may lead to more aerobic deterioration (Spoeltra and van Wikselaar, 1992). Therefore more research

Table 8-3. Effect of enzyme treatment and day of ensiling on water soluble carbohydrate contents of forage sorghum silage.

Item	Day of Ensiling	Treatment						SEM[a]	Probability		
		1	2	3	4	5	6		E[b]	D[c]	E*D[d]
Glucose	0	6.62	6.64	6.27	6.50	6.22	6.54	.386	.011	.001	.029
	40	1.66[e]	1.78[e]	2.42[f]	2.85[f]	2.43[f]	2.68[f]				
	100	0.94[e]	1.06[e]	1.94[f]	1.91[f]	2.91[g]	2.94[g]				
Fructose	0	6.44	6.33	6.67	6.17	6.86	6.12	.237	.597	.001	.833
	40	0.29	0.19	0.34	0.16	0.34	0.46				
	100	0.54	0.44	0.43	0.30	0.43	0.58				
Galactose	0	0.15	0.19	0.11	0.16	0.12	0.22	.045	.340	.006	.483
	40	0.23	0.28	0.28	0.12	0.26	0.26				
	100	0.24	0.25	0.18	0.25	0.24	0.27				
Xylose	0	0.07	0.05	0.06	0.05	0.06	0.07	.071	.001	.001	.033
	40	0.37[g]	0.32[g]	0.18[ef]	0.08[e]	0.20[ef]	0.24[fg]				
	100	0.68[g]	0.63[g]	0.34[f]	0.18[e]	0.45[f]	0.32[f]				
Arabinose	0	0.16	0.18	0.09	0.15	0.13	0.14	.062	.450	.009	.370
	40	0.09	0.09	0.07	0.06	0.07	0.03				
	100	0.13	0.14	0.10	0.11	0.10	0.13				

[a] Standard error of the mean
[b] Effect of enzyme
[c] Effect of day of ensiling
[d] Interaction of enzyme by day of ensiling
[efg] Means with unlike superscripts in the same row within an item heading differ ($P<0.05$)

Table 8-4. Effect of enzyme treatment and day of ensiling on structural carbohydrate contents of forage sorghum silage.

Item	Day of Ensiling	Treatment						SEM[a]	Probability		
		1	2	3	4	5	6		E[b]	D[c]	E*D[d]
NDF	0	61.64	63.04	60.62	60.54	60.03	61.86	1.034	.678	.001	.270
	40	58.08	56.10	57.44	58.81	58.17	57.34				
	100	59.53	56.33	58.12	57.42	57.17	56.81				
ADF	0	37.31	38.77	36.16	35.41	35.79	34.78	1.200	.087	.129	.321
	40	35.79	34.40	35.48	35.08	35.07	33.55				
	100	36.12	33.56	34.85	34.80	35.33	33.13				
Hemicellulose	0	24.32	24.26	24.36	24.12	24.57	25.75	1.379	.716	.003	.785
	40	22.28	21.69	21.96	23.03	23.10	24.79				
	100	23.41	22.76	22.59	22.61	21.84	23.88				
Cellulose	0	32.50	34.33	31.68	31.99	31.20	31.66	0.907	.338	.001	.491
	40	31.71	30.63	30.67	32.20	30.29	30.85				
	100	31.85	29.92	29.85	29.63	30.14	29.83				

[a] Standard error of the mean
[b] Effect of enzyme
[c] Effect of day of ensiling
[d] Interaction of enzyme by day of ensiling

is needed to evaluate the effects of different enzyme mixtures on the aerobic stability and nutritive value of silages.

Implications

Addition of enzyme preparations did not consistently affect pH, fermentation end-products or structural carbohydrate contents of forage sorghum silage. Enzyme mixtures differ in their ability to increase the residual soluble carbohydrates in forage sorghum silage. More research in needed (e.i. feeding trials) to justify the use of enzyme preparations as a silage additive.

CHAPTER 9

SUMMARY AND CONCLUSIONS

A series of studies were conducted to address the hypothesis that addition of silage additives (microbial inoculant and enzymes) improved the ensiling characteristics and aerobic stability of forages ensiled in temperate and tropical environments. In experiment one, a two year study was conducted to evaluate the effects of a lactic acid bacterial inoculant and a commercial enzyme mixture on the ensiling characteristics and aerobic stability of forage sorghum ensiled at 90 d of growth in temperate and tropical environments. The bacterial inoculant consisted of a homofermentative strain of *Lactobacillus plantarum* applied at 10^6 cfu/g of fresh material. The enzyme additive consisted of a multi-enzyme preparation containing arabinose, cellulase, ß-glucanase, hemicellulase, and xylanase and was applied at .1% of fresh material. In both locations, addition of the microbial inoculant alone or in combination with enzymes was effective in decreasing the pH, and increasing the lactic acid bacterial population and lactic acid content in forage sorghum silage at early stages of the fermentation process. In the temperate environment, inoculated sorghum also had greater fructose

content after 100 d post-ensiling as compared to sorghum silage without the microbial culture. However, for all treatments, fermentation characteristics in forage sorghum ensiled in the tropical environment after 100 d post-ensiling were similar. Results from Experiment 1, also indicated that inoculation may have a negative effect on the aerobic stability of forage sorghum exposure to air in the temperate environment. In the tropical environment, no effect of the microbial inoculant or enzyme on the aerobic stability of the resulting silage was observed. Findings from Experiment 1 also showed that the majority of the aerobic deterioration occurs within the first 3 d of aerobic exposure in the temperate silage, and within the first d in the tropical silage. In both environments, silage exposured to air after shorter fermentation periods (40 d) spoiled faster than silage exposed to air after 100 d post-ensiling, but a more pronounced effect due to length of fermentation was observed in the temperate environment. In Experiment 2, the differences in effectiveness of the microbial inoculant and enzyme mixture when forage sorghum was ensiled at 90 and 110 d of growth in a tropical environment was evaluated. Addition of the bacterial inoculant alone or in combination with enzymes improved the ensiling characteristics of forage sorghum, but a more beneficial response was observed in silage from the more immature forage. However, silage additives did not prevent the aerobic deterioration of forage sorghum silage, regardless of stage of maturity.

The fermentation characteristics and the resulting effects of a silage additive in Johnson grass (*Sorghum jalapense*) was evaluated in Experiment 3. Johnson grass was ensiled after 45 and 110 d of regrowth and treated with or without a combination of the microbial inoculant and commercial enzyme mixture. For both regrowth periods, Johnson grass treated with bacterial inoculant plus enzymes had lower pH and higher lactic acid bacterial populations and lactic acid content than control silage. Silage additives also decreased butyric acid levels in the more immature forage and ethanol in the Johnson grass ensiled at 110 d. However, fermentation characteristics of the resulting silage did not reach the recommended values for production of a stable silage. More research needs to be conducted to determine how to effectively ensile Johnson grass. In Experiment 4, the effects of a commercial enzyme preparation on cell-wall disappearance from forage sorghum and Johnson grass when harvested in a tropical environment were determined. Results from this experiment indicated that disappearance of the cell-wall fractions differs among forage types evaluated, and that application rates greater than recommended by the manufacturer are needed to ensure an improvement in cell-wall disappearance from forage sorghum and Johnson grass. The evaluation of commercial enzyme mixtures on NDF disappearance from forage sorghum harvested in a temperate environment were studied in Experiment 5. Findings from this experiment showed that; commercial enzyme preparations differ in their ability to degrade the NDF

fraction of forage sorghum, activity of the enzymes could by affected by pH and sample preparation (dried or frozen-thawed), and that application rates of enzyme mixtures greater than recommended did not improve NDF disappearance from forage sorghum.

Results from Experiment 6 demonstrated that enzyme mixtures did not consistently affect the ensiling characteristics of forage sorghum.

Conclusions from these experiments suggest that more research is needed to justify the use of microbial inoculant and enzymes as silage additives. In temperate environments, research must be concentrated on the implementation of biotechnology to develop microbial cultures capable of improving the fermentation characteristics while at the same, preventing aerobic deterioration. Studies to evaluate novel microorganisms and the introduction of bacteriocins or specific bacteriophages as silage additives should be considered. Additional alternatives include; the development of mathematical models for specific conditions (e.g. forage species, maturity) to predict the efficacy of use of silage additives on the preservation of nutrients from fresh crop through storage and the feeding phase. In tropical environments, future research needs to focus on the selection and isolation of specific strains of lactic acid bacteria from tropical forages. Strategies to decrease the populations of undesirable microorganisms at ensiling or during the silage fermentation (e.g. yeasts and molds) also need to the developed. Animal feeding trials should also be conducted to justify the use of silage

additives as a mechanisms to improved the quality of conserved feedstuffs. Economic studies to determine the feasibility of utilizing silage as a major feed component in ruminant feeding systems in tropical environments need to be conducted. Additional possibilities include; the evaluation of other grasses (e.g. *Pennisetum purpureum*) or legume (e.g. *Leucaena leucochephala*) species used alone or in combination as a potential feed resources, studies to determine the possible biological and economic benefits of industrial or agriculture by-products as silage additives, and implementation of management strategies (e.g. feeding silage only during specific season of the year) to evaluate the introduction of silage as a major feed component in ruminant production systems.

APPENDIX - A

Table A-1. Data used for analysis of organic acid content (g/ 100 g DM) in forage sorghum ensiled in a temperate environment (Chapter 2.)

Y	T	D	Citric	Malic	Oxaloacetic	Succinic
94	1	0	0.453	4.567	1.198	1.657
94	1	0	0.412	5.256	1.200	1.698
94	1	0	0.468	4.441	1.211	1.766
94	1	1	0.262	5.231	0.822	0.614
94	1	1	0.307	4.544	0.702	0.996
94	1	1	0.431	4.448	0.533	0.816
94	1	3	0.561	2.408	0.798	0.611
94	1	3	0.442	5.196	0.869	0.312
94	1	3	0.383	3.124	0.841	0.446
94	1	7	0.494	3.035	0.958	0.872
94	1	7	0.613	1.781	0.894	0.517
94	1	7	0.372	2.292	0.775	0.785
94	1	14	0.513	0.872	1.003	0.658
94	1	14	0.357	0.914	0.658	0.868
94	1	14	0.483	1.560	0.863	0.564
94	1	21	0.367	0.630	0.717	0.689
94	1	21	0.508	0.594	1.092	0.633
94	1	21	0.569	0.643	1.051	0.784
94	1	40	0.432	0.584	0.701	0.627
94	1	40	0.534	0.513	0.795	0.899
94	1	40	0.629	0.394	0.826	0.596
94	1	100	0.241	0.201	0.229	0.062
94	1	100	0.391	0.467	0.730	0.218
94	1	100	0.387	0.426	0.833	0.374
94	2	0	0.306	5.055	1.077	1.534
94	2	0	0.536	4.850	1.115	1.622
94	2	0	0.383	5.072	1.098	1.578
94	2	1	0.348	5.257	0.526	0.592
94	2	1	0.272	4.614	0.654	0.752
94	2	1	0.350	4.690	0.524	0.717
94	2	3	0.410	2.945	0.749	0.854
94	2	3	0.370	3.505	0.987	0.496
94	2	3	0.380	3.668	0.797	0.419
94	2	7	0.513	1.958	0.887	0.785
94	2	7	0.541	2.016	0.819	0.662
94	2	7	0.483	2.409	0.775	0.854
94	2	14	0.545	1.090	0.865	0.982
94	2	14	0.485	0.912	0.926	0.892
94	2	14	0.483	0.953	0.854	0.682
94	2	21	0.568	1.276	0.768	0.821
94	2	21	0.450	1.078	0.929	0.687
94	2	21	0.326	1.161	1.072	0.721
94	2	40	0.541	0.934	0.798	0.372
94	2	40	0.632	0.794	0.785	0.267
94	2	40	0.491	0.543	0.751	0.609
94	2	100	0.238	0.363	0.477	0.781
94	2	100	0.397	0.668	0.683	0.586
94	2	100	0.410	0.462	0.574	0.282
94	3	0	0.661	5.461	1.105	1.478
94	3	0	0.505	5.036	1.002	1.580
94	3	0	0.524	4.140	1.139	1.591
94	3	1	0.384	3.741	0.785	0.819
94	3	1	0.476	3.265	0.685	0.699
94	3	1	0.498	2.164	0.778	0.792
94	3	3	0.481	5.049	0.843	0.392
94	3	3	0.353	3.131	0.720	0.589
94	3	3	0.190	2.960	0.654	0.925
94	3	7	0.508	3.160	0.970	0.932
94	3	7	0.322	2.912	0.819	0.854
94	3	7	0.294	1.467	0.659	0.530
94	3	14	0.421	1.158	0.988	0.940
94	3	14	0.324	2.842	0.883	0.668
94	3	14	0.496	1.287	0.795	0.528
94	3	21	0.198	1.172	0.763	0.887
94	3	21	0.443	2.245	0.514	0.77

Table A-1. Cont.

Y	T	D	Citric	Malic	Oxaloacetic	Succinic
94	3	21	0.253	1.254	0.821	0.681
94	3	40	0.391	0.417	0.619	0.772
94	3	40	0.214	0.318	0.741	0.533
94	3	40	0.302	0.314	0.652	0.410
94	3	100	0.152	0.142	0.544	0.609
94	3	100	0.194	0.543	0.290	0.459
94	3	100	0.250	0.477	0.208	0.287
94	4	0	0.938	4.372	1.064	1.513
94	4	0	0.656	5.113	1.194	1.495
94	4	0	0.573	3.343	0.831	1.565
94	4	1	0.402	4.577	0.757	0.710
94	4	1	0.232	2.808	0.745	0.492
94	4	1	0.420	4.385	0.875	0.607
94	4	3	0.351	4.734	0.790	0.541
94	4	3	0.383	4.003	0.732	0.612
94	4	3	0.584	3.715	0.944	0.446
94	4	7	0.255	4.250	0.802	0.372
94	4	7	0.341	3.066	0.748	0.517
94	4	7	0.311	3.130	0.927	0.485
94	4	14	0.488	2.085	0.877	0.423
94	4	14	0.330	1.682	0.943	0.499
94	4	14	0.390	1.385	0.720	0.567
94	4	21	0.335	1.564	0.724	0.573
94	4	21	0.598	2.217	0.653	0.633
94	4	21	0.436	0.368	0.545	0.840
94	4	40	0.353	0.684	0.689	0.745
94	4	40	0.214	0.781	0.784	0.695
94	4	40	0.294	0.814	0.845	0.584
94	4	100	0.291	0.689	0.296	0.745
94	4	100	0.211	0.514	0.364	0.217
94	4	100	0.207	0.786	0.362	0.138
93	1	0	0.420	2.613	0.785	2.064
93	1	0	0.508	2.579	0.481	2.980
93	1	0	0.404	2.463	0.676	1.044
93	1	1	0.216	1.631	0.665	1.972
93	1	1	0.204	1.635	0.666	1.050
93	1	1	0.197	1.433	0.557	1.510
93	1	3	0.292	0.583	1.185	1.664
93	1	3	0.470	1.773	1.081	1.553
93	1	3	0.268	1.478	0.941	1.736
93	1	7	0.288	1.063	1.005	1.720
93	1	7	0.321	1.384	1.284	1.923
93	1	7	0.870	1.231	0.061	0.699
93	1	14	0.541	1.381	0.287	1.811
93	1	14	0.520	1.214	0.872	1.944
93	1	14	0.697	1.391	0.770	1.933
93	1	21	0.543	1.208	0.651	1.743
93	1	21	0.512	1.174	0.766	1.706
93	1	21	0.612	1.328	0.770	1.254
93	1	40	0.605	1.058	0.898	1.499
93	1	40	0.549	1.073	0.620	1.353
93	1	40	0.406	1.073	0.665	1.057
93	1	100	0.329	1.093	0.311	1.164
93	1	100	0.316	0.890	0.587	1.070
93	1	100	0.339	1.033	0.634	1.095
93	2	0	0.099	2.581	0.549	2.215
93	2	0	0.891	2.300	0.787	2.300
93	2	0	0.658	2.941	0.465	2.392
93	2	1	0.336	1.675	0.525	0.918
93	2	1	0.313	1.718	0.530	0.807
93	2	1	0.192	1.422	0.533	1.576
93	2	3	0.381	1.476	0.153	1.801
93	2	3	0.379	1.664	1.295	2.065
93	2	3	0.707	1.523	1.070	1.686
93	2	7	0.578	1.641	0.185	1.486

Table A-1. Cont.

Y	T	D	Citric	Malic	Oxaloacetic	Succinic
93	2	7	0.480	1.386	0.927	1.665
93	2	7	0.621	1.668	0.747	1.626
93	2	14	0.682	1.278	0.900	1.992
93	2	14	0.470	1.433	0.856	1.883
93	2	14	0.483	1.446	0.978	1.687
93	2	21	0.646	1.098	0.992	1.683
93	2	21	0.455	0.748	1.116	0.706
93	2	21	0.703	0.583	1.086	1.688
93	2	40	0.372	0.264	0.538	0.927
93	2	40	0.388	0.277	0.670	1.189
93	2	40	0.390	0.282	0.641	1.596
93	2	100	0.395	0.649	0.850	0.062
93	2	100	0.338	0.559	0.756	0.818
93	2	100	0.382	0.758	0.597	0.871
93	3	0	0.194	2.546	0.591	2.943
93	3	0	0.185	2.254	0.861	2.552
93	3	0	0.189	2.399	0.805	2.386
93	3	1	0.064	2.829	0.557	1.862
93	3	1	0.199	1.352	0.579	1.733
93	3	1	0.379	1.506	0.484	1.764
93	3	3	0.363	1.494	0.980	1.554
93	3	3	0.341	1.052	0.612	1.496
93	3	3	0.360	1.673	0.789	1.419
93	3	7	0.657	1.482	0.024	1.895
93	3	7	0.671	1.735	1.026	1.660
93	3	7	0.677	1.365	1.053	1.530
93	3	14	0.627	0.900	1.082	1.792
93	3	14	0.579	0.751	0.883	1.368
93	3	14	0.581	0.893	0.784	1.221
93	3	21	0.631	0.980	1.030	1.187
93	3	21	0.781	0.824	1.085	1.970
93	3	21	0.719	0.834	0.769	1.808
93	3	40	0.685	0.533	0.132	1.072
93	3	40	0.319	0.467	0.595	1.910
93	3	40	0.534	0.825	0.929	1.133
93	3	100	0.352	0.574	0.580	0.309
93	3	100	0.271	0.736	0.647	0.280
93	3	100	0.284	0.624	0.822	0.819
93	4	0	0.185	2.503	0.893	2.563
93	4	0	0.194	2.440	0.787	2.260
93	4	0	0.169	2.378	0.630	2.742
93	4	1	0.217	1.690	0.564	1.608
93	4	1	0.541	1.522	0.585	1.688
93	4	1	0.588	1.771	0.645	1.758
93	4	3	0.275	1.661	0.582	1.950
93	4	3	0.530	1.661	1.031	1.729
93	4	3	0.509	1.104	0.999	1.891
93	4	7	0.677	1.328	1.098	1.351
93	4	7	0.345	1.510	0.834	1.624
93	4	7	0.969	1.694	0.811	1.722
93	4	14	0.701	0.904	0.796	1.323
93	4	14	0.689	0.929	1.015	1.561
93	4	14	0.584	0.576	0.838	1.401
93	4	21	0.684	0.969	0.875	1.523
93	4	21	0.688	0.771	0.852	1.393
93	4	21	0.628	0.878	1.055	1.138
93	4	40	0.523	0.507	1.071	1.375
93	4	40	0.491	0.781	0.795	1.615
93	4	40	0.444	0.952	0.877	1.102
93	4	100	0.358	0.416	0.605	1.053
93	4	100	0.320	0.446	0.770	1.134
93	4	100	0.308	0.560	0.828	1.089

Abbreviations:
Y = Year; T = Treatment (1 = control, 2 = enzyme, 3 = inoculant, 4 = E + I); D = Day of ensiling.

Table A-2 . Data used for analysis of pH and microbial succession (cfu/g of fresh material) in forage sorghum ensiled in temperate and tropical environments. (Chapter 2)

Env	Year	Trt	D	Rep	pH	LAB	ENT	YM	LAY
1	93	1	0	1	5.40	3.94	5.18	3.38	2.30
1	93	1	0	2	5.40	3.07	4.83	2.00	1.00
1	93	1	0	3	5.40	3.44	4.72	2.00	1.00
1	93	1	1	1	4.50	6.15	5.53	5.33	3.52
1	93	1	1	2	4.50	6.85	5.73	2.60	2.60
1	93	1	1	3	4.50	7.39	6.05	3.40	2.60
1	93	1	3	1	3.90	7.01	3.60	3.87	2.60
1	93	1	3	2	4.00	6.68	3.30	3.40	3.25
1	93	1	3	3	4.00	6.88	3.10	3.70	0.00
1	93	1	7	1	3.9	8.27	1.00	3.40	2.90
1	93	1	7	2	3.90	7.74	1.00	3.30	2.60
1	93	1	7	3	3.95	7.73	0.00	3.30	2.60
1	93	1	14	1	3.65	8.17	2.90	4.39	2.60
1	93	1	14	2	3.68	7.95	3.07	4.32	1.00
1	93	1	14	3	3.65	7.32	3.44	4.26	2.60
1	93	1	21	1	3.56	7.48	1.00	4.39	2.60
1	93	1	21	2	3.58	7.54	0.00	4.52	2.60
1	93	1	21	3	3.55	7.84	2.60	4.13	3.68
1	93	1	40	1	3.60	7.80	0.00	4.87	5.22
1	93	1	40	2	3.60	7.60	3.40	2.60	1.00
1	93	1	40	3	3.60	7.53	3.60	4.49	4.20
1	93	1	100	1	3.75	8.07	2.60	5.75	5.31
1	93	1	100	2	3.80	7.73	2.30	4.40	3.58
1	93	1	100	3	3.75	7.35	2.60	5.65	4.58
1	93	2	0	1	5.30	3.14	5.03	3.57	2.30
1	93	2	0	2	5.40	3.31	4.83	2.73	0.00
1	93	2	0	3	5.40	3.86	4.68	3.02	2.80
1	93	2	1	1	4.60	7.29	5.73	4.38	3.07
1	93	2	1	2	4.55	7.18	5.59	2.60	2.60
1	93	2	1	3	4.65	6.65	5.49	2.90	2.60
1	93	2	3	1	4.00	7.28	2.90	3.55	2.60
1	93	2	3	2	4.00	6.97	3.07	3.40	3.40
1	93	2	3	3	4.00	7.19	3.45	3.38	0.00
1	93	2	7	1	3.85	8.03	0.00	3.38	2.60
1	93	2	7	2	3.90	7.78	2.60	3.20	2.60
1	93	2	7	3	3.90	7.97	2.60	3.44	1.00
1	93	2	14	1	3.65	7.88	2.90	4.37	2.90
1	93	2	14	2	3.65	7.35	3.62	4.22	2.60
1	93	2	14	3	3.70	7.43	3.64	4.37	0.00
1	93	2	21	1	3.50	7.31	0.00	4.35	3.55
1	93	2	21	2	3.50	8.06	2.30	3.99	4.18
1	93	2	21	3	3.50	7.33	3.67	4.30	3.99
1	93	2	40	1	3.60	7.76	2.90	3.82	3.55
1	93	2	40	2	3.70	6.67	0.00	4.66	4.55
1	93	2	40	3	3.65	6.69	2.30	4.12	5.85
1	93	2	100	1	3.70	6.70	2.60	3.66	3.64
1	93	2	100	2	3.65	6.60	2.90	5.13	4.86
1	93	2	100	3	3.65	6.90	2.60	4.19	3.53
1	93	3	0	1	5.50	4.99	4.54	3.04	2.73
1	93	3	0	2	5.40	5.66	4.96	2.47	2.30
1	93	3	0	3	5.50	5.25	4.77	3.43	2.30
1	93	3	1	1	4.80	7.62	6.10	3.50	3.10
1	93	3	1	2	4.60	7.66	6.11	3.94	2.60
1	93	3	1	3	4.50	7.48	6.02	2.90	2.60
1	93	3	3	1	3.90	7.79	3.45	3.20	3.40
1	93	3	3	2	3.90	8.14	0.00	4.03	0.00
1	93	3	3	3	3.90	8.06	3.07	3.70	2.60
1	93	3	7	1	3.65	8.29	0.00	4.09	3.07
1	93	3	7	2	3.70	8.21	2.60	2.60	2.60
1	93	3	7	3	3.70	8.23	2.30	2.90	0.00
1	93	3	14	1	3.55	7.75	3.44	4.22	1.00
1	93	3	14	2	3.55	8.24	2.90	4.21	2.60
1	93	3	14	3	3.50	7.93	0.00	3.99	0.00
1	93	3	21	1	3.50	7.53	2.90	4.53	2.90

Table A-2 . Cont.

Env	Year	Trt	D	Rep	pH	LAB	ENT	YM	LAY
1	93	3	21	2	3.50	7.54	3.49	4.15	1.00
1	93	3	21	3	3.50	6.87	3.44	4.44	0.00
1	93	3	40	1	3.60	7.38	2.60	4.41	4.13
1	93	3	40	2	3.58	7.87	1.00	4.51	3.75
1	93	3	40	3	3.60	7.58	3.90	4.30	4.56
1	93	3	100	1	3.60	6.94	2.60	5.04	4.55
1	93	3	100	2	3.65	7.71	3.50	5.65	4.21
1	93	3	100	3	3.60	6.95	2.90	4.55	3.61
1	93	4	0	1	5.40	4.95	4.66	3.49	2.65
1	93	4	0	2	5.40	5.28	4.73	2.77	2.30
1	93	4	0	3	5.40	5.06	4.71	3.24	2.30
1	93	4	1	1	4.70	7.60	6.11	2.90	3.38
1	93	4	1	2	4.75	7.57	6.25	3.25	2.60
1	93	4	1	3	4.65	7.92	6.05	3.07	3.58
1	93	4	3	1	3.85	8.51	2.60	4.04	2.60
1	93	4	3	2	3.90	8.14	3.07	3.40	2.60
1	93	4	3	3	3.95	8.18	2.60	4.43	2.90
1	93	4	7	1	3.60	8.59	2.60	3.38	2.90
1	93	4	7	2	3.65	8.83	2.60	3.07	2.60
1	93	4	7	3	3.65	8.76	0.00	2.60	1.00
1	93	4	14	1	3.48	7.87	2.90	4.26	2.60
1	93	4	14	2	3.50	7.37	2.60	4.15	0.00
1	93	4	14	3	3.45	7.90	3.76	3.64	2.30
1	93	4	21	1	3.50	6.80	3.44	5.11	3.62
1	93	4	21	2	3.51	6.79	3.60	4.75	3.55
1	93	4	21	3	3.50	7.03	4.02	5.02	0.00
1	93	4	40	1	3.65	6.05	3.07	4.75	4.19
1	93	4	40	2	3.60	6.57	2.90	4.52	4.57
1	93	4	40	3	3.65	6.40	2.90	5.07	4.22
1	93	4	100	1	3.65	6.75	2.60	5.25	5.22
1	93	4	100	2	3.60	6.74	2.90	4.16	4.60
1	93	4	100	3	3.60	6.80	2.60	4.45	3.52
1	94	1	0	1	5.30	3.82	3.92	5.22	2.90
1	94	1	0	2	5.10	2.90	4.48	5.10	2.60
1	94	1	0	3	5.30	3.30	4.45	4.84	3.20
1	94	1	1	1	4.30	7.34	5.55	5.44	3.38
1	94	1	1	2	4.30	7.10	5.65	5.24	3.07
1	94	1	1	3	4.35	6.97	5.72	5.23	3.20
1	94	1	3	1	3.70	8.11	5.09	4.49	4.06
1	94	1	3	2	3.70	7.51	5.14	4.38	4.16
1	94	1	3	3	3.50	8.00	5.06	4.45	3.45
1	94	1	7	1	3.45	9.07	4.20	4.85	3.40
1	94	1	7	2	3.50	8.86	3.90	5.32	3.64
1	94	1	7	3	3.60	8.69	3.35	4.55	3.07
1	94	1	14	1	3.55	8.34	0.00	4.62	2.60
1	94	1	14	2	3.50	8.74	2.90	4.40	2.90
1	94	1	14	3	3.55	8.51	2.60	5.20	4.20
1	94	1	21	1	3.50	8.04	3.07	4.79	2.90
1	94	1	21	2	3.50	7.65	2.60	5.67	2.30
1	94	1	21	3	3.60	8.09	2.60	4.55	3.38
1	94	1	40	1	3.50	6.70	2.60	4.16	3.40
1	94	1	40	2	3.50	7.24	2.30	4.70	3.97
1	94	1	40	3	3.60	7.33	2.60	4.30	3.15
1	94	1	100	1	3.50	6.30	2.60	4.60	3.20
1	94	1	100	2	3.50	6.87	2.30	4.50	3.85
1	94	1	100	3	3.50	6.89	2.45	4.70	3.60
1	94	2	0	1	5.30	3.40	4.48	5.66	3.07
1	94	2	0	2	5.10	3.38	3.62	4.98	2.90
1	94	2	0	3	5.10	2.90	5.40	5.92	2.60
1	94	2	1	1	4.30	7.19	5.38	5.43	3.50
1	94	2	1	2	4.35	7.63	5.65	5.20	3.07
1	94	2	1	3	4.30	7.57	4.76	5.22	2.60
1	94	2	3	1	3.60	8.41	4.84	4.30	4.10
1	94	2	3	2	3.70	8.01	5.19	4.49	2.60
1	94	2	3	3	3.70	8.13	4.85	4.63	3.45
1	94	2	7	1	3.50	9.18	3.98	5.29	3.49

Table A-2 . Cont.

Env	Year	Trt	D	Rep	pH	LAB	ENT	YM	LAY
1	94	2	7	2	3.50	8.76	3.78	4.58	3.20
1	94	2	7	3	3.60	9.14	3.65	4.62	3.40
1	94	2	14	1	3.45	8.67	2.60	4.40	3.44
1	94	2	14	2	3.55	8.74	0.00	4.33	2.60
1	94	2	14	3	3.60	8.65	2.60	5.16	3.40
1	94	2	21	1	3.50	8.39	2.60	4.64	3.20
1	94	2	21	2	3.60	7.70	2.60	3.60	2.90
1	94	2	21	3	3.60	7.76	2.30	5.77	3.07
1	94	2	40	1	3.50	7.18	2.30	5.23	3.40
1	94	2	40	2	3.50	7.50	2.60	5.01	3.94
1	94	2	40	3	3.50	7.06	2.60	4.64	4.36
1	94	2	100	1	3.55	6.89	3.00	4.80	3.30
1	94	2	100	2	3.50	5.24	2.30	4.65	3.78
1	94	2	100	3	3.50	6.45	2.45	4.30	3.10
1	94	3	0	1	5.20	5.03	4.22	5.29	2.60
1	94	3	0	2	5.10	5.18	5.42	4.85	2.90
1	94	3	0	3	5.10	5.23	3.95	5.30	3.20
1	94	3	1	1	4.40	8.54	5.13	5.40	3.44
1	94	3	1	2	4.35	8.58	5.47	5.46	3.20
1	94	3	1	3	4.30	8.54	5.30	4.80	2.60
1	94	3	3	1	3.40	8.60	4.97	4.23	3.49
1	94	3	3	2	3.50	9.43	5.16	4.50	2.60
1	94	3	3	3	3.50	9.48	5.15	4.39	3.34
1	94	3	7	1	3.35	9.00	3.84	4.52	3.07
1	94	3	7	2	3.40	9.25	3.80	4.74	3.62
1	94	3	7	3	3.35	9.00	3.73	4.97	3.82
1	94	3	14	1	3.50	8.03	2.90	4.44	3.92
1	94	3	14	2	3.50	8.82	2.60	5.16	3.90
1	94	3	14	3	3.45	8.40	0.00	3.60	2.60
1	94	3	21	1	3.50	7.91	3.07	5.87	3.30
1	94	3	21	2	3.45	7.16	2.60	4.20	3.07
1	94	3	21	3	3.55	7.63	0.00	5.05	2.30
1	94	3	40	1	3.50	7.06	2.30	4.99	3.89
1	94	3	40	2	3.53	7.24	2.90	4.49	4.10
1	94	3	40	3	3.50	7.42	2.30	4.22	2.90
1	94	3	100	1	3.50	6.80	2.60	4.78	3.20
1	94	3	100	2	3.50	6.90	2.30	4.67	3.00
1	94	3	100	3	3.50	7.02	2.60	4.58	2.80
1	94	4	0	1	5.30	5.01	3.95	5.81	2.60
1	94	4	0	2	5.10	5.11	4.79	5.39	2.58
1	94	4	0	3	5.10	4.85	5.41	5.06	3.20
1	94	4	1	1	4.40	8.56	5.34	5.55	3.38
1	94	4	1	2	4.30	8.51	5.06	5.37	3.07
1	94	4	1	3	4.30	8.38	5.18	5.45	3.07
1	94	4	3	1	3.50	9.04	5.15	4.64	2.60
1	94	4	3	2	3.55	8.63	5.02	4.52	3.45
1	94	4	3	3	3.50	9.14	5.04	4.36	3.40
1	94	4	7	1	3.35	9.09	3.60	4.55	2.90
1	94	4	7	2	3.40	8.37	3.80	4.84	3.52
1	94	4	7	3	3.40	8.40	4.15	4.91	3.74
1	94	4	14	1	3.40	8.39	2.60	5.25	2.60
1	94	4	14	2	3.45	8.31	2.60	5.12	3.19
1	94	4	14	3	3.45	8.46	3.30	4.64	2.60
1	94	4	21	1	3.50	7.08	2.90	5.66	2.90
1	94	4	21	2	3.50	7.44	2.60	4.40	3.20
1	94	4	21	3	3.50	7.60	2.60	4.07	3.07
1	94	4	40	1	3.50	7.25	2.30	4.66	3.82
1	94	4	40	2	3.45	7.63	2.60	4.65	4.00
1	94	4	40	3	3.50	7.43	2.60	4.65	4.00
1	94	4	100	1	3.50	6.90	2.60	4.50	2.90
1	94	4	100	2	3.50	7.01	2.30	4.64	2.60
1	94	4	100	3	3.55	7.10	2.30	4.67	2.70
2	93	1	0	1	5.57	4.36	6.86	6.12	2.94
2	93	1	0	2	5.70	4.76	7.45	6.37	2.55
2	93	1	0	3	5.82	3.67	7.19	6.13	2.55
2	93	1	1	1	5.22	5.41	7.59	6.81	4.10

Table A-2 . Cont.

Env	Year	Trt	D	Rep	pH	LAB	ENT	YM	LAY
2	93	1	1	2	4.84	5.42	7.45	7.25	4.05
2	93	1	1	3	4.76	5.46	7.77	7.11	3.99
2	93	1	3	1	4.78	6.89	5.87	6.73	5.55
2	93	1	3	2	4.81	7.37	5.29	7.24	5.64
2	93	1	3	3	4.52	7.80	5.78	6.78	6.21
2	93	1	7	1	4.93	7.73	3.85	6.25	4.46
2	93	1	7	2	4.88	7.77	4.03	7.12	7.01
2	93	1	7	3	4.50	7.02	3.99	5.94	5.21
2	93	1	14	1	4.59	6.44	2.90	5.94	5.51
2	93	1	14	2	4.51	6.96	2.84	6.41	5.39
2	93	1	14	3	4.24	7.04	2.60	5.80	5.49
2	93	1	21	1	4.77	7.09	2.00	6.63	6.54
2	93	1	21	2	4.57	6.99	3.31	7.70	6.71
2	93	1	21	3	4.45	6.48	1.00	6.76	6.55
2	93	1	40	1	4.30	7.30	3.26	6.10	5.35
2	93	1	40	2	4.27	7.09	2.73	7.38	5.43
2	93	1	40	3	4.10	6.44	0.00	6.77	6.51
2	93	1	100	1	4.19	6.50	3.00	6.30	5.20
2	93	1	100	2	4.19	6.75	2.30	6.20	5.10
2	93	1	100	3	4.16	6.43	1.00	7.30	6.20
2	93	2	0	1	5.55	4.01	7.29	5.91	2.74
2	93	2	0	2	5.26	4.34	7.37	6.02	2.25
2	93	2	0	3	6.02	3.48	7.09	5.84	2.58
2	93	2	1	1	4.73	6.04	7.65	6.90	4.00
2	93	2	1	2	4.89	5.12	7.71	6.99	4.21
2	93	2	1	3	4.74	5.99	8.20	6.95	3.98
2	93	2	3	1	4.85	6.92	5.77	6.28	4.53
2	93	2	3	2	4.86	8.01	4.98	6.75	4.93
2	93	2	3	3	4.80	7.76	4.83	6.77	5.36
2	93	2	7	1	4.54	7.74	3.50	7.18	6.69
2	93	2	7	2	4.61	7.74	3.49	7.58	6.10
2	93	2	7	3	4.46	7.47	3.70	6.40	5.33
2	93	2	14	1	4.39	7.10	3.83	6.35	5.89
2	93	2	14	2	4.46	7.76	4.24	6.87	6.65
2	93	2	14	3	4.31	7.25	4.34	5.81	4.46
2	93	2	21	1	4.64	5.72	2.73	5.57	5.56
2	93	2	21	2	4.79	7.20	4.30	7.95	7.49
2	93	2	21	3	4.63	7.38	3.93	6.68	5.76
2	93	2	40	1	4.21	6.16	2.30	5.41	5.29
2	93	2	40	2	4.25	7.09	2.60	7.70	7.23
2	93	2	40	3	4.23	6.78	2.00	7.18	6.57
2	93	2	100	1	4.17	6.34	2.30	6.54	5.78
2	93	2	100	2	4.31	6.65	2.30	6.70	6.20
2	93	2	100	3	4.19	6.70	1.00	7.20	6.85
2	93	3	0	1	4.99	5.43	6.59	6.25	2.51
2	93	3	0	2	5.26	5.69	6.96	6.15	2.23
2	93	3	0	3	5.95	5.55	6.29	6.18	2.61
2	93	3	1	1	4.66	6.57	6.09	5.01	3.86
2	93	3	1	2	4.56	6.53	6.10	6.32	4.05
2	93	3	1	3	4.62	6.24	5.81	5.55	3.87
2	93	3	3	1	4.44	6.66	5.37	5.70	4.44
2	93	3	3	2	4.48	8.21	5.42	6.66	4.65
2	93	3	3	3	4.30	7.64	4.67	5.23	4.19
2	93	3	7	1	4.40	7.99	3.71	6.39	6.22
2	93	3	7	2	4.55	7.71	3.49	7.11	7.09
2	93	3	7	3	4.50	7.27	3.20	7.19	5.32
2	93	3	14	1	4.35	7.26	5.04	6.90	6.67
2	93	3	14	2	4.20	7.26	4.70	7.33	7.28
2	93	3	14	3	4.26	7.01	3.47	7.34	6.00
2	93	3	21	1	4.37	7.44	3.24	6.48	6.28
2	93	3	21	2	4.43	6.70	1.00	5.77	5.63
2	93	3	21	3	4.40	7.54	3.55	6.88	6.74
2	93	3	40	1	4.05	6.57	4.38	6.45	4.65
2	93	3	40	2	4.03	7.06	4.68	8.43	5.67
2	93	3	40	3	4.05	7.08	4.21	8.27	6.94

Table A-2 . Cont.

Env	Year	Trt	D	Rep	pH	LAB	ENT	YM	LAY
2	93	3	100	1	4.15	6.54	2.00	6.54	5.78
2	93	3	100	2	4.21	7.65	2.30	7.68	6.34
2	93	3	100	3	4.04	6.67	2.90	8.12	7.45
2	93	4	0	1	5.33	5.58	7.04	6.18	2.43
2	93	4	0	2	5.42	5.81	7.17	6.39	2.72
2	93	4	0	3	5.88	5.39	6.90	5.87	2.58
2	93	4	1	1	4.57	6.26	5.90	5.22	3.65
2	93	4	1	2	4.72	6.44	6.57	5.74	3.96
2	93	4	1	3	4.64	6.09	6.75	5.81	4.24
2	93	4	3	1	4.41	8.35	4.78	5.91	4.16
2	93	4	3	2	4.24	8.32	5.54	6.02	3.72
2	93	4	3	3	4.29	8.50	4.92	6.01	3.34
2	93	4	7	1	4.40	7.76	3.50	6.75	5.96
2	93	4	7	2	4.50	7.85	3.78	7.81	6.63
2	93	4	7	3	4.42	7.83	1.00	6.99	6.55
2	93	4	14	1	4.16	6.92	3.32	5.57	4.98
2	93	4	14	2	4.44	7.64	3.48	7.28	7.21
2	93	4	14	3	4.19	6.55	2.69	6.80	5.83
2	93	4	21	1	4.33	6.56	3.54	6.91	4.85
2	93	4	21	2	4.50	6.94	3.75	7.01	6.83
2	93	4	21	3	4.34	6.34	3.23	6.75	4.11
2	93	4	40	1	4.01	6.51	4.64	5.21	4.56
2	93	4	40	2	3.96	6.74	4.03	6.94	5.63
2	93	4	40	3	3.95	6.83	3.07	6.03	5.64
2	93	4	100	1	4.07	6.75	2.30	6.30	5.90
2	93	4	100	2	3.96	7.45	2.60	5.98	5.64
2	93	4	100	3	4.10	6.43	3.20	7.12	6.87
2	94	1	0	1	5.67	2.30	4.15	3.68	2.69
2	94	1	0	2	5.76	3.07	4.03	2.84	2.30
2	94	1	0	3	5.89	3.41	6.49	3.34	3.29
2	94	1	1	1	5.24	7.73	7.89	5.51	5.23
2	94	1	1	2	4.74	7.72	7.99	6.06	4.33
2	94	1	1	3	4.82	8.17	7.55	5.66	5.21
2	94	1	3	1	4.74	8.35	6.52	6.68	6.25
2	94	1	3	2	4.78	8.01	6.68	6.95	6.04
2	94	1	3	3	4.53	8.42	6.19	7.39	5.45
2	94	1	7	1	4.70	7.16	7.84	8.00	7.25
2	94	1	7	2	4.72	7.18	7.57	7.03	6.48
2	94	1	7	3	4.60	7.40	7.41	7.53	7.32
2	94	1	14	1	4.48	7.65	4.05	7.68	7.68
2	94	1	14	2	4.54	7.31	3.70	6.31	6.26
2	94	1	14	3	4.20	7.21	4.10	5.89	6.03
2	94	1	21	1	4.58	6.35	2.30	4.95	5.10
2	94	1	21	2	4.53	7.61	6.60	6.57	6.96
2	94	1	21	3	4.38	6.54	6.60	5.89	5.91
2	94	1	40	1	4.16	6.84	3.79	5.20	5.17
2	94	1	40	2	4.15	6.98	3.23	5.28	5.04
2	94	1	40	3	4.12	6.63	4.02	5.56	5.33
2	94	1	100	1	4.09	5.54	3.00	5.40	5.29
2	94	1	100	2	4.19	6.24	4.65	4.30	4.60
2	94	1	100	3	4.16	6.36	4.62	5.32	4.85
2	94	2	0	1	5.65	2.90	4.33	2.77	2.90
2	94	2	0	2	5.35	3.98	5.25	3.38	3.49
2	94	2	0	3	6.10	4.16	5.84	3.70	3.62
2	94	2	1	1	4.86	7.43	8.05	5.15	4.45
2	94	2	1	2	4.87	8.27	7.72	5.12	5.16
2	94	2	1	3	4.73	8.22	7.38	5.77	5.58
2	94	2	3	1	4.70	8.02	6.91	6.68	6.25
2	94	2	3	2	4.75	8.31	6.42	6.95	6.04
2	94	2	3	3	4.72	8.45	6.09	7.39	6.45
2	94	2	7	1	4.70	8.03	6.22	7.98	6.34
2	94	2	7	2	4.80	8.07	6.17	7.96	7.90
2	94	2	7	3	4.74	8.26	6.29	7.74	7.29
2	94	2	14	1	4.47	7.26	2.30	5.04	4.80
2	94	2	14	2	4.45	7.26	5.45	6.23	6.76
2	94	2	14	3	4.50	7.00	3.69	6.07	6.05
2	94	2	21	1	4.50	6.72	3.71	6.13	5.82

Table A-2 . Cont.

Env	Year	Trt	D	Rep	pH	LAB	ENT	YM	LAY
2	94	2	21	2	4.65	5.92	1.00	5.55	5.15
2	94	2	21	3	4.60	6.77	6.72	6.31	6.79
2	94	2	40	1	4.20	5.65	2.47	4.69	4.30
2	94	2	40	2	4.05	6.74	4.06	5.69	4.60
2	94	2	40	3	4.11	7.37	3.88	5.77	4.60
2	94	2	100	1	4.20	6.05	3.62	4.84	4.69
2	94	2	100	2	4.12	6.12	5.10	4.77	4.47
2	94	2	100	3	4.19	5.82	2.47	4.60	4.60
2	94	3	0	1	5.23	5.37	4.98	3.59	2.69
2	94	3	0	2	5.24	5.14	6.87	3.55	2.69
2	94	3	0	3	5.87	5.27	6.08	3.59	3.04
2	94	3	1	1	4.62	8.83	8.85	5.14	5.10
2	94	3	1	2	4.57	9.12	8.94	5.70	5.52
2	94	3	1	3	4.56	9.07	9.09	5.90	5.14
2	94	3	3	1	4.35	9.16	5.71	6.03	5.97
2	94	3	3	2	4.40	9.19	6.60	6.78	6.42
2	94	3	3	3	4.28	8.75	6.59	6.80	6.75
2	94	3	7	1	4.15	7.85	5.29	7.59	7.30
2	94	3	7	2	4.25	8.41	4.25	7.69	7.32
2	94	3	7	3	4.40	8.55	3.19	6.36	6.23
2	94	3	14	1	4.42	6.91	4.02	6.21	6.12
2	94	3	14	2	4.15	6.70	3.51	6.65	6.65
2	94	3	14	3	4.22	6.65	2.90	5.86	5.54
2	94	3	21	1	4.25	6.77	5.84	6.25	6.02
2	94	3	21	2	4.35	6.47	3.30	6.10	5.97
2	94	3	21	3	4.35	6.36	4.09	5.89	5.66
2	94	3	40	1	4.14	6.30	2.00	5.12	5.55
2	94	3	40	2	4.10	5.80	4.48	4.77	4.84
2	94	3	40	3	4.05	6.46	4.04	5.17	5.28
2	94	3	100	1	4.20	5.92	4.08	5.04	4.00
2	94	3	100	2	4.08	4.88	3.00	4.95	4.30
2	94	3	100	3	4.10	6.02	3.38	4.77	4.30
2	94	4	0	1	5.43	4.45	5.31	2.00	2.69
2	94	4	0	2	5.32	5.20	6.02	2.77	2.60
2	94	4	0	3	5.88	5.36	6.36	2.47	3.49
2	94	4	1	1	4.52	8.85	7.53	5.34	4.49
2	94	4	1	2	4.70	8.94	7.44	5.86	5.04
2	94	4	1	3	4.64	9.09	7.09	5.38	5.15
2	94	4	3	1	4.35	8.94	6.02	7.07	6.03
2	94	4	3	2	4.22	8.71	5.94	6.90	6.71
2	94	4	3	3	4.29	9.13	6.41	7.00	6.59
2	94	4	7	1	4.25	8.09	5.96	7.10	7.24
2	94	4	7	2	4.25	8.01	5.60	7.26	7.00
2	94	4	7	3	4.25	7.52	6.00	7.46	7.41
2	94	4	14	1	4.11	6.92	3.43	6.60	6.54
2	94	4	14	2	4.39	7.22	3.38	6.04	6.09
2	94	4	14	3	4.20	6.55	2.84	5.96	5.90
2	94	4	21	1	4.15	6.62	6.00	6.22	6.02
2	94	4	21	2	4.35	6.54	3.40	6.07	5.47
2	94	4	21	3	4.35	6.63	3.00	5.71	5.04
2	94	4	40	1	4.02	5.87	1.00	5.91	5.97
2	94	4	40	2	3.91	5.98	5.14	5.27	5.73
2	94	4	40	3	3.87	6.78	3.86	5.57	4.99
2	94	4	100	1	4.07	6.00	4.20	5.26	4.47
2	94	4	100	2	3.96	5.65	4.45	4.80	4.77
2	94	4	100	3	4.10	4.95	3.92	4.47	4.80

Abbreviations:
Env = Environment, 1 = Temperate, 2 = Tropical; Trt = treatment, 1 = control, 2 = enzyme, 3 = inoculant, 4 = E + I; D = Day of ensiling; Rep = Replicate, Ent = Enterobacteriaceae, LAB = Lactic acid bacteria, YM = Yeasts and molds, LAY = Lactate assimilating yeast.

Table A-3. Data used for analysis of fermentation end-products (g/100 g DM) in forage sorghum ensiled in a temperate environment (Chapter 2)

Year	Trt	Day	Acetic acid	Lactic acid	Propionic acid	Ethanol	Butyric Acid
93	1	0	0.294	0.9676	0	0.171	0
93	1	0	0.232	0.0660	0	0.066	0
93	1	0	0.350	0.6988	0	0.187	0
93	1	1	1.007	3.5647	0	0.691	0
93	1	1	1.091	2.7387	0.007	0.423	0.006
93	1	1	1.000	3.5671	0.004	0.543	0.005
93	1	3	1.001	5.6033	0.005	1.798	0.009
93	1	3	1.283	5.3286	0.006	1.255	0.003
93	1	3	1.298	5.7318	0.018	1.177	0.004
93	1	7	1.190	6.6049	0.019	1.290	0.003
93	1	7	1.208	5.8368	0.009	1.999	0.008
93	1	7	1.795	6.3193	0.008	1.351	0.009
93	1	14	1.554	9.2949	0.008	1.652	0.014
93	1	14	1.786	10.138	0	1.513	0.017
93	1	14	1.735	9.3815	0.009	1.036	0.016
93	1	21	1.826	9.0176	0.010	1.449	0
93	1	21	1.802	9.4047	0.010	1.239	0.014
93	1	21	1.879	9.9177	0.009	1.599	0.014
93	1	40	2.096	7.6580	0.012	1.511	0.008
93	1	40	2.322	9.9165	0.012	1.171	0.009
93	1	40	1.258	6.5395	0.011	1.020	0.016
93	1	100	2.152	5.4800	0.015	1.074	0.013
93	1	100	2.050	6.3880	0.016	1.038	0.008
93	1	100	1.896	6.4170	0.014	1.295	0.006
93	2	0	0.182	0.6081	0	0.377	0
93	2	0	0.266	0.5401	0	0.173	0
93	2	0	0.180	0.6720	0	0.150	0
93	2	1	0.917	3.5891	0.004	0.461	0.007
93	2	1	1.185	4.5328	0.003	0.558	0.006
93	2	1	1.088	3.6239	0.007	0.493	0.005
93	2	3	1.445	6.1924	0.017	0.914	0.019
93	2	3	1.172	5.0116	0	1.123	0
93	2	3	1.046	5.8457	0.013	1.241	0.004
93	2	7	1.287	6.5637	0.009	1.206	0.003
93	2	7	1.473	5.5841	0.009	1.876	0.018
93	2	7	1.264	5.9151	0.008	1.673	0.009
93	2	14	1.770	10.487	0.008	1.799	0.014
93	2	14	1.647	9.3887	0	1.381	0.017
93	2	14	1.869	8.8697	0.009	1.397	0.016
93	2	21	1.999	9.1399	0.010	1.465	0
93	2	21	1.692	10.234	0.010	1.511	0.014
93	2	21	1.692	8.8331	0.009	1.629	0.014
93	2	40	1.788	7.6000	0.012	1.207	0.008
93	2	40	1.878	8.5684	0.012	1.161	0.009
93	2	40	1.788	7.3643	0.011	1.253	0.016
93	2	100	1.850	6.3830	0.015	1.103	0.013
93	2	100	2.072	5.6060	0.016	1.203	0.008
93	2	100	2.117	6.0330	0.014	0.841	0.006
93	3	0	0.198	0.4999	0	0.194	0
93	3	0	0.175	0.7758	0	0.113	0
93	3	0	0.217	0.4045	0	0.356	0
93	3	1	1.147	5.4511	0.004	0.677	0.007
93	3	1	1.018	4.8699	0.003	0.416	0.006
93	3	1	1.057	4.8720	0.007	0.409	0.005
93	3	3	1.561	6.9722	0.017	0.971	0.009
93	3	3	0.830	8.4206	0.011	1.548	0.023
93	3	3	1.101	7.2709	0.003	1.360	0.004
93	3	7	1.614	8.7409	0.009	2.223	0.003
93	3	7	1.257	9.0480	0.009	1.692	0.018
93	3	7	1.456	9.2408	0.008	1.067	0.009
93	3	14	1.754	9.1518	0.008	1.269	0.014
93	3	14	1.884	8.5351	0	1.728	0.017
93	3	14	1.394	9.4757	0.009	1.283	0.016
93	3	21	1.688	8.5716	0.010	1.428	0.011

Table A-3. Cont.

Year	Trt	Day	Acetic acid	Lactic acid	Propionic acid	Ethanol	Butyric Acid
93	3	21	1.809	8.0559	0.010	1.602	0.014
93	3	21	1.768	9.2927	0.009	0.986	0.014
93	3	40	1.022	8.4511	0.012	0.471	0.008
93	3	40	1.778	10.037	0.012	1.845	0.009
93	3	40	1.980	7.0457	0.011	1.237	0.016
93	3	100	1.845	6.2230	0.015	1.003	0.013
93	3	100	1.663	7.7210	0.016	1.045	0.008
93	3	100	1.823	6.0430	0.014	1.271	0.006
93	4	0	0.197	0.7992	0	0.112	0
93	4	0	0.228	0.3512	0	0.260	0
93	4	0	0.203	0.7304	0	0.138	0
93	4	1	1.150	4.4840	0.004	0.741	0.007
93	4	1	1.066	4.8741	0.003	0.400	0.006
93	4	1	1.093	5.6152	0.007	0.514	0.005
93	4	3	1.302	7.1165	0.017	0.870	0.019
93	4	3	1.289	7.5338	0.011	0.994	0.023
93	4	3	1.185	6.6505	0.013	1.258	0.004
93	4	7	1.390	10.258	0.009	2.043	0.033
93	4	7	1.467	10.068	0.009	1.161	0.018
93	4	7	1.404	12.431	0.008	1.975	0.009
93	4	14	1.599	10.038	0.008	1.301	0.014
93	4	14	1.971	9.4834	0	1.204	0.017
93	4	14	1.640	9.0985	0.009	1.558	0.016
93	4	21	1.949	9.3011	0.010	1.564	0
93	4	21	1.840	7.0156	0.010	1.426	0.014
93	4	21	1.558	10.775	0.009	1.527	0.014
93	4	40	1.833	9.1831	0.012	1.361	0.008
93	4	40	1.312	9.8370	0.012	1.401	0.009
93	4	40	1.716	7.6850	0.011	1.010	0.016
93	4	100	1.618	7.2010	0.015	1.048	0.013
93	4	100	1.918	6.8280	0.016	1.190	0.008
93	4	100	1.754	6.8770	0.014	1.384	0.006
94	1	0	0.148	0.3370	0.002	0.074	0.007
94	1	0	0.385	1.0840	0.002	0.183	0.008
94	1	0	0.234	0.9160	0.003	0.135	0.006
94	1	1	0.721	3.8650	0.000	0.507	0.004
94	1	1	0.655	5.0300	0.001	0.544	0.005
94	1	1	1.179	5.0060	0.001	0.581	0.002
94	1	3	0.883	6.8580	0.007	0.473	0.005
94	1	3	1.035	4.6170	0	0.618	0.011
94	1	3	0.881	6.8590	0.003	0.514	0.007
94	1	7	1.136	5.7070	0	0.628	0.009
94	1	7	1.206	7.5880	0	0.590	0.011
94	1	7	1.159	8.3570	0.006	0.642	0.007
94	1	14	1.310	10.870	0.011	0.634	0.010
94	1	14	1.307	8.6270	0.010	0.404	0.009
94	1	14	0.984	7.5260	0.010	0.433	0.007
94	1	21	1.274	10.420	0.002	0.391	0.003
94	1	21	1.377	9.4210	0.004	0.480	0.004
94	1	21	1.529	8.9720	0.002	0.400	0.016
94	1	40	1.632	8.7450	0.008	0.374	0.015
94	1	40	1.592	7.9420	0.009	0.385	0.009
94	1	40	1.601	8.1200	0.007	0.354	0.014
94	1	100	0.328	2.9530	0	0.210	0.001
94	1	100	0.809	7.8640	0.003	0.349	0.015
94	1	100	1.931	8.0620	0.004	0.573	0.004
94	2	0	0.158	0.4493	0.000	0.137	0.006
94	2	0	0.238	0.7046	0	0.444	0.004
94	2	0	0.381	1.2497	0.001	0.332	0.008
94	2	1	0.830	4.8035	0	0.672	0.004
94	2	1	0.540	2.9258	0	0.373	0.009
94	2	1	0.813	4.4997	0.000	0.569	0.009
94	2	3	0.994	5.9238	0	0.586	0.008
94	2	3	1.013	4.7179	0	0.540	0.009
94	2	3	1.161	4.6471	0	0.503	0.006
94	2	7	1.288	9.4148	0	0.629	0.007

Table A-3. Cont.

Year	Trt	Day	Acetic acid	Lactic acid	Propionic acid	Ethanol	Butyric Acid
94	2	7	1.289	6.9143	0	0.646	0.008
94	2	7	1.283	6.4027	0	0.615	0.006
94	2	14	1.480	9.2147	0	0.527	0.012
94	2	14	1.375	9.1636	0.001	0.536	0.011
94	2	14	1.564	9.0432	0	0.628	0.015
94	2	21	1.444	10.108	0.001	0.579	0.012
94	2	21	1.491	9.7346	0.001	0.404	0.011
94	2	21	1.673	9.4630	0.001	0.582	0.014
94	2	40	1.741	8.4710	0.004	0.408	0.009
94	2	40	1.652	8.5410	0.001	0.374	0.011
94	2	40	1.592	8.9640	0.000	0.359	0.009
94	2	100	1.747	6.4388	0.011	0.580	0.004
94	2	100	1.805	7.6517	0.002	0.611	0.013
94	2	100	1.928	6.3361	0.010	0.365	0.005
94	3	0	0.180	0.1639	0	0.124	0
94	3	0	0.160	0.4408	0	0.206	0
94	3	0	0.990	0.4963	0	0.072	0
94	3	1	0.968	3.2186	0	0.279	0.003
94	3	1	0.920	8.2372	0	0.584	0.003
94	3	1	0.794	3.6380	0	0.436	0.003
94	3	3	0.852	6.3183	0	0.442	0
94	3	3	0.836	8.3982	0	0.532	0.006
94	3	3	0.992	8.0765	0	0.649	0.009
94	3	7	1.211	5.0781	0	0.275	0.007
94	3	7	0.586	12.273	0	1.202	0.002
94	3	7	1.359	7.0210	0	0.436	0.002
94	3	14	1.574	10.853	0	0.816	0.007
94	3	14	1.225	10.302	0.006	0.666	0.007
94	3	14	1.478	8.6945	0	0.171	0.006
94	3	21	1.231	11.002	0.006	0.675	0.007
94	3	21	1.038	11.673	0.001	1.623	0.008
94	3	21	1.113	11.822	0.008	1.367	0.007
94	3	40	1.342	8.4890	0.007	0.415	0.009
94	3	40	1.456	8.8740	0.006	0.408	0.007
94	3	40	1.547	8.1120	0.003	0.374	0.006
94	3	100	1.694	7.8180	0.002	1.747	0.008
94	3	100	1.885	6.8002	0.011	0.748	0.008
94	3	100	1.276	6.7070	0.002	0.251	0.010
94	4	0	0.189	0.1486	0	0.126	0
94	4	0	0.188	0.4257	0	0.164	0.001
94	4	0	0.207	0.6338	0	0.199	0.001
94	4	1	0.838	6.6372	0	0.694	0.002
94	4	1	0.709	4.9831	0	0.408	0.001
94	4	1	0.564	5.9001	0	0.435	0.002
94	4	3	0.730	5.9855	0	0.511	0.001
94	4	3	1.271	5.2142	0	0.295	0.001
94	4	3	0.749	7.7104	0	0.894	0.005
94	4	7	1.476	8.5867	0	0.566	0.000
94	4	7	0.874	9.6164	0	0.496	0.002
94	4	7	0.820	6.9639	0.005	0.548	0.008
94	4	14	1.471	8.9556	0	0.634	0.009
94	4	14	1.238	9.9145	0.013	0.619	0.008
94	4	14	1.592	8.8557	0	0.508	0.013
94	4	21	2.058	10.161	0.008	0.434	0.002
94	4	21	1.082	11.194	0.002	0.721	0.008
94	4	21	1.266	11.134	0.004	0.669	0.015
94	4	40	1.420	8.2460	0.005	0.425	0.017
94	4	40	1.350	8.0490	0.003	0.419	0.009
94	4	40	1.320	8.1060	0.001	0.368	0.007
94	4	100	1.783	8.7458	0	0.431	0.006
94	4	100	1.502	7.2142	0.003	0.399	0.011
94	4	100	1.837	9.0262	0.004	0.482	0.012

Abbreviations:
Trt = treatment, 1 = control, 2 = enzyme, 3 = inoculant, 4 = E+I

Table A-4. Data used for analysis of water soluble carbohydrate contents (g/ 100 g DM) in forage sorghum ensiled in a temperate environment. (Chapter 2)

Year	Trt	Day	Glucose	Xylose	Galactose	Arabinose	Fructose
94	1	0	6.6879	0.0819	0.1607	0.1407	6.7163
94	1	0	6.4145	0.0813	0.1654	0.1911	6.5177
94	1	0	6.7808	0.0731	0.1461	0.1709	6.0887
94	1	1	2.4132	0.0605	0.1994	0.1854	1.8905
94	1	1	4.4269	0.0799	0.1543	0.1565	2.3232
94	1	1	4.1811	0.1333	0.3757	0.0836	1.9839
94	1	3	3.6399	0.1025	0.1531	0.1100	1.7617
94	1	3	3.6536	0.1013	0.3878	0.1333	2.6824
94	1	3	3.215	0.0956	0.3179	0.0911	1.0260
94	1	7	2.3878	0.1045	0.3493	0.1290	0.9943
94	1	7	2.8747	0.0899	0.2898	0.1527	0.8412
94	1	7	2.3307	0.1216	0.3377	0.1333	1.2874
94	1	14	2.4242	0.1066	0.2973	0.1298	0.2015
94	1	14	1.5771	0.1219	0.1422	0.1024	0.3635
94	1	14	3.3724	0.1763	0.3006	0.0945	0.6848
94	1	21	1.1581	0.1316	0.3388	0.1105	0.3042
94	1	21	2.9685	0.1292	0.3564	0.1025	0.3575
94	1	21	2.9967	0.1786	0.3201	0.0978	0.2083
94	1	40	0.5887	0.4534	0.1056	0.0892	0.4341
94	1	40	2.4398	0.3058	0.2488	0.0928	0.2762
94	1	40	1.9578	0.3757	0.3371	0.0892	0.1874
94	1	100	0.5618	0.6077	0.1566	0.1302	0.533
94	1	100	1.6095	0.7751	0.3698	0.1824	0.4577
94	1	100	0.6489	0.6778	0.1938	0.1053	0.6363
94	2	0	7.6812	0.0829	0.2457	0.2755	6.9904
94	2	0	5.9582	0.0367	0.1224	0.0864	6.9629
94	2	0	6.2934	0.0568	0.2155	0.1931	5.0525
94	2	1	4.4307	0.0847	0.3214	0.1924	2.4638
94	2	1	3.0357	0.0587	0.2981	0.2065	2.4628
94	2	1	3.1035	0.0751	0.2339	0.1524	1.0844
94	2	3	3.8543	0.1022	0.2947	0.1600	1.995
94	2	3	3.6213	0.1305	0.2574	0.2589	1.4749
94	2	3	3.5923	0.1492	0.3473	0.1257	1.4183
94	2	7	2.2063	0.1115	0.3168	0.1506	1.2017
94	2	7	2.7472	0.0972	0.2823	0.1100	1.0554
94	2	7	2.5622	0.1373	0.3332	0.0472	1.1091
94	2	14	2.3462	0.0822	0.2868	0.0993	0.9612
94	2	14	2.1325	0.1169	0.3698	0.0852	0.5525
94	2	14	2.7685	0.1666	0.3786	0.0564	0.4178
94	2	21	2.2000	0.0873	0.2884	0.0929	0.1624
94	2	21	2.0478	0.1178	0.3341	0.0576	0.3576
94	2	21	1.6387	0.189	0.2821	0.0821	0.2562
94	2	40	1.9103	0.1998	0.1514	0.0719	0.2413
94	2	40	1.6598	0.5619	0.3974	0.0783	0.1668
94	2	40	1.7923	0.2044	0.3088	0.1426	0.1873
94	2	100	1.0835	0.7965	0.2437	0.1900	0.5372
94	2	100	1.0164	0.5045	0.2306	0.0979	0.3795
94	2	100	1.0821	0.6157	0.2711	0.1425	0.4239
94	3	0	6.7086	0.0810	0.1389	0.1133	5.9171
94	3	0	6.5924	0.0651	0.1611	0.1787	6.9507
94	3	0	5.9609	0.0742	0.1372	0.1411	6.0836
94	3	1	2.4768	0.0978	0.2159	0.1606	2.1429
94	3	1	3.7012	0.0782	0.2388	0.1534	2.8333
94	3	1	4.7862	0.0871	0.2251	0.1502	2.3082
94	3	3	2.4827	0.1225	0.3679	0.1232	3.1375
94	3	3	3.5628	0.1475	0.2967	0.1331	1.9854
94	3	3	3.2666	0.1543	0.2545	0.1327	2.1921
94	3	7	2.6455	0.1162	0.3952	0.1045	2.3433
94	3	7	2.2517	0.0875	0.2779	0.0978	2.9988
94	3	7	1.8983	0.1452	0.1314	0.1245	1.3822
94	3	14	1.8122	0.1123	0.1958	0.0978	1.7653
94	3	14	1.7139	0.1732	0.1864	0.0711	2.3658
94	3	14	1.9402	0.1309	0.149	0.0648	1.9722

Table A-4. Cont.

Year	Trt	Day	Glucose	Xylose	Galactose	Arabinose	Fructose
94	3	21	1.9295	0.1545	0.3358	0.1028	2.5835
94	3	21	1.2091	0.0588	0.1542	0.1245	1.986
94	3	21	0.8331	0.1402	0.2086	0.0096	0.9835
94	3	40	1.1481	0.7376	0.3098	0.0880	1.8983
94	3	40	1.215	0.1354	0.2224	0.0845	1.6506
94	3	40	1.5847	0.0296	0.1031	0.0837	0.8336
94	3	100	1.5171	0.6443	0.2638	0.142	1.9073
94	3	100	0.7371	0.6975	0.1366	0.1875	0.9296
94	3	100	0.8589	0.5891	0.2987	0.1148	1.2468
94	4	0	5.7601	0.0499	0.1498	0.2084	6.7471
94	4	0	6.8008	0.0482	0.2101	0.1626	5.7127
94	4	0	5.3619	0.0568	0.2202	0.1113	5.9492
94	4	1	3.4581	0.0589	0.2187	0.1145	2.1441
94	4	1	3.8524	0.0845	0.2245	0.1063	2.4799
94	4	1	3.6469	0.0762	0.2268	0.1169	2.5926
94	4	3	3.8251	0.1093	0.3309	0.1245	1.9941
94	4	3	3.4259	0.1185	0.3729	0.0845	2.5770
94	4	3	2.8729	0.129	0.3784	0.1045	2.6354
94	4	7	2.3417	0.0499	0.2506	0.1375	2.047
94	4	7	2.2885	0.0571	0.2917	0.1398	2.5763
94	4	7	1.2781	0.1185	0.3248	0.1224	2.3316
94	4	14	1.2819	0.0919	0.2673	0.1478	2.0412
94	4	14	1.8227	0.1189	0.3084	0.1777	2.5929
94	4	14	1.9182	0.0977	0.1995	0.1473	1.3662
94	4	21	1.4666	0.22	0.2085	0.1013	2.0739
94	4	21	1.3347	0.1749	0.232	0.1481	0.9769
94	4	21	1.1693	0.8433	0.2345	0.1836	2.5709
94	4	40	1.6874	0.2177	0.1206	0.0945	0.8779
94	4	40	1.3422	0.3514	0.3407	0.1752	2.4007
94	4	40	0.9827	0.2439	0.3525	0.1043	1.3389
94	4	100	0.853	0.8938	0.2193	0.2253	1.0434
94	4	100	1.2293	0.3956	0.194	0.2022	1.7544
94	4	100	0.9305	0.2106	0.2312	0.1605	1.1445
93	1	0	5.4839	0.0737	0.0509	0.0286	5.515
93	1	0	5.1204	0.0731	0.0555	0.0982	4.5249
93	1	0	5.511	0.0813	0.0363	0.0812	4.4652
93	1	1	3.4366	0.0865	0.0515	0.0612	1.6133
93	1	1	3.1497	0.0753	0.0423	0.0546	1.0696
93	1	1	3.2705	0.0556	0.0424	0.0728	2.5593
93	1	3	0.0352	0.0997	0.0697	0.7992	1.0245
93	1	3	3.2145	0.0183	0.1644	0.0767	1.2530
93	1	3	1.4657	0.0147	0.1425	0.0645	1.1124
93	1	7	1.8815	0.1269	0.1341	0.096	0.6248
93	1	7	1.7031	0.1358	0.1023	0.067	0.7376
93	1	7	1.7183	0.1558	0.0688	0.0674	0.6059
93	1	14	1.5531	0.1662	0.0824	0.0877	0.5029
93	1	14	1.5607	0.1456	0.0974	0.0708	0.5951
93	1	14	1.6144	0.0341	0.0945	0.0321	0.304
93	1	21	1.2426	0.1044	0.1165	0.0773	0.2651
93	1	21	1.1912	0.1184	0.1207	0.0523	0.2695
93	1	21	1.2288	0.1234	0.0967	0.059	0.4343
93	1	40	1.1453	0.2134	0.0635	0.0906	0.3964
93	1	40	1.1724	0.3438	0.0852	0.0303	0.4524
93	1	40	1.1098	0.3351	0.0748	0.0202	0.2246
93	1	100	1.0692	0.5034	0.0652	0.125	0.5598
93	1	100	1.0819	0.5266	0.0583	0.1086	0.2038
93	1	100	1.1177	0.5243	0.0765	0.1156	0.313
93	2	0	5.777	0.0678	0.087	0.0852	4.734
93	2	0	4.821	0.0761	0.0697	0.1116	4.800
93	2	0	5.453	0.0682	0.1068	0.076	4.6269
93	2	1	3.261	0.0466	0.0416	0.0597	1.6789
93	2	1	2.963	0.0437	0.0207	0.0326	1.8449
93	2	1	3.522	0.0855	0.0748	0.0935	1.5431
93	2	3	2.764	0.1532	0.2784	0.0756	0.9149
93	2	3	2.690	0.1073	0.2452	0.0676	0.8153
93	2	3	2.964	0.0938	0.0756	0.0795	0.9365

Table A-4. Cont.

Year	Trt	Day	Glucose	Xylose	Galactose	Arabinose	Fructose
93	2	7	1.709	0.1088	0.0957	0.0776	0.5132
93	2	7	1.7363	0.1146	0.0385	0.0658	0.7352
93	2	7	1.8890	0.1684	0.1117	0.1102	0.4842
93	2	14	1.724	0.0968	0.0865	0.0809	0.4261
93	2	14	1.587	0.1151	0.1351	0.0631	0.5669
93	2	14	1.654	0.1104	0.054	0.078	0.4559
93	2	21	1.1824	0.1555	0.0717	0.0615	0.5222
93	2	21	1.1083	0.1773	0.1337	0.0907	0.3643
93	2	21	1.1967	0.1383	0.0963	0.0234	0.3782
93	2	40	1.0723	0.2791	0.0978	0.1241	0.3577
93	2	40	1.0876	0.3163	0.0945	0.0234	0.3077
93	2	40	1.1678	0.388	0.0602	0.1163	0.2683
93	2	100	1.0307	0.7206	0.0456	0.0919	0.3998
93	2	100	1.0567	0.4026	0.0546	0.0945	0.4812
93	2	100	1.1346	0.654	0.0840	0.099	0.3219
93	3	0	5.346	0.0675	0.0654	0.0670	4.0605
93	3	0	5.512	0.0578	0.0756	0.0897	4.3955
93	3	0	4.821	0.098	0	0.0768	6.0704
93	3	1	2.968	0.047	0.0691	0.0476	1.7822
93	3	1	3.096	0.05	0	0.0633	2.5845
93	3	1	3.621	0.1028	0.2001	0.095	1.9657
93	3	3	2 .8062	0.0293	0.1724	0.0735	1.9338
93	3	3	2.153	0.122	0.1084	0.0648	2.2976
93	3	3	2.2278	0.1028	0.1101	0.045	1.9657
93	3	7	1.5909	0.0991	0.072	0.1033	1.0955
93	3	7	1.6305	0.1214	0.0908	0.099	0.9764
93	3	7	1.6427	0.1291	0.1284	0.1175	1.4269
93	3	14	1.515	0.1072	0.0872	0.0732	0.6677
93	3	14	1.5337	0.1402	0.1229	0.0811	0.7688
93	3	14	1.4934	0.1384	0.1309	0.0833	1.3313
93	3	21	1.143	0.1688	0.1232	0.0784	1.4571
93	3	21	1.0642	0.0961	0.0912	0.0813	0.9488
93	3	21	1.0312	0.1389	0.1235	0.0723	0.9058
93	3	40	1.0616	0.1022	0.0701	0.0754	1.2853
93	3	40	1.05	0.2792	0.0554	0.0645	0.6572
93	3	40	1.087	0.234	0.085	0.0875	1.2414
93	3	100	1.064	0.6832	0.056	0.1059	0.7358
93	3	100	1.043	0.6126	0.0702	0.1043	1.2982
93	3	100	1.0564	0.5378	0.0614	0.0849	1.2905
93	4	0	5.7980	0.0450	0.0412	0.087	5.0312
93	4	0	4.9056	0.0558	0.051	0.0441	4.8943
93	4	0	5.2174	0.062	0.060	0.0376	4.9033
93	4	1	3.3459	0.0457	0.0344	0.0939	2.5085
93	4	1	3.2665	0.0897	0.0461	0.0222	2.7199
93	4	1	3.0549	0.0723	0.06	0.0785	1.0508
93	4	3	2.1067	0.115	0.1869	0.0913	1.9539
93	4	3	2.2901	0.0995	0.1177	0.0767	1.7007
93	4	3	2.5549	0.0872	0.1376	0.048	1.4512
93	4	7	1.3115	0.1493	0.092	0.1109	1.6654
93	4	7	1.6792	0.1867	0.0695	0.1246	1.5782
93	4	7	1.7661	0.1879	0.248	0.0935	0.9544
93	4	14	1.5327	0.1855	0.0902	0.1329	1.1198
93	4	14	1.5981	0.1528	0.0836	0.1273	1.9394
93	4	14	1.4517	0.1134	0.0735	0.0679	0.4591
93	4	21	1.0874	0.2751	0.0733	0.1242	0.6335
93	4	21	1.2208	0.2636	0.1211	0.0592	1.2019
93	4	21	1.1208	0.2383	0.0654	0.0737	1.3910
93	4	40	1.1934	0.2942	0.1085	0.0875	1.0978
93	4	40	1.1108	0.3342	0.1115	0.122	0.4510
93	4	40	1.0973	0.3961	0.0676	0.1062	1.3480
93	4	100	1.0561	0.5011	0.0572	0.0717	0.5188
93	4	100	1.0712	0.6037	0.0595	0.0912	1.1095
93	4	100	1.1229	0.4936	0.0611	0.0841	1.1504

Abbreviations:
Trt = treatment; 1 = control, 2 = enzyme, 3 = inoculant, 4 = E +I

Table A-5. Data used for analysis of structural carbohydrate content (g/ 100 g DM) in forage sorghum ensiled in a temperate environment (Chapter 2)

Year	Trt	Day	NDF	Hemicellulose	ADF	Cellulose
93	1	0	54.2300	21.380	32.849	28.234
93	1	0	57.6362	22.080	35.555	30.485
93	1	0	58.2502	22.256	35.993	32.751
93	2	0	54.7903	20.925	33.864	28.609
93	2	0	55.5685	22.776	32.791	27.861
93	2	0	54.2495	22.559	31.690	27.338
93	3	0	55.8190	22.738	33.080	28.158
93	3	0	56.2967	22.496	33.800	26.438
93	3	0	54.8387	22.145	32.693	28.280
93	4	0	59.7958	20.494	39.301	33.427
93	4	0	58.1439	18.611	39.532	33.906
93	4	0	57.4471	22.778	34.668	28.732
93	1	40	57.1159	22.469	34.646	29.142
93	1	40	58.8908	22.765	36.125	30.599
93	1	40	58.5276	22.085	36.441	30.715
93	2	40	55.7812	20.269	35.511	29.599
93	2	40	58.0203	22.046	35.974	30.281
93	2	40	52.2267	28.125	24.101	30.613
93	3	40	57.5228	21.705	35.817	30.335
93	3	40	55.8277	21.412	34.415	29.087
93	3	40	57.0564	22.369	34.686	30.021
93	4	40	55.6318	20.954	34.677	28.186
93	4	40	52.1092	19.754	32.354	27.244
93	4	40	55.3821	23.029	32.352	28.639
93	1	100	59.5819	24.164	35.417	30.630
93	1	100	58.8200	22.045	36.774	32.094
93	1	100	58.1256	23.310	34.815	29.710
93	2	100	55.8905	20.946	34.944	29.093
93	2	100	55.3435	21.452	33.891	28.927
93	2	100	58.2150	22.652	35.562	29.282
93	3	100	57.2047	20.550	36.654	30.890
93	3	100	54.4737	21.378	33.094	28.566
93	3	100	56.5530	22.178	34.375	29.753
93	4	100	56.4775	20.703	35.773	29.611
93	4	100	55.0066	20.938	34.068	28.975
93	4	100	55.4520	21.730	33.721	29.005
94	1	0	63.7135	25.156	38.556	26.984
94	1	0	61.5940	24.536	37.058	24.106
94	1	0	59.6221	23.282	36.340	31.042
94	2	0	64.3458	24.985	39.359	32.753
94	2	0	62.5435	24.610	37.933	27.931
94	3	0	60.9197	25.139	35.779	25.446
94	3	0	59.3350	24.508	34.826	27.488
94	3	0	54.6195	22.742	31.877	27.561
94	4	0	55.6124	23.132	32.480	28.138
94	4	0	57.3484	25.738	31.609	26.969
94	4	0	56.6637	23.511	33.151	26.672
94	1	40	57.2093	21.860	35.348	30.697
94	1	40	57.9513	22.434	35.517	30.405
94	1	40	59.0942	22.571	36.523	31.574
94	2	40	54.5032	20.586	33.917	29.699
94	2	40	59.4520	22.524	36.927	30.430
94	2	40	54.3561	21.973	32.382	28.551
94	3	40	59.5751	25.160	34.414	28.536
94	3	40	60.9979	24.844	36.153	31.888
94	3	40	57.0667	21.182	35.884	30.983
94	4	40	58.7621	21.591	37.170	32.679
94	4	40	57.9078	21.689	36.218	31.381
94	4	40	58.6982	20.836	37.861	33.248
94	1	100	60.7209	24.112	36.608	31.359
94	1	100	59.0873	23.291	35.796	31.272
94	1	100	58.8054	22.844	35.960	31.260
94	2	100	56.4969	25.970	30.526	26.660
94	2	100	55.8740	19.168	36.706	32.321

Table A-5. Cont.

Year	Trt	Day	NDF	Hemicellulose	ADF	Cellulose
94	2	100	56.6293	23.158	33.470	32.842
94	3	100	55.3300	20.847	34.482	27.645
94	3	100	59.1692	22.047	37.122	28.944
94	3	100	59.8640	22.666	37.197	30.141
94	4	100	58.2208	22.201	36.019	29.576
94	4	100	57.4539	22.926	34.527	29.063
94	4	100	56.5914	22.723	33.867	25.851

Abbreviations:
Trt = treatment; 1 = control, 2 = enzyme, 3 = inoculant, 4 = E+I

Table A-6. Data used for analysis of organic acid contents (g/ 100 g DM) of forage sorghum ensiled in a tropical environment (Chapter 2)

Year	Trt	Day	Citric	Malic	Oxal	Succinic
94	1	0	0.875	4.277	1.076	4.416
94	1	0	0.742	4.918	1.150	2.212
94	1	0	0.968	4.491	1.147	2.006
94	1	1	0.265	2.805	1.275	3.513
94	1	1	0.321	3.697	1.301	3.109
94	1	1	0.285	2.407	1.122	2.537
94	1	3	0.28	2.98	1.171	3.236
94	1	3	0.188	3.663	1.135	1.833
94	1	3	0.279	1.353	1.741	2.006
94	1	7	0.204	2.755	1.471	2.880
94	1	7	0.412	2.315	0.254	2.217
94	1	7	0.336	3.007	1.224	3.359
94	1	14	0.417	2.799	0.388	2.059
94	1	14	0.597	1.968	1.525	3.71
94	1	14	0.643	1.897	0.636	3.798
94	1	21	0.509	3.753	1.047	3.138
94	1	21	0.287	2.922	1.074	2.630
94	1	21	0.379	2.225	1.163	1.867
94	1	40	0.433	3.108	2.051	2.653
94	1	40	0.515	2.162	2.133	2.873
94	1	40	0.356	2.856	1.008	1.818
94	1	100	0.125	1.033	0.914	1.078
94	1	100	0.108	1.167	0.479	0.831
94	1	100	0.173	1.369	0.527	1.198
94	2	0	0.798	4.700	1.678	3.431
94	2	0	0.612	4.773	1.085	3.724
94	2	0	0.744	5.612	1.160	2.844
94	2	1	0.359	2.838	1.831	4.289
94	2	1	0.785	2.163	1.486	4.419
94	2	1	0.407	2.386	0.424	2.824
94	2	3	0.237	2.091	1.138	3.275
94	2	3	0.169	3.988	1.111	2.560
94	2	3	0.403	1.937	1.148	2.910
94	2	7	0.393	1.849	1.580	3.761
94	2	7	0.507	2.282	1.701	3.366
94	2	7	0.354	2.101	1.072	2.097
94	2	14	0.586	3.095	1.467	1.232
94	2	14	0.258	2.223	1.667	3.109
94	2	14	0.531	3.544	1.082	2.632
94	2	21	0.456	1.102	1.074	2.509
94	2	21	0.387	2.414	1.250	1.005
94	2	21	0.308	1.052	0.755	1.858
94	2	40	0.456	1.359	1.303	2.027
94	2	40	0.364	1.536	0.797	1.780
94	2	40	0.297	2.060	0.545	1.457
94	2	100	0.347	1.344	1.065	1.045
94	2	100	0.184	1.396	0.529	1.134
94	2	100	0.237	2.047	0.507	1.251
94	3	0	0.511	4.560	1.19	3.607
94	3	0	0.802	4.345	1.380	3.553
94	3	0	0.371	3.998	1.123	2.685
94	3	1	0.163	2.548	1.366	3.322
94	3	1	0.824	3.611	1.235	2.474
94	3	1	0.797	2.393	1.010	3.258
94	3	3	0.799	2.592	1.410	2.484
94	3	3	0.728	2.724	0.995	2.536
94	3	3	0.730	2.583	1.266	2.488
94	3	7	0.638	3.685	1.123	2.740
94	3	7	0.773	3.081	1.224	2.043
94	3	7	0.249	2.829	1.169	0.930
94	3	14	0.309	4.249	0.996	2.670
94	3	14	0.274	3.476	0.850	2.161
94	3	14	0.262	2.747	1.063	1.806
94	3	21	0.177	2.247	1.225	1.734

Table A-6. Cont.

Year	Trt	Day	Citric	Malic	Oxal	Succinic
94	3	21	0.304	2.708	1.068	1.932
94	3	21	0.181	1.795	0.983	1.433
94	3	40	0.273	2.499	1.319	1.757
94	3	40	0.279	2.352	0.766	1.677
94	3	40	0.264	1.863	0.800	1.517
94	3	100	0.124	1.753	0.943	1.316
94	3	100	0.177	1.998	0.852	1.919
94	3	100	0.169	1.934	0.987	1.459
94	4	0	0.720	5.001	1.034	3.565
94	4	0	0.306	4.567	1.311	2.966
94	4	0	0.987	4.221	1.355	4.856
94	4	1	0.497	3.080	1.58	2.282
94	4	1	0.336	2.889	1.318	2.658
94	4	1	0.306	2.143	1.57	2.445
94	4	3	0.283	3.035	1.691	2.567
94	4	3	0.639	2.603	0.948	3.821
94	4	3	0.121	3.839	1.353	1.879
94	4	7	0.736	3.515	1.108	1.923
94	4	7	0.469	3.104	1.705	2.187
94	4	7	0.516	2.858	0.947	1.984
94	4	14	0.234	3.473	0.984	3.511
94	4	14	0.44	2.123	1.149	1.687
94	4	14	0.596	2.092	0.787	2.883
94	4	21	0.314	3.934	1.296	1.873
94	4	21	0.312	3.430	0.984	1.858
94	4	21	0.110	2.453	1.436	1.253
94	4	40	0.216	2.018	0.849	1.091
94	4	40	0.194	2.251	0.585	1.265
94	4	40	0.332	3.348	0.891	1.717
94	4	100	0.137	1.949	0.914	1.870
94	4	100	0.104	1.006	0.768	1.656
94	4	100	0.104	2.329	0.905	0.996
93	1	0	0.813	4.560	0.883	2.137
93	1	0	0.633	3.456	1.040	1.999
93	1	0	0.617	4.327	0.941	2.103
93	1	1	0.457	2.809	0.823	0.875
93	1	1	0.876	2.974	0.432	1.298
93	1	1	0.654	2.647	0.201	1.020
93	1	3	0.435	1.096	0.473	1.606
93	1	3	0.397	3.421	0.745	1.073
93	1	3	0.172	2.518	0.693	0.976
93	1	7	0.916	3.363	0.912	0.997
93	1	7	0.747	2.339	0.734	1.600
93	1	7	0.096	1.635	0.827	1.876
93	1	14	0.843	3.629	1.002	1.141
93	1	14	0.204	2.568	0.797	1.301
93	1	14	0.452	2.415	1.057	1.571
93	1	21	0.687	1.229	0.942	0.881
93	1	21	0.373	2.787	0.748	1.132
93	1	21	0.960	1.415	0.765	1.175
93	1	40	0.078	1.895	0.512	1.592
93	1	40	0.717	1.590	0.626	0.987
93	1	40	0.443	1.787	0.426	0.968
93	1	100	0.113	1.342	0.574	0.637
93	1	100	0.311	1.245	0.174	1.162
93	1	100	0.543	0.831	0.339	1.673
93	2	0	0.647	4.105	0	0.975
93	2	0	0.229	4.356	1.112	2.320
93	2	0	0.810	5.000	1.606	0.876
93	2	1	0.501	2.405	0.843	1.271
93	2	1	0.599	3.041	0.675	1.006
93	2	1	0.493	2.534	0.567	0.876
93	2	3	0.128	3.646	0.216	1.124
93	2	3	0.691	2.268	0.435	1.175
93	2	3	0.88	1.276	0.448	1.218
93	2	7	0.638	2.556	0.896	2.309

Table A-6. Cont.

year	trt	day	citric	malic	oxal	succinic
93	2	7	0.099	3.581	0.685	1.159
93	2	7	0.560	1.243	0.524	1.087
93	2	14	0.122	1.966	1.070	1.582
93	2	14	0.183	2.745	0.978	1.148
93	2	14	0.312	1.852	0.459	2.859
93	2	21	0.597	1.850	0.698	1.840
93	2	21	0.521	1.906	0.997	1.199
93	2	21	0.479	2.996	1.691	2.407
93	2	40	0.703	2.575	0.947	1.936
93	2	40	0.136	2.024	0.747	1.096
93	2	40	0.400	2.004	0	1.082
93	2	100	0.344	1.243	0.085	0.531
93	2	100	0.407	1.092	0.077	0.482
93	2	100	0.563	1.106	0.206	1.040
93	3	0	0.744	5.310	0.512	2.638
93	3	0	0.529	4.650	0.903	1.237
93	3	0	0.947	4.316	0.738	1.244
93	3	1	0.522	1.937	0.645	0.856
93	3	1	0.536	2.686	0.943	0.861
93	3	1	0.387	3.172	0.432	1.331
93	3	3	0.395	2.223	0.724	1.175
93	3	3	0.345	2.042	0.62	0.912
93	3	3	0.467	1.836	0.735	1.459
93	3	7	0.778	1.713	0.498	1.837
93	3	7	0.400	2.340	0.641	1.405
93	3	7	0.699	2.007	0.678	1.045
93	3	14	0.439	2.286	0.337	1.168
93	3	14	0.677	1.805	0.253	0.751
93	3	14	0.693	2.054	1.220	1.299
93	3	21	0.596	2.067	0.605	1.261
93	3	21	0.838	1.569	0.760	1.124
93	3	21	0.281	2.026	0.787	1.391
93	3	40	0.946	1.328	0.372	1.022
93	3	40	0.741	1.521	0.261	0.910
93	3	40	0.463	2.054	0.519	1.347
93	3	100	0.184	1.748	0.231	0.710
93	3	100	0.231	1.750	0.155	0.686
93	3	100	0.250	1.440	0.203	1.144
93	4	0	0.650	5.588	1.020	2.580
93	4	0	0.501	4.946	0.997	1.716
93	4	0	0.653	3.271	0.409	1.083
93	4	1	0.765	3.354	0.842	0.878
93	4	1	0.602	2.196	0.635	0.695
93	4	1	0.876	3.345	0.523	0.979
93	4	3	0.952	1.915	0.643	0.994
93	4	3	0.355	2.956	0.243	1.142
93	4	3	0.425	2.517	0.645	1.208
93	4	7	0.253	1.954	0.397	1.003
93	4	7	0.369	2.341	0.466	1.012
93	4	7	0.882	3.078	0.255	1.875
93	4	14	0.415	2.017	0.487	1.978
93	4	14	0.209	2.172	0.587	1.602
93	4	14	0.188	2.856	0.309	2.816
93	4	21	0.381	2.099	0.299	0.867
93	4	21	0.247	1.339	0.223	1.279
93	4	21	0.486	1.942	0.391	2.130
93	4	40	0.292	2.596	0.514	0.876
93	4	40	0.687	1.731	0.418	1.056
93	4	40	0.576	1.019	0.164	0.695
93	4	100	0.489	1.762	0.878	0.975
93	4	100	0.378	1.438	0.199	1.432
93	4	100	0.453	1.836	0.791	0.891

Abbreviations:
Trt = treatment; 1 = control, 2 = enzyme, 3 = inoculant, 4 = E +I; Oxa = Oxaloacetic acid

Table A-7. Data used for analysis of fermentation end-products (g/100 g DM) of forage sorghum ensiled in a tropical environment (Chapter 2)

Year	Trt	Day	Ace	Lac	Prop	Etoh	But
94	1	0	0.083	0.146	0	0.061	0
94	1	0	0.027	0.053	0	0.057	0
94	1	0	0.126	0.027	0	0.132	0
94	1	1	0.128	1.348	0.005	0.480	0.020
94	1	1	0.256	1.268	0.000	0.510	0.030
94	1	1	0.230	1.298	0	0.466	0.030
94	1	3	0.917	5.550	0.007	0.516	0.086
94	1	3	0.606	6.809	0.003	0.659	0.033
94	1	3	0.656	3.485	0.006	0.546	0.077
94	1	7	0.795	5.890	0.000	0.665	0.050
94	1	7	0.642	6.991	0.006	0.856	0.048
94	1	7	0.850	8.596	0.008	0.934	0.073
94	1	14	0.418	5.845	0.030	1.217	0.028
94	1	14	0.971	8.218	0.051	1.371	0.080
94	1	14	1.182	6.422	0.051	1.167	0.022
94	1	21	0.614	5.830	0.039	0.627	0.052
94	1	21	0.907	6.850	0.041	0.704	0.018
94	1	21	0.599	5.401	0.042	0.413	0.071
94	1	40	0.822	4.109	0.029	0.453	0.050
94	1	40	0.761	5.593	0.036	0.529	0.027
94	1	40	0.304	3.877	0.026	0.294	0.043
94	1	100	0.714	2.902	0.020	0.202	0.053
94	1	100	0.803	2.585	0.040	0.283	0.076
94	1	100	0.732	2.125	0	0.103	0.011
94	2	0	0.037	0.077	0	0.096	0
94	2	0	0.082	0.099	0	0.029	0
94	2	0	0.089	0.092	0	0.099	0
94	2	1	0.223	0.581	0	0.540	0.024
94	2	1	0.259	0.249	0.005	0.627	0.031
94	2	1	0.172	0.242	0	0.478	0.067
94	2	3	0.609	4.877	0.003	0.715	0.071
94	2	3	1.141	6.614	0.009	0.623	0.077
94	2	3	0.795	6.717	0.003	0.831	0.049
94	2	7	1.175	6.540	0.004	1.089	0.003
94	2	7	0.886	7.374	0.006	0.884	0.053
94	2	7	0.534	6.926	0.007	0.835	0.052
94	2	14	1.106	6.721	0.054	1.038	0.001
94	2	14	0.808	7.826	0.031	0.922	0.039
94	2	14	0.814	7.877	0.030	0.918	0.024
94	2	21	0.722	5.541	0.038	0.651	0.097
94	2	21	0.767	6.411	0.038	0.638	0.031
94	2	21	0.686	5.945	0.036	0.740	0.042
94	2	40	0.561	5.949	0.021	0.431	0.059
94	2	40	0.350	4.038	0	0.431	0.035
94	2	40	0.817	4.097	0	0.317	0.025
94	2	100	0.775	2.791	0.024	0.227	0.000
94	2	100	0.733	1.875	0.020	0.391	0.092
94	2	100	0.874	2.514	0.040	0.126	0.079
94	3	0	0.106	0.092	0	0.081	0
94	3	0	0.082	0.023	0	0.154	0
94	3	0	0.117	0.158	0.001	0.022	0
94	3	1	0.288	2.281	0.000	0.148	0.029
94	3	1	0.406	1.189	0.000	0.191	0.056
94	3	1	0.312	1.137	0.005	0.962	0.052
94	3	3	0.556	9.318	0.005	0.594	0.085
94	3	3	0.573	8.720	0.007	0.715	0.060
94	3	3	0.792	8.692	0.007	0.749	0.078
94	3	7	0.705	8.730	0.006	0.811	0.091
94	3	7	0.756	7.874	0.005	0.817	0.037
94	3	7	0.786	5.850	0	0.83	0.099
94	3	14	0.636	8.431	0.068	0.893	0.071
94	3	14	0.845	8.120	0	1.485	0.047
94	3	14	0.670	6.067	0	0.942	0.067
94	3	21	0.726	5.617	0	0.436	0.080

Table A-7. Cont.

Year	Trt	Day	Ace	Lac	Prop	Etoh	But
94	3	21	0.469	7.282	0.061	0.588	0.047
94	3	21	0.628	6.201	0	0.595	0.038
94	3	40	0.235	5.180	0.255	0.406	0.024
94	3	40	0.701	4.823	0	0.327	0.096
94	3	40	0.416	4.621	0	0.462	0.083
94	3	100	0.319	3.287	0.039	0.296	0.019
94	3	100	0.795	5.102	0.020	0.331	0.014
94	3	100	0.769	3.460	0	0.250	0.071
94	4	0	0.023	0.158	0	0.055	0
94	4	0	0.105	0.149	0	0.139	0
94	4	0	0.127	0.041	0.007	0.072	0
94	4	1	0.205	2.255	0.000	0.936	0.060
94	4	1	0.215	1.329	0.009	0.009	0.050
94	4	1	0.342	0.519	0	0.402	0.034
94	4	3	0.770	9.463	0.002	0.822	0.007
94	4	3	0.759	8.517	0.004	0.669	0.008
94	4	3	0.354	8.161	0.007	0.553	0.097
94	4	7	0.557	7.642	0.009	0.639	0.098
94	4	7	0.447	7.877	0	0.859	0.057
94	4	7	0.844	6.509	0.007	0.571	0.015
94	4	14	0.606	9.799	0.037	0.987	0.066
94	4	14	0.573	7.334	0.030	1.448	0.006
94	4	14	0.923	8.225	0.045	0.852	0.077
94	4	21	0.672	6.923	0.028	0.666	0.064
94	4	21	0.776	6.269	0.060	0.694	0.097
94	4	21	0.755	4.335	0	0.090	0.089
94	4	40	1.354	3.421	0	0.235	0.001
94	4	40	0.881	3.834	0.093	0.346	0.065
94	4	40	0.840	5.649	0.083	0.592	0.094
94	4	100	0.782	4.263	0.021	0.401	0.065
94	4	100	0.793	4.224	0.02	0.328	0.036
94	4	100	0.637	3.274	0	0.149	0.032
93	1	0	0.04	0.222	0	0.033	0
93	1	0	0.03	0.232	0	0.025	0
93	1	0	0.026	0.084	0	0.009	0
93	1	1	0.192	0.937	0	0.172	0.038
93	1	1	0.232	1.764	0.006	0.143	0.041
93	1	1	0.204	1.634	0.006	0.076	0.049
93	1	3	0.088	0.695	0	0.089	0
93	1	3	0.327	2.3	0	0.314	0.075
93	1	3	0.261	2.142	0	0.327	0.062
93	1	7	0.27	1.909	0	0.372	0.083
93	1	7	0.228	1.957	0	0.53	0.007
93	1	7	0.466	4.189	0	0.667	0.081
93	1	14	0.489	2.765	0.430	0.772	0.083
93	1	14	0.218	2.117	0.052	1.585	0.064
93	1	14	0.317	3.607	0.056	0.872	0.051
93	1	21	0.297	1.064	0.059	0.313	0.084
93	1	21	0.497	2.999	0.053	1.079	0.078
93	1	21	0.397	3.446	0.054	0.846	0.084
93	1	40	0.352	1.484	0.084	0.337	0.107
93	1	40	0.298	1.39	0	0.79	0.032
93	1	40	0.291	1.613	0	0.41	0.012
93	1	100	0.724	1.767	0.039	0.463	0.047
93	1	100	0.459	1.165	0.060	0.470	0.039
93	1	100	0.398	1.384	0.111	0.921	0.030
93	2	0	0.015	0.109	0	0.009	0
93	2	0	0.055	0.26	0	0.028	0
93	2	0	0.033	0.155	0	0.009	0
93	2	1	0.326	1.81	0	0.048	0.033
93	2	1	0.308	1.551	0	0.092	0.071
93	2	1	0.154	0.994	0	0.228	0.055
93	2	3	0.236	1.511	0	0.224	0.056
93	2	3	0.293	2.172	0	0.286	0.054
93	2	3	0.227	1.362	0	0.139	0.078
93	2	7	0.373	2.215	0	0.543	0.05

Table A-7. Cont.

Year	Trt	Day	Ace	Lac	Prop	Etoh	But
93	2	7	0.365	2.026	0	0.805	0.06
93	2	7	0.292	3.081	0	0.636	0.059
93	2	14	0.26	3.317	0.084	0.495	0.091
93	2	14	0.271	1.901	0.09	0.401	0.035
93	2	14	0.343	2.837	0.08	1.765	0.073
93	2	21	0.305	2.373	0.064	0.59	0.064
93	2	21	0.403	2.245	0.09	0.508	0.062
93	2	21	0.557	1.99	0.079	0.646	0.067
93	2	40	0.452	2.407	0.056	1.09	0.066
93	2	40	0.342	1.702	0.06	0.459	0.06
93	2	40	0.539	1.758	0.05	0.459	0.06
93	2	100	0.269	1.850	0.088	0.310	0.047
93	2	100	0.733	1.466	0.080	0.969	0.057
93	2	100	0.586	1.085	0.030	0.990	0.026
93	3	0	0.068	0.423	0	0.085	0
93	3	0	0.030	0.213	0	0.031	0
93	3	0	0.040	0.196	0	0.018	0
93	3	1	0.183	2.529	0	0.154	0.027
93	3	1	0.160	1.818	0	0.154	0.049
93	3	1	0.210	1.215	0	0.071	0.032
93	3	3	0.147	1.525	0	0.333	0.029
93	3	3	0.239	1.703	0	0.098	0.038
93	3	3	0.293	4.167	0	0.38	0.031
93	3	7	0.345	3.813	0	0.466	0.066
93	3	7	0.262	3.486	0	0.654	0.043
93	3	7	0.279	3.868	0	0.815	0.082
93	3	14	0.275	2.606	0.08	1.25	0.062
93	3	14	0.294	4.44	0.062	1.049	0.059
93	3	14	0.46	6.074	0.071	1.359	0.093
93	3	21	0.40	2.492	0.05	0.422	0.09
93	3	21	0.394	2.027	0.08	0.506	0.010
93	3	21	0.301	2.727	0.04	0.489	0.084
93	3	40	0.488	1.979	0.027	0.521	0.027
93	3	40	0.364	1.318	0.06	0.49	0.026
93	3	40	0.357	1.886	0.03	0.49	0.031
93	3	100	0.409	1.206	0.044	0.903	0.037
93	3	100	0.721	1.592	0.071	0.558	0.028
93	3	100	0.309	1.745	0.021	0.854	0.034
93	4	0	0.068	0.083	0	0.048	0
93	4	0	0.058	0.215	0	0.04	0
93	4	0	0.026	0.206	0	0.002	0
93	4	1	0.195	0.625	0	0.154	0.039
93	4	1	0.237	1.561	0	0.175	0.056
93	4	1	0.212	2.103	0	0.148	0.048
93	4	3	0.218	3.492	0	0.59	0.032
93	4	3	0.258	3.076	0	0.314	0.032
93	4	3	0.237	2.15	0	0.379	0.061
93	4	7	0.265	3.718	0	0.554	0.050
93	4	7	0.269	3.443	0	0.746	0.073
93	4	7	0.311	4.425	0	0.835	0.045
93	4	14	0.334	4.395	0.073	1.272	0.084
93	4	14	0.379	3.872	0.06	1.161	0.075
93	4	14	0.43	4.67	0.04	1.422	0.055
93	4	21	0.408	2.926	0.04	0.513	0.042
93	4	21	0.314	2.414	0.03	0.506	0.092
93	4	21	0.367	3.067	0.044	0.842	0.03
93	4	40	0.453	2.011	0.06	0.366	0.02
93	4	40	0.38	2.367	0.032	0.618	0.03
93	4	40	0.244	1.915	0.04	0.6	0.02
93	4	100	0.485	1.581	0.019	0.652	0.05
93	4	100	0.640	1.915	0.045	0.735	0.02
93	4	100	0.439	1.429	0.077	0.692	0.03

Abbreviations:
Trt = treatment; 1 = control, 2 = enzyme, 3 = inoculant, 4 = E+I; Ace = Acetic acid, Lac = Lactic acid; Pro = Propionic Acid, Etoh = Ethanol, But = Butyric acid.

Table A-8. Data used for analysis of water soluble carbhydrate contents (g/100 g DM) in forage sorghum ensiled in a tropical environment (Chapter 2)

Year	Trt	Day	Glucose	Xylose	Galactose	Arabinose	Fructose
94	1	0	2.9064	0.6815	0.24762	0.08609	4.0702
94	1	0	2.8024	0.7724	0.25851	0.07615	3.0249
94	1	0	2.6933	0.691	0.16691	0.07598	3.3164
94	1	1	0.6062	0.7453	0.07224	0.06379	0.2663
94	1	1	0.5814	0.6331	0.06507	0.06465	0.4373
94	1	1	0.7615	0.8191	0.07307	0.06448	0.6003
94	1	3	0.8515	0.0783	0.04748	0.06007	0.1671
94	1	3	0.6889	0.5515	0.02737	0.04668	0.2082
94	1	3	0.2315	0.7076	0.01843	0.05878	0.4683
94	1	7	0.4146	0.7375	1.04511	0.14902	0.06
94	1	7	0.5275	0.8673	0.16049	0.07995	0.39
94	1	7	0.6406	0.5537	0.18413	0.06915	0.4368
94	1	14	0.5541	0.8	0.13461	0.05904	0.2663
94	1	14	0.5321	0.919	0.27964	0.10174	0.3079
94	1	14	0.4858	0.9187	0.32749	0.13173	0.4864
94	1	21	0.4466	0.7754	0.10575	0.14953	0.3078
94	1	21	0.5003	0.5391	0.31306	0.12222	0.2948
94	1	21	0.3622	0.615	0.08573	0.0873	0.1455
94	1	40	0.5022	0.3022	0.21839	0.10692	0.2824
94	1	40	0.0528	0.4267	0.2182	0.13424	0.3366
94	1	40	0.4962	0.2451	0.15825	0.09439	0.1502
94	1	100	0.2896	0.1704	0.08611	0.04279	0.019
94	1	100	0.4211	0.2432	0.22649	0.09849	0.1012
94	1	100	0.1382	0.1262	0.08816	0.16318	0.0718
94	2	0	2.8664	0.8115	0.237	0.08618	3.678
94	2	0	2.9519	0.6731	0.24799	0.1587	3.1008
94	2	0	2.7922	0.6345	0.24706	0.07753	2.9228
94	2	1	0.5484	0.8199	0.08797	0.06353	0.5357
94	2	1	0.8977	0.4275	0.37701	0.06552	0.4588
94	2	1	0.5571	0.7336	0.10342	0.11384	0.3206
94	2	3	0.4545	0.8103	0.08387	0.08445	0.2997
94	2	3	0.6408	0.6698	0.12139	0.08687	0.2092
94	2	3	0.7086	0.7993	0.09113	0.07753	0.3568
94	2	7	0.5258	0.9812	0.16747	0.0561	0.6429
94	2	7	0.4827	0.8742	0.23207	0.04477	0.175
94	2	7	0.4194	0.1579	0.13451	0.06656	0.2769
94	2	14	0.4282	0.8965	0.27415	0.1676	0.2994
94	2	14	0.8612	0.9383	3.21706	0.15472	0.5599
94	2	14	0.3994	0.568	0.20517	0.1383	0.3049
94	2	21	0.3491	0.5469	0.23589	0.0536	0.2729
94	2	21	0.3793	0.3092	0.20303	0.0821	0.2842
94	2	21	0.4169	0.5888	0.16607	0.09439	0.3447
94	2	40	0.2757	0.2195	0.18571	0.1249	0.1371
94	2	40	0.4957	0.2167	0.21047	0.11254	0.1932
94	2	40	0.3	0.1878	0.30989	0.13813	0.2465
94	2	100	0.2121	0.1628	0.12753	0.08626	0.0768
94	2	100	0.3327	0.1979	0.14866	0.14419	0.0686
94	2	100	0.2739	0.2872	0.20656	0.11314	0.078
94	3	0	3.1978	0.5785	0.22881	0.08825	3.2119
94	3	0	2.6928	0.6637	0.2263	0.11133	3.0657
94	3	0	2.9932	0.6295	0.16654	0.08134	3.0611
94	3	1	0.9742	0.8044	0.09318	0.08073	0.359
94	3	1	0.3538	0.6233	0.0916	0.07183	0.4791
94	3	1	0.6421	0.6645	0.05222	0.07727	0.9844
94	3	3	0.4378	0.1275	0.11683	0.05826	0.2879
94	3	3	0.3523	0.6434	0.07931	0.03043	0.2416
94	3	3	0.436	0.7105	0.07065	0.04719	0.5252
94	3	7	0.4238	0.7954	0.12576	0.09836	0.3201
94	3	7	0.3842	0.5368	0.13591	0.04814	0.642
94	3	7	0.4169	0.7888	0.16607	0.09439	0.4447
94	3	14	0.457	0.7142	0.20517	0.14971	0.3148
94	3	14	0.4486	0.1818	0.17184	0.13061	0.3404
94	3	14	0.4164	0.3523	0.15155	0.11496	0.2852
94	3	21	0.3658	0.1137	0.1023	0.09344	0.474

Table A-8. Cont.

Year	Trt	Day	Glucose	Xylose	Galactose	Arabinose	Fructose
94	3	21	0.3441	0.6401	0.13768	0.11496	0.2368
94	3	21	0.3315	0.6586	0.13517	0.09707	0.1973
94	3	40	0.0953	0.1151	0.12288	0.05895	0.1689
94	3	40	0.419	0.1177	0.13321	0.06889	0.2329
94	3	40	0.3922	0.0988	0.10184	0.06146	0.1614
94	3	100	0.3761	0.1038	0.09197	0.08842	0.3964
94	3	100	0.2575	0.1537	0.12586	0.12092	0.1084
94	3	100	0.0394	0.2163	0.15592	0.16233	0.2267
94	4	0	3.0534	0.6229	0.1293	0.1141	3.1424
94	4	0	2.9757	0.6937	0.1159	0.0994	3.2766
94	4	0	2.4756	0.6785	0.10321	0.1023	3.2456
94	4	1	0.7802	0.7281	0.07764	0.11652	0.2876
94	4	1	0.3777	0.72	0.08667	0.09568	0.8953
94	4	1	0.6557	0.6412	0.07633	.12637	0.3732
94	4	3	0.4445	0.8332	0.02346	0.13207	0.3029
94	4	3	0.5464	0.7191	0.30468	0.14305	0.5443
94	4	3	0.3837	0.3073	0.07429	0.07477	0.7283
94	4	7	0.4256	0.2252	0.18999	0.18575	0.2194
94	4	7	0.2291	0.4868	0.17706	0.15801	0.3631
94	4	7	0.402	0.9052	0.1024	0.02602	0.5666
94	4	14	0.4554	1.0151	0.96524	0.17262	0.6213
94	4	14	0.3056	0.9044	0.30012	0.14228	0.7012
94	4	14	0.3202	0.8979	0.29975	0.09693	0.4869
94	4	21	0.3731	0.6085	0.19884	0.11249	0.3622
94	4	21	0.2602	0.6843	0.25637	0.1829	0.3471
94	4	21	0.2815	0.5713	0.23589	0.09716	0.3601
94	4	40	0.0726	0.0937	0.1091	0.08073	0.141
94	4	40	0.4111	0.1496	0.12511	0.13484	0.4148
94	4	40	0.1175	0.1682	0.15769	0.11297	0.2087
94	4	100	0.0501	0.1198	0.11683	0.10977	0.0918
94	4	100	0.2904	0.3121	0.18664	0.17503	0.22
94	4	100	0.1489	0.2048	0.15946	0.15809	0.2088
93	1	0	3.2714	0.4054	0.0947	0.0464	2.63159
93	1	0	3.3068	0.3155	0.0844	0.0335	2.66163
93	1	0	3.1332	0.3238	0.0949	0.0428	2.6027
93	1	1	0.9576	0.3238	0.0709	0.1366	0.3035
93	1	1	1.07	0.3281	0.1223	0.1588	0.37289
93	1	1	1.1101	0.226	0.0947	0.1226	0.64594
93	1	3	0.6311	0.3311	0.0432	0.0812	0.14883
93	1	3	0.605	0.418	0.0596	0.0545	0.212
93	1	3	0.6418	0.2117	0.071	0.034	0.18781
93	1	7	0.5702	0.0874	0.083	0.074	0.12899
93	1	7	0.5744	0.1058	0.0991	0.0632	0.09276
93	1	7	0.5404	0.1796	0.1719	0.0745	0.23356
93	1	14	0.2079	0.1573	0.0714	0.0356	0.1219
93	1	14	0.1353	0.0955	0.0922	0.0428	0.0227
93	1	14	0.1255	0.107	0.0819	0.0308	0.16087
93	1	21	0.0858	0.116	0.067	0.043	0.02786
93	1	21	0.2704	0.1024	0.0646	0.0287	0.0897
93	1	21	0.1012	0.3209	0.2815	0.0402	0.11225
93	1	40	0.1229	0.0999	0.0567	0.0171	0.01754
93	1	40	0.1064	0.0723	0.0307	0.0207	0.07843
93	1	40	0.0792	0.0671	0.0428	0.0233	0.02626
93	1	100	0.0552	0.034	0.0125	0.1443	0.01995
93	1	100	0.0335	0.0729	0.0432	0.1884	0.01754
93	1	100	0.0334	0.0619	0.1066	0.0974	0.02007
93	2	0	3.2752	0.4298	0	0.0171	2.17692
93	2	0	3.3139	0.4855	0	0.1002	2.87811
93	2	0	3.1122	0.3315	0	0	2.71357
93	2	1	1.2348	0.4483	0	0	0.29146
93	2	1	0.9514	0.5354	0.0507	0.0902	0.28665
93	2	1	1.1996	0.2151	0	0	0.73299
93	2	3	0.5032	0.5232	0	0	0.61477
93	2	3	0.7473	0.0255	0	0.0686	0.06364
93	2	3	0.6497	0.2398	0.1091	0.1017	0.39895
93	2	7	0.453	0.1511	0.1701	0.1145	0.16064

Table A-8. Cont.

Year	Trt	Day	Glucose	Xylose	Galactose	Arabinose	Fructose
93	2	7	0.4606	0.1224	0.2136	0	0.14493
93	2	7	0.464	0.1428	0.2064	0.1112	0.13163
93	2	14	0.1007	0.1598	0.1165	0.0518	0.09459
93	2	14	0.3456	0.2804	0.202	0.073	0.46861
93	2	14	0.2928	0.1304	0.1561	0.0604	0.28103
93	2	21	0.3185	0.2308	0.1731	0.0912	0.0665
93	2	21	0.1383	0.1934	0.0827	0.0977	0.05056
93	2	21	0.1059	0.0392	0.1953	0.101	0.13007
93	2	40	0.1735	0.1066	0.1795	0.1442	0.07602
93	2	40	0.1145	0.2032	0.0699	0.1198	0.11513
93	2	40	0.1141	0.0421	0.0448	0	0.09903
93	2	100	0.0417	0.0577	0.069	0.0875	0.09929
93	2	100	0.0606	0.0531	0.0669	0.0589	0.13037
93	2	100	0.0808	0.0392	0.0794	0.1083	2.46632
93	2	0	2.9862	0.2364	0.0637	0.1130	2.74653
93	3	0	3.3268	0.3412	0.0684	0.098	0.91416
93	3	0	3.2974	0.2956	0.0567	0.0764	2.6681
93	3	1	1.2168	0.4343	0	0.0607	0.608
93	3	1	1.225	0.4643	0	0.0727	0.6547
93	3	1	1.0331	0.1361	0	0.0404	0.7012
93	3	3	0.3251	0.6523	0	0.056	0.5430
93	3	3	0.3919	0.2015	0	0.0528	0.5210
93	3	3	0.4969	0.1123	0.1666	0.0801	0.4956
93	3	7	0.374	0.1455	0.1408	0.0618	0.4235
93	3	7	0.2659	0.1329	0.1729	0.0625	0.3214
93	3	7	0.4588	0.1347	0.1662	0.0678	0.25959
93	3	14	0.089	0.1594	0.1327	0.0638	0.26131
93	3	14	0.1604	0.1511	0.143	0.1221	0.33067
93	3	14	0.0543	0.0912	0.1490	0	0.28516
93	3	21	0.0846	0.0733	0.0685	0.051	0.26807
93	3	21	0.1245	0.0564	0.037	0.0183	0.26521
93	3	21	0.0631	0.0595	0.1063	0.0523	0.25041
93	3	40	0.0826	0.1282	0.0585	0.0813	0.26658
93	3	40	0.0902	0.1109	0.0901	0.0342	0.25374
93	3	40	0.0659	0.0636	0.0276	0.0343	0.24858
93	3	100	0.0267	0.0325	0	0.1279	0.06627
93	3	100	0.0168	0.0664	0.036	0.0633	0.01949
93	3	100	0.0686	0.2193	0	0.1283	0.10147
93	4	0	3.2568	0.2961	0.0661	0.0849	2.44131
93	4	0	3.3836	0.5988	0.0686	0.0748	2.91134
93	4	0	3.3923	0.107	0.0561	0.0416	2.59243
93	4	1	1.083	0.467	0.0426	0.0681	0.50989
93	4	1	1.2456	0.3261	0.0561	0.0679	0.90278
93	4	1	1.1866	0.1012	0.0603	0.0482	0.48512
93	4	3	0.4234	0.4527	0.0241	0	0.24571
93	4	3	0.3876	0.387	0.0324	0.2415	0.2248
93	4	3	0.3670	0.3705	0.0331	0.0831	0.48722
93	4	7	0.2836	0.1357	0	0.0991	0.27071
93	4	7	0.3846	0.0198	0	0.042	0.25649
93	4	7	0.1349	0.1123	0.064	0.0332	0.31921
93	4	14	0.0366	0.2889	0.2708	0.2745	0.26303
93	4	14	0.1234	0.1621	0	0.0604	0.26991
93	4	14	0.0997	0.0864	0.0873	0.0379	0.25007
93	4	21	0.1042	0.1227	0	0.0643	0.24491
93	4	21	0.0465	0.0212	0	0.022	0.25821
93	4	21	0.0478	0.213	0.2228	0.1952	0.27885
93	4	40	0.0487	0.1663	0.0671	0.098	0.13025
93	4	40	0.0309	0.1221	0.0782	0.0617	0.16512
93	4	40	0.0417	0.1051	0.069	0.0686	0.21603
93	4	100	0.0456	0.1411	0.0437	0.1384	0.05183
93	4	100	0.0234	0.0518	0.1513	0.026	0.03419
93	4	100	0.0523	0.0234	0	0.1184	0.09621

Abbreviations:
Trt = treatment; 1 = control, 2 = enzyme, 3 = inoculant, 4 = E + I

Table A-9. Data used for analysis of structural carbohydrate content (g/100 g DM) in forage sorghum ensiled in a tropical environment (Chapter 2)

Year	Trt	Day	NDF	Hemic	ADF	Cellulose
93	1	0	65.5870	28.822	36.764	29.4389
93	1	0	67.7001	29.127	38.572	30.3120
93	1	0	64.3585	28.523	35.834	28.3479
93	2	0	64.7438	27.700	37.043	35.7929
93	2	0	68.8884	29.763	39.124	31.2407
93	2	0	63.7272	27.694	36.032	27.9144
93	3	0	66.1694	29.000	37.169	29.3878
93	3	0	66.9667	29.203	37.763	29.2620
93	3	0	66.6251	30.240	36.384	29.1822
93	4	0	66.7888	29.659	37.129	30.5561
93	4	0	67.3527	28.992	38.360	30.6154
93	4	0	63.9930	27.409	36.583	29.3517
93	1	40	66.6034	26.626	39.976	32.9252
93	1	40	65.3147	25.749	39.564	32.6542
93	1	40	61.9175	26.595	35.322	29.9292
93	2	40	62.5651	23.548	39.016	32.6953
93	2	40	60.0752	23.382	36.692	30.8002
93	2	40	62.4105	23.826	38.583	31.7820
93	3	40	64.3368	25.554	38.781	32.8563
93	3	40	62.8742	25.207	37.666	31.2922
93	3	40	61.9003	26.146	35.753	29.7062
93	4	40	62.1656	25.274	36.891	31.0693
93	4	40	62.4101	25.109	37.301	31.3432
93	4	40	63.7251	24.884	38.840	31.1634
93	1	100	65.5959	23.584	42.011	34.3195
93	1	100	68.9363	24.446	44.490	33.3961
93	1	100	65.0183	24.245	40.772	34.1466
93	2	100	61.9078	22.586	39.321	33.0114
93	2	100	62.3421	23.345	41.234	34.236
93	2	100	63.6119	23.953	39.658	33.4576
93	3	100	64.8948	24.692	40.202	33.8738
93	3	100	63.9285	24.627	39.301	31.6502
93	3	100	63.6148	24.400	39.213	31.4224
93	4	100	63.1383	24.181	38.956	33.1839
93	4	100	62.5746	24.389	38.185	32.3295
93	4	100	63.8606	25.025	38.834	32.0754
94	1	0	66.5734	26.437	40.135	32.1485
94	1	0	66.3692	26.377	39.992	31.6718
94	1	0	72.8442	27.457	45.386	37.5477
94	2	0	64.5333	26.479	38.054	28.9805
94	2	0	64.8948	26.120	38.774	32.0309
94	2	0	65.7161	26.878	38.838	31.8578
94	3	0	63.1873	25.854	37.332	29.7740
94	3	0	65.0458	26.164	38.881	31.4381
94	3	0	66.8678	27.992	38.874	31.7111
94	4	0	66.9349	26.976	39.958	32.9776
94	4	0	66.1408	25.182	40.958	33.5128
94	4	0	69.5535	25.285	44.267	36.4107
94	1	40	66.4813	26.577	39.903	32.8649
94	1	40	65.1917	25.701	39.490	32.5927
94	1	40	62.0361	26.646	35.389	29.9865
94	2	40	61.4489	23.128	38.320	32.1120
94	2	40	59.9544	23.335	36.619	30.7382
94	2	40	62.5348	23.874	38.660	31.8453
94	3	40	64.2190	25.508	38.710	32.7961
94	3	40	62.9959	25.256	37.739	31.3528
94	3	40	61.7862	26.098	35.687	29.6514
94	4	40	62.0402	25.223	36.816	31.0066
94	4	40	62.2953	25.062	37.232	31.2856
94	4	40	63.5992	24.835	38.763	31.1018
94	1	100	65.7627	23.644	42.118	34.4067
94	1	100	68.8513	24.415	44.435	33.3549
94	1	100	65.0881	24.271	40.816	34.1832
94	2	100	61.7926	22.544	39.247	32.9499

Table A-9. Cont.

Year	Trt	Day	NDF	Hemic	ADF	Cellulose
94	2	100	62.8349	23.889	38.945	32.4114
94	2	100	63.7313	23.998	39.732	33.5204
94	3	100	64.9811	24.725	40.256	33.9188
94	3	100	64.0663	24.680	39.385	31.7184
94	3	100	63.7047	24.435	39.269	31.4668
94	4	100	63.2942	24.241	39.053	33.2658
94	4	100	62.6684	24.425	38.242	32.3780
94	4	100	63.7681	24.989	38.778	32.0289

Abbreviations:
Trt = Treatments, 1 = control, 2 = enzyme, 3 = inoculant, 4 = E + I; Hemic = hemicellulose

Table A-10 Data used for analysis of pH, temperature, and microbial populations in forage sorghum silage exposed to air in temperate and tropical environments (Chapter 3)

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
1	93	1	1	0	1	3.60	4.87	7.84	22.77
1	93	1	1	0	2	3.60	4.60	7.73	21.11
1	93	1	1	0	3	ND	ND	ND	22.22
1	93	1	1	1	1	3.85	4.24	7.57	23.88
1	93	1	1	1	2	3.75	6.69	7.86	36.11
1	93	1	1	1	3	ND	ND	ND	22.22
1	93	1	1	2	1	ND	ND	ND	26.11
1	93	1	1	2	2	ND	ND	ND	27.77
1	93	1	1	2	3	ND	ND	ND	29.11
1	93	1	1	3	1	7.10	8.24	8.23	25.55
1	93	1	1	3	2	6.90	7.98	8.29	21.67
1	93	1	1	3	3	ND	ND	ND	26.11
1	93	1	1	4	1	ND	ND	ND	23.88
1	93	1	1	4	2	ND	ND	ND	21.66
1	93	1	1	4	3	ND	ND	ND	22.22
1	93	1	1	5	1	7.00	8.22	8.14	23.33
1	93	1	1	5	2	7.00	8.00	8.64	22.22
1	93	1	1	5	3	ND	ND	ND	22.22
1	93	1	1	6	1	ND	ND	ND	22.22
1	93	1	1	6	2	ND	ND	ND	22.22
1	93	1	1	6	3	ND	ND	ND	22.22
1	93	1	1	7	1	8.10	7.66	7.90	22.22
1	93	1	1	7	2	8.40	7.58	7.20	22.50
1	93	1	1	7	3	ND	ND	ND	22.77
1	93	1	2	0	1	3.60	3.82	7.76	18.88
1	93	1	2	0	2	3.70	4.66	7.70	23.33
1	93	1	2	0	3	ND	ND	ND	16.66
1	93	1	2	1	1	3.70	4.36	7.49	21.11
1	93	1	2	1	2	3.85	4.16	7.22	35.55
1	93	1	2	1	3	ND	ND	ND	22.77
1	93	1	2	2	1	ND	ND	ND	28.66
1	93	1	2	2	2	ND	ND	ND	26.11
1	93	1	2	2	3	ND	ND	ND	23.88
1	93	1	2	3	1	6.50	8.40	9.20	21.11
1	93	1	2	3	2	7.20	8.61	9.01	25.55
1	93	1	2	3	3	ND	ND	ND	20.55
1	93	1	2	4	1	ND	ND	ND	20.00
1	93	1	2	4	2	ND	ND	ND	23.88
1	93	1	2	4	3	ND	ND	ND	18.33
1	93	1	2	5	1	6.70	7.81	7.14	20.00
1	93	1	2	5	2	7.10	8.52	7.74	23.33
1	93	1	2	5	3	ND	ND	ND	17.77
1	93	1	2	6	1	ND	ND	ND	20.00
1	93	1	2	6	2	ND	ND	ND	24.44
1	93	1	2	6	3	ND	ND	ND	17.77
1	93	1	2	7	1	8.90	6.87	8.60	20.00
1	93	1	2	7	2	8.50	7.40	8.13	24.44
1	93	1	2	7	3	ND	ND	ND	18.33
1	93	1	3	0	1	3.60	4.41	7.38	20.55
1	93	1	3	0	2	3.58	4.51	6.67	21.11
1	93	1	3	0	3	ND	ND	ND	22.22
1	93	1	3	1	1	3.78	6.29	7.64	21.38
1	93	1	3	1	2	4.40	6.57	6.85	23.33
1	93	1	3	1	3	ND	ND	ND	37.22
1	93	1	3	2	1	ND	ND	ND	30.00
1	93	1	3	2	2	ND	ND	ND	31.11
1	93	1	3	2	3	ND	ND	ND	30.55
1	93	1	3	3	1	4.50	7.44	8.37	21.11
1	93	1	3	3	2	6.50	8.64	8.98	23.88
1	93	1	3	3	3	ND	ND	ND	23.33
1	93	1	3	4	1	ND	ND	ND	21.11
1	93	1	3	4	2	ND	ND	ND	22.77
1	93	1	3	4	3	ND	ND	ND	22.22
1	93	1	3	5	1	7.40	7.85	6.62	21.11
1	93	1	3	5	2	8.00	7.86	7.02	22.22
1	93	1	3	5	3	ND	ND	ND	22.22

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
1	93	1	3	6	1	ND	ND	ND	21.11
1	93	1	3	6	2	ND	ND	ND	22.22
1	93	1	3	6	3	ND	ND	ND	22.22
1	93	1	3	7	1	7.90	8.30	7.64	21.11
1	93	1	3	7	2	8.00	7.80	7.74	22.22
1	93	1	3	7	3	ND	ND	ND	22.77
1	93	1	4	0	1	3.65	4.75	6.05	21.11
1	93	1	4	0	2	3.60	4.52	6.57	21.11
1	93	1	4	0	3	ND	ND	ND	22.22
1	93	1	4	1	1	4.00	4.23	7.10	23.33
1	93	1	4	1	2	3.80	5.56	6.47	30.55
1	93	1	4	1	3	ND	ND	ND	25.55
1	93	1	4	2	1	ND	ND	ND	32.50
1	93	1	4	2	2	ND	ND	ND	27.22
1	93	1	4	2	3	ND	ND	ND	28.88
1	93	1	4	3	1	4.90	6.98	8.09	23.33
1	93	1	4	3	2	6.90	7.63	8.60	24.44
1	93	1	4	3	3	ND	ND	ND	24.44
1	93	1	4	4	1	ND	ND	ND	21.66
1	93	1	4	4	2	ND	ND	ND	22.22
1	93	1	4	4	3	ND	ND	ND	22.22
1	93	1	4	5	1	7.60	8.43	6.95	21.11
1	93	1	4	5	2	7.60	8.02	6.90	22.22
1	93	1	4	5	3	ND	ND	ND	22.22
1	93	1	4	6	1	ND	ND	ND	21.11
1	93	1	4	6	2	ND	ND	ND	22.22
1	93	1	4	6	3	ND	ND	ND	22.77
1	93	1	4	7	1	7.80	8.21	7.92	21.11
1	93	1	4	7	2	7.90	8.15	8.20	22.77
1	93	1	4	7	3	ND	ND	ND	23.33
1	93	2	1	0	1	3.75	5.35	8.23	17.77
1	93	2	1	0	2	3.80	4.15	7.42	20.00
1	93	2	1	0	3	ND	ND	ND	18.88
1	93	2	1	1	1	3.60	5.35	6.46	18.88
1	93	2	1	1	2	3.60	5.39	6.35	21.11
1	93	2	1	1	3	ND	ND	ND	19.44
1	93	2	1	2	1	ND	ND	ND	20.55
1	93	2	1	2	2	ND	ND	ND	33.88
1	93	2	1	2	3	ND	ND	ND	23.33
1	93	2	1	3	1	3.70	7.09	6.95	31.22
1	93	2	1	3	2	4.80	6.81	6.40	25.00
1	93	2	1	3	3	ND	ND	ND	25.55
1	93	2	1	4	1	ND	ND	ND	22.22
1	93	2	1	4	2	ND	ND	ND	22.77
1	93	2	1	4	3	ND	ND	ND	22.22
1	93	2	1	5	1	5.65	7.42	8.27	22.22
1	93	2	1	5	2	6.20	7.11	7.38	23.33
1	93	2	1	5	3	ND	ND	ND	22.22
1	93	2	1	6	1	ND	ND	ND	22.22
1	93	2	1	6	2	ND	ND	ND	23.33
1	93	2	1	6	3	ND	ND	ND	22.22
1	93	2	1	7	1	6.40	7.59	7.23	21.11
1	93	2	1	7	2	4.50	7.15	6.39	22.22
1	93	2	1	7	3	ND	ND	ND	21.11
1	93	2	2	0	1	3.70	3.62	6.78	16.66
1	93	2	2	0	2	3.65	5.13	6.71	17.77
1	93	2	2	0	3	ND	ND	ND	18.88
1	93	2	2	1	1	3.60	5.32	6.03	17.77
1	93	2	2	1	2	3.60	5.12	6.22	19.44
1	93	2	2	1	3	ND	ND	ND	19.44
1	93	2	2	2	1	ND	ND	ND	17.77
1	93	2	2	2	2	ND	ND	ND	20.00
1	93	2	2	2	3	ND	ND	ND	22.77
1	93	2	2	3	1	3.70	6.49	6.98	18.33
1	93	2	2	3	2	3.70	5.85	6.40	20.00
1	93	2	2	3	3	ND	ND	ND	23.88

Table A-10 Cont.

Evn	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
1	93	2	2	4	1	ND	ND	ND	20.55
1	93	2	2	4	2	ND	ND	ND	20.55
1	93	2	2	4	3	ND	ND	ND	37.22
1	93	2	2	5	1	3.75	6.20	7.00	25.55
1	93	2	2	5	2	4.25	4.77	6.95	23.33
1	93	2	2	5	3	ND	ND	ND	21.66
1	93	2	2	6	1	ND	ND	ND	21.66
1	93	2	2	6	2	ND	ND	ND	25.55
1	93	2	2	6	3	ND	ND	ND	23.33
1	93	2	2	7	1	4.30	6.13	6.51	21.11
1	93	2	2	7	2	4.70	7.18	7.43	21.11
1	93	2	2	7	3	ND	ND	ND	21.66
1	93	2	3	0	1	3.60	5.04	6.81	18.88
1	93	2	3	0	2	3.65	5.65	7.87	18.88
1	93	2	3	0	3	ND	ND	ND	15.55
1	93	2	3	1	1	3.70	5.19	5.46	18.33
1	93	2	3	1	2	3.70	4.00	5.95	15.00
1	93	2	3	1	3	ND	ND	ND	15.55
1	93	2	3	2	1	ND	ND	ND	20.00
1	93	2	3	2	2	ND	ND	ND	22.22
1	93	2	3	2	3	ND	ND	ND	15.55
1	93	2	3	3	1	3.70	4.60	6.22	20.00
1	93	2	3	3	2	3.65	6.27	6.41	23.33
1	93	2	3	3	3	ND	ND	ND	17.22
1	93	2	3	4	1	ND	ND	ND	20.00
1	93	2	3	4	2	ND	ND	ND	22.22
1	93	2	3	4	3	ND	ND	ND	18.33
1	93	2	3	5	1	3.75	7.12	6.52	22.77
1	93	2	3	5	2	3.90	6.99	6.46	21.66
1	93	2	3	5	3	ND	ND	ND	22.77
1	93	2	3	6	1	ND	ND	ND	22.22
1	93	2	3	6	2	ND	ND	ND	22.22
1	93	2	3	6	3	ND	ND	ND	20.00
1	93	2	3	7	1	5.30	6.03	7.01	21.11
1	93	2	3	7	2	5.25	7.99	7.32	21.11
1	93	2	3	7	3	ND	ND	ND	17.77
1	93	2	4	0	1	3.65	5.25	7.14	20.00
1	93	2	4	0	2	3.60	4.16	6.78	17.77
1	93	2	4	0	3	ND	ND	ND	15.55
1	93	2	4	1	1	3.70	5.39	6.31	21.11
1	93	2	4	1	2	3.70	5.46	7.01	18.88
1	93	2	4	1	3	ND	ND	ND	16.66
1	93	2	4	2	1	ND	ND	ND	22.77
1	93	2	4	2	2	ND	ND	ND	19.44
1	93	2	4	2	3	ND	ND	ND	17.77
1	93	2	4	3	1	3.60	6.98	6.31	22.22
1	93	2	4	3	2	3.60	6.41	6.55	22.77
1	93	2	4	3	3	ND	ND	ND	23.33
1	93	2	4	4	1	ND	ND	ND	22.22
1	93	2	4	4	2	ND	ND	ND	25.00
1	93	2	4	4	3	ND	ND	ND	25.00
1	93	2	4	5	1	4.00	6.60	7.08	22.22
1	93	2	4	5	2	3.75	6.17	6.97	21.11
1	93	2	4	5	3	ND	ND	ND	20.00
1	93	2	4	6	1	ND	ND	ND	22.22
1	93	2	4	6	2	ND	ND	ND	21.11
1	93	2	4	6	3	ND	ND	ND	20.00
1	93	2	4	7	1	4.00	8.05	8.08	21.66
1	93	2	4	7	2	5.00	7.55	7.31	20.55
1	93	2	4	7	3	ND	ND	ND	18.88
1	94	1	1	0	1	3.50	4.56	7.40	21.11
1	94	1	1	0	2	3.50	4.30	7.27	22.22
1	94	1	1	0	3	ND	ND	ND	23.33
1	94	1	1	1	1	3.50	5.61	7.23	23.88
1	94	1	1	1	2	3.50	4.99	7.16	25.55
1	94	1	1	1	3	ND	ND	ND	26.11

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
1	94	1	1	2	1	ND	ND	ND	28.88
1	94	1	1	2	2	ND	ND	ND	23.33
1	94	1	1	2	3	ND	ND	ND	25.55
1	94	1	1	3	1	3.60	5.82	8.15	24.44
1	94	1	1	3	2	3.70	6.10	8.04	22.77
1	94	1	1	3	3	ND	ND	ND	28.33
1	94	1	1	4	1	ND	ND	ND	23.33
1	94	1	1	4	2	ND	ND	ND	25.55
1	94	1	1	4	3	ND	ND	ND	23.88
1	94	1	1	5	1	5.80	8.81	8.73	22.22
1	94	1	1	5	2	4.00	7.47	8.51	25.55
1	94	1	1	5	3	ND	ND	ND	24.44
1	94	1	1	6	1	ND	ND	ND	22.22
1	94	1	1	6	2	ND	ND	ND	21.11
1	94	1	1	6	3	ND	ND	ND	23.33
1	94	1	1	7	1	6.50	8.60	8.78	22.22
1	94	1	1	7	2	6.80	8.72	8.66	21.11
1	94	1	1	7	3	ND	ND	ND	23.33
1	94	1	2	0	1	3.50	5.01	7.31	22.22
1	94	1	2	0	2	3.50	4.46	7.53	21.11
1	94	1	2	0	3	ND	ND	ND	22.22
1	94	1	2	1	1	3.50	6.25	7.51	25.00
1	94	1	2	1	2	3.60	6.13	7.53	23.33
1	94	1	2	1	3	ND	ND	ND	23.88
1	94	1	2	2	1	ND	ND	ND	22.22
1	94	1	2	2	2	ND	ND	ND	21.66
1	94	1	2	2	3	ND	ND	ND	23.88
1	94	1	2	3	1	5.20	6.38	8.24	22.22
1	94	1	2	3	2	3.70	6.33	8.11	22.77
1	94	1	2	3	3	ND	ND	ND	27.77
1	94	1	2	4	1	ND	ND	ND	22.22
1	94	1	2	4	2	ND	ND	ND	24.44
1	94	1	2	4	3	ND	ND	ND	23.88
1	94	1	2	5	1	6.30	8.84	8.22	22.77
1	94	1	2	5	2	5.20	8.74	8.43	23.33
1	94	1	2	5	3	ND	ND	ND	22.22
1	94	1	2	6	1	ND	ND	ND	26.66
1	94	1	2	6	2	ND	ND	ND	22.22
1	94	1	2	6	3	ND	ND	ND	22.22
1	94	1	2	7	1	6.6	8.72	8.70	26.66
1	94	1	2	7	2	6.50	8.39	8.73	22.22
1	94	1	2	7	3	ND	ND	ND	21.11
1	94	1	3	0	1	3.50	4.22	7.36	22.22
1	94	1	3	0	2	3.55	4.49	7.01	22.22
1	94	1	3	0	3	ND	ND	ND	22.22
1	94	1	3	1	1	3.50	6.09	8.03	38.33
1	94	1	3	1	2	3.70	6.08	8.16	32.77
1	94	1	3	1	3	ND	ND	ND	42.22
1	94	1	3	2	1	ND	ND	ND	26.66
1	94	1	3	2	2	ND	ND	ND	31.11
1	94	1	3	2	3	ND	ND	ND	30.00
1	94	1	3	3	1	6.90	8.28	7.89	23.33
1	94	1	3	3	2	6.20	7.74	8.70	25.55
1	94	1	3	3	3	ND	ND	ND	24.44
1	94	1	3	4	1	ND	ND	ND	22.77
1	94	1	3	4	2	ND	ND	ND	24.44
1	94	1	3	4	3	ND	ND	ND	24.44
1	94	1	3	5	1	6.40	8.80	8.81	23.33
1	94	1	3	5	2	6.70	8.71	8.86	25.00
1	94	1	3	5	3	ND	ND	ND	24.44
1	94	1	3	6	1	ND	ND	ND	23.33
1	94	1	3	6	2	ND	ND	ND	24.44
1	94	1	3	6	3	ND	ND	ND	24.44
1	94	1	3	7	1	7.40	8.76	8.81	23.33
1	94	1	3	7	2	7.50	8.73	8.72	23.88
1	94	1	3	7	3	ND	ND	ND	24.44

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
1	94	1	4	0	1	3.50	4.66	7.49	23.33
1	94	1	4	0	2	3.50	4.55	7.94	21.11
1	94	1	4	0	3	ND	ND	ND	23.33
1	94	1	4	1	1	3.50	6.23	8.19	42.22
1	94	1	4	1	2	3.60	6.17	8.20	30.00
1	94	1	4	1	3	ND	ND	ND	36.11
1	94	1	4	2	1	ND	ND	ND	25.55
1	94	1	4	2	2	ND	ND	ND	27.22
1	94	1	4	2	3	ND	ND	ND	26.11
1	94	1	4	3	1	6.20	8.10	9.13	22.77
1	94	1	4	3	2	6.90	7.81	7.80	23.33
1	94	1	4	3	3	ND	ND	ND	23.33
1	94	1	4	4	1	ND	ND	ND	23.33
1	94	1	4	4	2	ND	ND	ND	22.22
1	94	1	4	4	3	ND	ND	ND	24.44
1	94	1	4	5	1	7.00	8.60	8.81	23.33
1	94	1	4	5	2	7.30	8.81	8.72	22.22
1	94	1	4	5	3	ND	ND	ND	23.33
1	94	1	4	6	1	ND	ND	ND	23.33
1	94	1	4	6	2	ND	ND	ND	22.22
1	94	1	4	6	3	ND	ND	ND	24.44
1	94	1	4	7	1	8.00	8.72	8.69	24.55
1	94	1	4	7	2	7.80	8.82	8.72	24.44
1	94	1	4	7	3	ND	ND	ND	24.44
1	94	2	1	0	1	3.50	4.60	7.30	20.00
1	94	2	1	0	2	3.60	4.50	7.40	21.11
1	94	2	1	0	3	ND	ND	ND	21.11
1	94	2	1	1	1	3.50	8.12	7.42	21.11
1	94	2	1	1	2	3.50	7.92	7.50	21.66
1	94	2	1	1	3	ND	ND	ND	23.88
1	94	2	1	2	1	ND	ND	ND	22.22
1	94	2	1	2	2	ND	ND	ND	22.77
1	94	2	1	2	3	ND	ND	ND	33.33
1	94	2	1	3	1	3.50	7.46	7.82	25.55
1	94	2	1	3	2	3.50	7.37	7.66	25.55
1	94	2	1	3	3	ND	ND	ND	21.66
1	94	2	1	4	1	ND	ND	ND	26.66
1	94	2	1	4	2	ND	ND	ND	24.44
1	94	2	1	4	3	ND	ND	ND	24.44
1	94	2	1	5	1	6.30	8.12	8.12	25.27
1	94	2	1	5	2	4.70	7.10	8.21	25.00
1	94	2	1	5	3	ND	ND	ND	23.88
1	94	2	1	6	1	ND	ND	ND	23.33
1	94	2	1	6	2	ND	ND	ND	25.55
1	94	2	1	6	3	ND	ND	ND	23.33
1	94	2	1	7	1	5.60	7.62	7.98	22.22
1	94	2	1	7	2	6.10	7.60	7.80	23.77
1	94	2	1	7	3	ND	ND	ND	23.66
1	94	2	2	0	1	3.70	4.80	7.20	20.00
1	94	2	2	0	2	3.60	4.65	7.40	20.00
1	94	2	2	0	3	ND	ND	ND	20.00
1	94	2	2	1	1	3.50	7.95	7.55	21.11
1	94	2	2	1	2	3.50	8.15	7.38	21.11
1	94	2	2	1	3	ND	ND	ND	21.11
1	94	2	2	2	1	ND	ND	ND	23.33
1	94	2	2	2	2	ND	ND	ND	21.66
1	94	2	2	2	3	ND	ND	ND	21.11
1	94	2	2	3	1	3.50	7.47	7.72	20.00
1	94	2	2	3	2	3.50	7.13	7.28	23.33
1	94	2	2	3	3	ND	ND	ND	22.77
1	94	2	2	4	1	ND	ND	ND	23.33
1	94	2	2	4	2	ND	ND	ND	22.22
1	94	2	2	4	3	ND	ND	ND	26.66
1	94	2	2	5	1	3.60	7.74	8.20	22.22
1	94	2	2	5	2	3.70	7.18	7.64	22.22
1	94	2	2	5	3	ND	ND	ND	23.05

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
1	94	2	2	6	1	ND	ND	ND	21.66
1	94	2	2	6	2	ND	ND	ND	24.44
1	94	2	2	6	3	ND	ND	ND	23.33
1	94	2	2	7	1	6.50	7.81	8.22	21.11
1	94	2	2	7	2	6.30	8.33	8.29	23.33
1	94	2	2	7	3	ND	ND	ND	21.66
1	94	2	3	0	1	3.55	4.78	7.15	20.00
1	94	2	3	0	2	3.50	4.67	7.35	20.00
1	94	2	3	0	3	ND	ND	ND	22.22
1	94	2	3	1	1	3.80	8.27	7.59	25.00
1	94	2	3	1	2	3.50	8.37	7.30	21.11
1	94	2	3	1	3	ND	ND	ND	22.77
1	94	2	3	2	1	ND	ND	ND	27.22
1	94	2	3	2	2	ND	ND	ND	33.33
1	94	2	3	2	3	ND	ND	ND	36.66
1	94	2	3	3	1	6.10	7.41	7.35	20.00
1	94	2	3	3	2	6.40	7.38	7.02	20.55
1	94	2	3	3	3	ND	ND	ND	20.00
1	94	2	3	4	1	ND	ND	ND	21.66
1	94	2	3	4	2	ND	ND	ND	23.33
1	94	2	3	4	3	ND	ND	ND	23.33
1	94	2	3	5	1	6.90	8.27	8.14	22.77
1	94	2	3	5	2	6.50	8.56	9.13	23.33
1	94	2	3	5	3	ND	ND	ND	23.33
1	94	2	3	6	1	ND	ND	ND	21.66
1	94	2	3	6	2	ND	ND	ND	23.33
1	94	2	3	6	3	ND	ND	ND	23.33
1	94	2	3	7	1	6.90	8.12	8.28	22.22
1	94	2	3	7	2	7.50	8.03	7.80	21.66
1	94	2	3	7	3	ND	ND	ND	22.77
1	94	2	4	0	1	3.55	4.67	7.23	20.00
1	94	2	4	0	2	3.50	4.64	7.35	18.88
1	94	2	4	0	3	ND	ND	ND	21.11
1	94	2	4	1	1	3.60	8.27	7.60	21.38
1	94	2	4	1	2	3.60	7.88	6.84	27.22
1	94	2	4	1	3	ND	ND	ND	21.11
1	94	2	4	2	1	ND	ND	ND	32.77
1	94	2	4	2	2	ND	ND	ND	23.33
1	94	2	4	2	3	ND	ND	ND	30.00
1	94	2	4	3	1	6.40	7.43	7.86	20.55
1	94	2	4	3	2	6.40	7.58	7.13	18.33
1	94	2	4	3	3	ND	ND	ND	20.00
1	94	2	4	4	1	ND	ND	ND	23.33
1	94	2	4	4	2	ND	ND	ND	21.11
1	94	2	4	4	3	ND	ND	ND	24.44
1	94	2	4	5	1	6.20	8.50	8.29	23.05
1	94	2	4	5	2	6.90	8.39	8.46	21.66
1	94	2	4	5	3	ND	ND	ND	24.44
1	94	2	4	6	1	ND	ND	ND	22.22
1	94	2	4	6	2	ND	ND	ND	21.66
1	94	2	4	6	3	ND	ND	ND	23.88
1	94	2	4	7	1	7.00	8.46	8.58	21.66
1	94	2	4	7	2	7.60	7.79	8.57	20.55
1	94	2	4	7	3	ND	ND	ND	23.33
2	93	1	1	0	1	4.27	6.10	6.40	28.05
2	93	1	1	0	2	4.10	7.38	6.09	28.61
2	93	1	1	0	3	ND	ND	ND	29.72
2	93	1	1	1	1	4.05	7.84	7.45	29.44
2	93	1	1	1	2	6.17	8.20	7.58	37.22
2	93	1	1	1	3	ND	ND	ND	41.66
2	93	1	1	2	1	ND	ND	ND	34.44
2	93	1	1	2	2	ND	ND	ND	32.77
2	93	1	1	2	3	ND	ND	ND	33.33
2	93	1	1	3	1	6.79	8.03	8.07	32.22
2	93	1	1	3	2	7.39	8.34	8.31	31.66
2	93	1	1	3	3	ND	ND	ND	32.77

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
2	93	1	1	4	1	ND	ND	ND	32.22
2	93	1	1	4	2	ND	ND	ND	31.11
2	93	1	1	4	3	ND	ND	ND	33.33
2	93	1	1	5	1	6.80	8.24	8.23	30.55
2	93	1	1	5	2	7.25	8.33	8.44	30.55
2	93	1	1	5	3	ND	ND	ND	31.66
2	93	1	1	6	1	ND	ND	ND	28.88
2	93	1	1	6	2	ND	ND	ND	28.33
2	93	1	1	6	3	ND	ND	ND	32.22
2	93	1	1	7	1	6.97	8.38	8.06	27.77
2	93	1	1	7	2	7.37	8.41	8.56	27.50
2	93	1	1	7	3	ND	ND	ND	32.22
2	93	1	2	0	1	4.21	5.41	6.44	28.88
2	93	1	2	0	2	4.25	7.70	6.12	28.05
2	93	1	2	0	3	ND	ND	ND	30.27
2	93	1	2	1	1	4.96	7.96	8.32	37.77
2	93	1	2	1	2	4.05	7.16	8.46	36.66
2	93	1	2	1	3	ND	ND	ND	40.00
2	93	1	2	2	1	ND	ND	ND	32.77
2	93	1	2	2	2	ND	ND	ND	33.88
2	93	1	2	2	3	ND	ND	ND	37.77
2	93	1	2	3	1	6.48	7.77	8.24	35.55
2	93	1	2	3	2	6.91	7.98	7.40	33.88
2	93	1	2	3	3	ND	ND	ND	33.33
2	93	1	2	4	1	ND	ND	ND	34.44
2	93	1	2	4	2	ND	ND	ND	33.33
2	93	1	2	4	3	ND	ND	ND	33.33
2	93	1	2	5	1	6.70	7.81	8.31	33.33
2	93	1	2	5	2	7.10	8.00	8.02	32.77
2	93	1	2	5	3	ND	ND	ND	30.55
2	93	1	2	6	1	ND	ND	ND	29.44
2	93	1	2	6	2	ND	ND	ND	30.55
2	93	1	2	6	3	ND	ND	ND	29.44
2	93	1	2	7	1	7.41	8.09	8.65	29.44
2	93	1	2	7	2	7.59	8.22	8.57	30.00
2	93	1	2	7	3	ND	ND	ND	28.88
2	93	1	3	0	1	4.03	6.45	6.49	29.44
2	93	1	3	0	2	4.05	8.43	6.58	29.44
2	93	1	3	0	3	ND	ND	ND	28.33
2	93	1	3	1	1	3.94	7.64	7.47	33.88
2	93	1	3	1	2	4.22	7.55	7.46	40.55
2	93	1	3	1	3	ND	ND	ND	33.88
2	93	1	3	2	1	ND	ND	ND	36.66
2	93	1	3	2	2	ND	ND	ND	32.77
2	93	1	3	2	3	ND	ND	ND	31.11
2	93	1	3	3	1	5.89	7.44	7.40	36.11
2	93	1	3	3	2	5.93	7.89	7.87	34.44
2	93	1	3	3	3	ND	ND	ND	30.55
2	93	1	3	4	1	ND	ND	ND	33.88
2	93	1	3	4	2	ND	ND	ND	32.22
2	93	1	3	4	3	ND	ND	ND	31.11
2	93	1	3	5	1	6.17	8.02	7.88	32.77
2	93	1	3	5	2	6.34	8.25	8.05	31.11
2	93	1	3	5	3	ND	ND	ND	30.83
2	93	1	3	6	1	ND	ND	ND	31.66
2	93	1	3	6	2	ND	ND	ND	30.00
2	93	1	3	6	3	ND	ND	ND	30.00
2	93	1	3	7	1	7.59	8.48	8.43	31.66
2	93	1	3	7	2	7.59	8.07	7.92	28.88
2	93	1	3	7	3	ND	ND	ND	28.88
2	93	1	4	0	1	4.01	5.21	5.45	28.88
2	93	1	4	0	2	3.96	6.94	5.29	29.44
2	93	1	4	0	3	ND	ND	ND	30.00
2	93	1	4	1	1	3.89	7.89	7.58	35.55
2	93	1	4	1	2	5.35	7.99	7.80	34.44
2	93	1	4	1	3	ND	ND	ND	38.88

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
2	93	1	4	2	1	ND	ND	ND	32.22
2	93	1	4	2	2	ND	ND	ND	32.22
2	93	1	4	2	3	ND	ND	ND	31.11
2	93	1	4	3	1	6.20	8.03	8.11	31.66
2	93	1	4	3	2	6.08	8.10	8.21	31.11
2	93	1	4	3	3	ND	ND	ND	31.11
2	93	1	4	4	1	ND	ND	ND	32.22
2	93	1	4	4	2	ND	ND	ND	32.22
2	93	1	4	4	3	ND	ND	ND	32.22
2	93	1	4	5	1	6.46	8.34	8.21	31.11
2	93	1	4	5	2	6.64	8.21	8.17	31.66
2	93	1	4	5	3	ND	ND	ND	31.11
2	93	1	4	6	1	ND	ND	ND	29.44
2	93	1	4	6	2	ND	ND	ND	29.44
2	93	1	4	6	3	ND	ND	ND	31.11
2	93	1	4	7	1	7.51	8.47	8.81	30.00
2	93	1	4	7	2	7.27	8.16	8.64	27.77
2	93	1	4	7	3	ND	ND	ND	30.00
2	93	2	1	0	1	4.19	5.58	6.12	30.00
2	93	2	1	0	2	4.16	5.31	5.77	29.44
2	93	2	1	0	3	ND	ND	ND	30.55
2	93	2	1	1	1	4.61	6.58	6.73	31.11
2	93	2	1	1	2	5.22	6.31	6.26	35.55
2	93	2	1	1	3	ND	ND	ND	34.44
2	93	2	1	2	1	ND	ND	ND	31.11
2	93	2	1	2	2	ND	ND	ND	37.50
2	93	2	1	2	3	ND	ND	ND	35.00
2	93	2	1	3	1	5.26	6.35	7.08	31.11
2	93	2	1	3	2	5.93	5.85	7.06	36.11
2	93	2	1	3	3	ND	ND	ND	33.88
2	93	2	1	4	1	ND	ND	ND	37.77
2	93	2	1	4	2	ND	ND	ND	33.33
2	93	2	1	4	3	ND	ND	ND	36.11
2	93	2	1	5	1	7.22	6.21	7.12	36.66
2	93	2	1	5	2	6.98	6.02	6.89	32.22
2	93	2	1	5	3	ND	ND	ND	34.44
2	93	2	1	6	1	ND	ND	ND	33.88
2	93	2	1	6	2	ND	ND	ND	31.11
2	93	2	1	6	3	ND	ND	ND	31.66
2	93	2	1	7	1	6.03	6.43	7.19	32.22
2	93	2	1	7	2	6.70	6.29	6.96	31.11
2	93	2	1	7	3	ND	ND	ND	31.66
2	93	2	2	0	1	4.17	5.03	5.04	26.66
2	93	2	2	0	2	4.31	5.21	5.84	33.88
2	93	2	2	0	3	ND	ND	ND	30.55
2	93	2	2	1	1	4.90	6.00	6.72	27.77
2	93	2	2	1	2	4.84	6.00	6.18	41.38
2	93	2	2	1	3	ND	ND	ND	38.88
2	93	2	2	2	1	ND	ND	ND	35.00
2	93	2	2	2	2	ND	ND	ND	39.44
2	93	2	2	2	3	ND	ND	ND	36.11
2	93	2	2	3	1	5.59	6.21	7.35	36.66
2	93	2	2	3	2	5.98	5.73	7.22	37.22
2	93	2	2	3	3	ND	ND	ND	36.66
2	93	2	2	4	1	ND	ND	ND	36.66
2	93	2	2	4	2	ND	ND	ND	35.00
2	93	2	2	4	3	ND	ND	ND	35.55
2	93	2	2	5	1	7.05	6.30	7.04	33.33
2	93	2	2	5	2	6.87	6.09	6.92	32.22
2	93	2	2	5	3	ND	ND	ND	32.22
2	93	2	2	6	1	ND	ND	ND	32.22
2	93	2	2	6	2	ND	ND	ND	32.22
2	93	2	2	6	3	ND	ND	ND	31.66
2	93	2	2	7	1	7.14	6.50	7.25	31.11
2	93	2	2	7	2	7.00	6.52	6.84	32.22
2	93	2	2	7	3	ND	ND	ND	32.22

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
2	93	2	3	0	1	4.15	5.50	5.93	30.55
2	93	2	3	0	2	4.21	5.89	6.01	30.00
2	93	2	3	0	3	ND	ND	ND	29.44
2	93	2	3	1	1	4.71	6.50	6.08	37.77
2	93	2	3	1	2	4.71	6.89	6.73	41.11
2	93	2	3	1	3	ND	ND	ND	40.00
2	93	2	3	2	1	ND	ND	ND	36.11
2	93	2	3	2	2	ND	ND	ND	32.77
2	93	2	3	2	3	ND	ND	ND	32.22
2	93	2	3	3	1	5.87	5.90	7.59	34.44
2	93	2	3	3	2	5.93	6.20	7.37	32.22
2	93	2	3	3	3	ND	ND	ND	32.22
2	93	2	3	4	1	ND	ND	ND	33.33
2	93	2	3	4	2	ND	ND	ND	32.22
2	93	2	3	4	3	ND	ND	ND	32.22
2	93	2	3	5	1	7.29	6.28	7.31	32.77
2	93	2	3	5	2	7.25	6.32	7.27	32.22
2	93	2	3	5	3	ND	ND	ND	32.22
2	93	2	3	6	1	ND	ND	ND	32.22
2	93	2	3	6	2	ND	ND	ND	32.22
2	93	2	3	6	3	ND	ND	ND	32.22
2	93	2	3	7	1	6.96	6.39	7.01	31.11
2	93	2	3	7	2	7.15	6.58	7.09	31.11
2	93	2	3	7	3	ND	ND	ND	32.22
2	93	2	4	0	1	4.07	5.67	5.41	26.66
2	93	2	4	0	2	3.96	5.89	5.89	26.66
2	93	2	4	0	3	ND	ND	ND	26.66
2	93	2	4	1	1	4.82	6.67	6.83	26.66
2	93	2	4	1	2	4.51	6.89	6.71	35.55
2	93	2	4	1	3	ND	ND	ND	38.88
2	93	2	4	2	1	ND	ND	ND	31.66
2	93	2	4	2	2	ND	ND	ND	34.44
2	93	2	4	2	3	ND	ND	ND	33.33
2	93	2	4	3	1	6.71	6.95	7.92	33.33
2	93	2	4	3	2	6.76	7.04	7.01	31.66
2	93	2	4	3	3	ND	ND	ND	37.22
2	93	2	4	4	1	ND	ND	ND	34.44
2	93	2	4	4	2	ND	ND	ND	31.11
2	93	2	4	4	3	ND	ND	ND	31.11
2	93	2	4	5	1	7.07	6.70	7.42	31.66
2	93	2	4	5	2	7.10	6.79	7.00	31.11
2	93	2	4	5	3	ND	ND	ND	31.11
2	93	2	4	6	1	ND	ND	ND	32.22
2	93	2	4	6	2	ND	ND	ND	31.11
2	93	2	4	6	3	ND	ND	ND	31.11
2	93	2	4	7	1	7.23	6.50	7.10	31.11
2	93	2	4	7	2	7.24	6.42	7.04	31.11
2	93	2	4	7	3	ND	ND	ND	31.11
2	94	1	1	0	1	4.16	5.25	6.84	30.75
2	94	1	1	0	2	4.15	5.28	7.07	30.75
2	94	1	1	0	3	ND	ND	ND	30.75
2	94	1	1	1	1	4.20	5.28	6.57	31.00
2	94	1	1	1	2	4.20	5.02	6.57	31.00
2	94	1	1	1	3	ND	ND	ND	38.00
2	94	1	1	2	1	ND	ND	ND	30.50
2	94	1	1	2	2	ND	ND	ND	31.25
2	94	1	1	2	3	ND	ND	ND	32.75
2	94	1	1	3	1	4.20	4.17	6.32	30.31
2	94	1	1	3	2	4.40	4.06	6.25	38.00
2	94	1	1	3	3	ND	ND	ND	37.00
2	94	1	1	4	1	ND	ND	ND	31.00
2	94	1	1	4	2	ND	ND	ND	36.25
2	94	1	1	4	3	ND	ND	ND	34.75
2	94	1	1	5	1	4.20	4.50	5.96	30.50
2	94	1	1	5	2	4.20	4.66	6.29	33.00
2	94	1	1	5	3	ND	ND	ND	31.50

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
2	94	1	1	6	1	ND	ND	ND	35.25
2	94	1	1	6	2	ND	ND	ND	33.75
2	94	1	1	6	3	ND	ND	ND	32.50
2	94	1	1	7	1	4.20	4.47	5.91	36.00
2	94	1	1	7	2	4.40	4.17	5.50	32.00
2	94	1	1	7	3	ND	ND	ND	32.50
2	94	1	2	0	1	4.20	5.23	6.49	30.50
2	94	1	2	0	2	4.05	5.77	6.02	30.50
2	94	1	2	0	3	ND	ND	ND	30.75
2	94	1	2	1	1	4.20	4.88	6.70	31.00
2	94	1	2	1	2	4.20	5.50	6.84	32.25
2	94	1	2	1	3	ND	ND	ND	39.50
2	94	1	2	2	1	ND	ND	ND	38.50
2	94	1	2	2	2	ND	ND	ND	39.00
2	94	1	2	2	3	ND	ND	ND	36.00
2	94	1	2	3	1	4.25	4.30	6.26	38.00
2	94	1	2	3	2	4.30	4.56	5.39	37.25
2	94	1	2	3	3	ND	ND	ND	35.25
2	94	1	2	4	1	ND	ND	ND	34.75
2	94	1	2	4	2	ND	ND	ND	33.75
2	94	1	2	4	3	ND	ND	ND	33.25
2	94	1	2	5	1	4.25	4.69	6.30	31.75
2	94	1	2	5	2	4.30	4.60	6.88	32.25
2	94	1	2	5	3	ND	ND	ND	32.00
2	94	1	2	6	1	ND	ND	ND	32.50
2	94	1	2	6	2	ND	ND	ND	32.75
2	94	1	2	6	3	ND	ND	ND	33.00
2	94	1	2	7	1	4.30	4.57	6.20	30.50
2	94	1	2	7	2	4.40	4.82	5.55	30.75
2	94	1	2	7	3	ND	ND	ND	31.00
2	94	1	3	0	1	4.14	5.12	6.28	30.75
2	94	1	3	0	2	4.10	4.77	6.73	30.75
2	94	1	3	0	3	ND	ND	ND	30.75
2	94	1	3	1	1	4.20	5.62	6.04	37.00
2	94	1	3	1	2	4.20	5.69	6.00	47.00
2	94	1	3	1	3	ND	ND	ND	46.00
2	94	1	3	2	1	ND	ND	ND	35.00
2	94	1	3	2	2	ND	ND	ND	35.00
2	94	1	3	2	3	ND	ND	ND	35.00
2	94	1	3	3	1	4.45	5.64	6.41	35.00
2	94	1	3	3	2	4.40	5.21	6.19	34.75
2	94	1	3	3	3	ND	ND	ND	34.75
2	94	1	3	4	1	ND	ND	ND	32.50
2	94	1	3	4	2	ND	ND	ND	32.75
2	94	1	3	4	3	ND	ND	ND	33.00
2	94	1	3	5	1	4.45	5.35	5.69	30.50
2	94	1	3	5	2	4.60	5.09	5.70	30.75
2	94	1	3	5	3	ND	ND	ND	32.75
2	94	1	3	6	1	ND	ND	ND	32.00
2	94	1	3	6	2	ND	ND	ND	32.50
2	94	1	3	6	3	ND	ND	ND	33.75
2	94	1	3	7	1	4.42	4.66	5.55	30.00
2	94	1	3	7	2	4.50	4.72	5.43	30.75
2	94	1	3	7	3	ND	ND	ND	30.75
2	94	1	4	0	1	3.91	5.91	6.57	31.00
2	94	1	4	0	2	3.87	5.57	6.78	31.00
2	94	1	4	0	3	ND	ND	ND	30.50
2	94	1	4	1	1	4.20	4.88	6.47	34.00
2	94	1	4	1	2	4.20	4.87	6.24	44.00
2	94	1	4	1	3	ND	ND	ND	50.00
2	94	1	4	2	1	ND	ND	ND	39.00
2	94	1	4	2	2	ND	ND	ND	36.00
2	94	1	4	2	3	ND	ND	ND	37.50
2	94	1	4	3	1	4.40	4.70	5.50	38.75
2	94	1	4	3	2	4.30	5.02	6.00	34.75
2	94	1	4	3	3	ND	ND	ND	35.00

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
2	94	1	4	4	1	ND	ND	ND	35.75
2	94	1	4	4	2	ND	ND	ND	33.00
2	94	1	4	4	3	ND	ND	ND	33.00
2	94	1	4	5	1	4.35	5.15	6.16	32.50
2	94	1	4	5	2	4.45	5.25	5.88	33.00
2	94	1	4	5	3	ND	ND	ND	32.00
2	94	1	4	6	1	ND	ND	ND	33.00
2	94	1	4	6	2	ND	ND	ND	33.50
2	94	1	4	6	3	ND	ND	ND	32.50
2	94	1	4	7	1	4.50	4.49	5.50	30.75
2	94	1	4	7	2	4.25	5.21	5.62	30.75
2	94	1	4	7	3	ND	ND	ND	31.00
2	94	2	1	0	1	4.19	5.04	6.05	29.50
2	94	2	1	0	2	4.09	4.95	6.15	29.50
2	94	2	1	0	3	ND	ND	ND	30.00
2	94	2	1	1	1	4.30	5.02	6.18	29.00
2	94	2	1	1	2	4.25	5.37	6.62	30.00
2	94	2	1	1	3	ND	ND	ND	29.00
2	94	2	1	2	1	ND	ND	ND	30.44
2	94	2	1	2	2	ND	ND	ND	29.33
2	94	2	1	2	3	ND	ND	ND	32.94
2	94	2	1	3	1	4.30	5.27	6.18	29.44
2	94	2	1	3	2	4.35	5.40	6.43	28.61
2	94	2	1	3	3	ND	ND	ND	27.22
2	94	2	1	4	1	ND	ND	ND	29.44
2	94	2	1	4	2	ND	ND	ND	28.05
2	94	2	1	4	3	ND	ND	ND	27.50
2	94	2	1	5	1	4.50	5.71	6.00	30.00
2	94	2	1	5	2	5.50	5.17	6.46	28.88
2	94	2	1	5	3	ND	ND	ND	27.77
2	94	2	1	6	1	ND	ND	ND	31.11
2	94	2	1	6	2	ND	ND	ND	30.00
2	94	2	1	6	3	ND	ND	ND	29.16
2	94	2	1	7	1	4.28	5.70	6.29	31.38
2	94	2	1	7	2	4.33	5.54	6.46	29.72
2	94	2	1	7	3	ND	ND	ND	29.72
2	94	2	2	0	1	4.20	4.88	6.17	29.50
2	94	2	2	0	2	4.12	4.77	6.34	29.50
2	94	2	2	0	3	ND	ND	ND	29.50
2	94	2	2	1	1	4.25	5.39	6.10	37.50
2	94	2	2	1	2	4.15	5.40	6.11	32.00
2	94	2	2	1	3	ND	ND	ND	31.00
2	94	2	2	2	1	ND	ND	ND	31.94
2	94	2	2	2	2	ND	ND	ND	28.88
2	94	2	2	2	3	ND	ND	ND	28.61
2	94	2	2	3	1	4.30	5.77	6.30	31.11
2	94	2	2	3	2	6.00	5.66	6.28	29.16
2	94	2	2	3	3	ND	ND	ND	29.16
2	94	2	2	4	1	ND	ND	ND	32.22
2	94	2	2	4	2	ND	ND	ND	28.88
2	94	2	2	4	3	ND	ND	ND	29.16
2	94	2	2	5	1	6.50	5.37	6.09	32.22
2	94	2	2	5	2	4.30	5.58	6.24	29.16
2	94	2	2	5	2	4.30	5.58	6.24	29.16
2	94	2	2	5	2	4.30	5.58	6.24	29.16
2	94	2	2	5	3	ND	ND	ND	32.77
2	94	2	2	6	1	ND	ND	ND	33.33
2	94	2	2	6	2	ND	ND	ND	30.55
2	94	2	2	6	3	ND	ND	ND	29.16
2	94	2	2	7	1	4.30	5.48	6.13	32.50
2	94	2	2	7	2	4.80	5.69	6.33	30.83
2	94	2	2	7	3	ND	ND	ND	34.72
2	94	2	3	0	1	4.20	5.40	5.48	29.50
2	94	2	3	0	2	4.08	5.32	5.36	29.50
2	94	2	3	0	3	ND	ND	ND	29.50
2	94	2	3	1	1	4.15	5.27	6.19	33.00

Table A-10 Cont.

Env	Yr	Fer	Trt	D	Rep	pH	YM	TB	Tem
2	94	2	3	1	2	4.25	5.34	6.17	33.00
2	94	2	3	1	3	ND	ND	ND	32.00
2	94	2	3	2	1	ND	ND	ND	28.88
2	94	2	3	2	2	ND	ND	ND	30.55
2	94	2	3	2	3	ND	ND	ND	29.50
2	94	2	3	3	1	4.50	5.24	6.46	32.88
2	94	2	3	3	2	4.25	5.48	6.30	32.22
2	94	2	3	3	3	ND	ND	ND	31.05
2	94	2	3	4	1	ND	ND	ND	33.88
2	94	2	3	4	2	ND	ND	ND	33.61
2	94	2	3	4	3	ND	ND	ND	28.05
2	94	2	3	5	1	5.30	5.49	6.37	31.38
2	94	2	3	5	2	6.20	5.61	6.40	30.55
2	94	2	3	5	3	ND	ND	ND	28.05
2	94	2	3	6	1	ND	ND	ND	35.55
2	94	2	3	6	2	ND	ND	ND	31.94
2	94	2	3	6	3	ND	ND	ND	28.88
2	94	2	3	7	1	5.80	5.46	6.26	35.00
2	94	2	3	7	2	4.20	5.30	6.13	31.38
2	94	2	3	7	3	ND	ND	ND	28.88
2	94	2	4	0	1	4.07	5.26	5.72	30.00
2	94	2	4	0	2	3.96	4.80	5.76	30.00
2	94	2	4	0	3	ND	ND	ND	30.00
2	94	2	4	1	1	4.20	5.22	6.18	33.00
2	94	2	4	1	2	4.20	5.57	6.31	38.00
2	94	2	4	1	3	ND	ND	ND	35.00
2	94	2	4	2	1	ND	ND	ND	33.88
2	94	2	4	2	2	ND	ND	ND	31.94
2	94	2	4	2	3	ND	ND	ND	33.05
2	94	2	4	3	1	6.80	5.31	6.46	31.94
2	94	2	4	3	2	4.40	5.41	6.45	29.72
2	94	2	4	3	3	ND	ND	ND	40.00
2	94	2	4	4	1	ND	ND	ND	32.22
2	94	2	4	4	2	ND	ND	ND	30.83
2	94	2	4	4	3	ND	ND	ND	33.33
2	94	2	4	5	1	6.50	5.34	6.39	30.83
2	94	2	4	5	2	4.80	5.48	6.48	31.11
2	94	2	4	5	3	ND	ND	ND	32.77
2	94	2	4	6	1	ND	ND	ND	31.38
2	94	2	4	6	2	ND	ND	ND	33.33
2	94	2	4	6	3	ND	ND	ND	34.16
2	94	2	4	7	1	5.50	5.49	6.22	31.38
2	94	2	4	7	2	5.50	5.52	6.36	32.50
2	94	2	4	7	3	ND	ND	ND	33.05

Abbreviations:
Env = Environment; 1 = Temperate , 2 = Tropical
Yr = Year
Fer = Fermentation length; 1 = 40 d; 2 = 100 d
Trt = Treatmet; 1 = control, 2 = enzyme , 3 = Inoculant , 4 = E+I
D = Day of aerobic exposure
Rep = replicate
YM = Yeast and mold population (cfu/g of fresh material)
TB = Total bacteria population (cfu/g of fresh material)
Tem = Temperature

Table A-11. Data used for analysis of fermentation end-products (g/100 g DM) in forage sorghum exposed to air in a temperate environment (Chapter 3)

Year	Fer	Trt	Day	Lac	Ace	Pro	Etoh	But
94	1	1	0	8.745	1.632	0.008	0.374	0.015
94	1	1	0	7.942	1.592	0.009	0.485	0.009
94	1	1	1	3.540	0.558	0.007	0.195	0.025
94	1	1	1	2.942	0.400	0.006	0.136	0.027
94	1	1	3	2.291	0.429	0.003	0.225	0.030
94	1	1	3	1.432	0.077	0	0.064	0.019
94	1	1	5	1.258	0.198	0.005	0.293	0.018
94	1	1	5	1.279	0.060	0.005	0.325	0.032
94	1	1	7	0.948	0.292	0.006	0.590	0.015
94	1	1	7	0.971	0.299	0	0.568	0.021
94	1	2	0	8.471	1.741	0.001	0.374	0.011
94	1	2	0	7.541	1.652	0.000	0.359	0.009
94	1	2	1	1.546	0.836	0.004	1.299	0.053
94	1	2	1	3.457	0.970	0.003	1.339	0.041
94	1	2	3	2.617	0.602	0.006	0.861	0.049
94	1	2	3	1.865	0.597	0.001	0.525	0.054
94	1	2	5	0.791	0.143	0.006	0.451	0.042
94	1	2	5	1.853	0.137	0.004	0.530	0.051
94	1	2	7	0.801	0.604	0	0.560	0.044
94	1	2	7	1.107	0.258	0.002	0.365	0.031
94	1	3	0	8.489	1.456	0.007	0.415	0.009
94	1	3	0	8.874	1.547	0.006	0.408	0.007
94	1	3	1	7.066	0.955	0.003	0.839	0.072
94	1	3	1	4.241	0.820	0.005	0.542	0.044
94	1	3	3	0.664	0.403	0.010	0.524	0.043
94	1	3	3	0.798	0.607	0.028	0.516	0.031
94	1	3	5	0.404	0.282	0.005	0.440	0.084
94	1	3	5	0.404	0.273	0	0.459	0
94	1	3	7	0.260	0.301	0	0.437	0.045
94	1	3	7	0.115	0.282	0	0.172	0.041
94	1	4	0	8.246	1.420	0.005	0.425	0.017
94	1	4	0	8.049	1.350	0.003	0.419	0.009
94	1	4	1	8.285	0.175	0.005	0.877	0.010
94	1	4	1	3.518	0.106	0.005	0.787	0.037
94	1	4	3	0.553	0.874	0.010	0.430	0.018
94	1	4	3	0.603	0.430	0.008	0.331	0.012
94	1	4	5	0.394	0.292	0.008	0.272	0.022
94	1	4	5	0.221	0.372	0.009	0.530	0.084
94	1	4	7	0.056	0.337	0.005	0.362	0.070
94	1	4	7	0.077	0.326	0.005	0.386	0.055
94	2	1	0	3.953	1.328	0	0.510	0.001
94	2	1	0	7.864	0.809	0.003	0.449	0.015
94	2	1	1	1.720	0.262	0.012	0.252	0.003
94	2	1	1	6.845	0.891	0.008	0.416	0.071
94	2	1	3	5.715	0.613	0.006	0.474	0.073
94	2	1	3	6.824	0.594	0.009	0.694	0.071
94	2	1	5	0.867	0.157	0.008	0.274	0.028
94	2	1	5	1.904	0.141	0.011	0.151	0.084
94	2	1	7	5.615	0.606	0.006	0.221	0.079
94	2	1	7	1.357	0.248	0.007	0.113	0.048
94	2	2	0	6.438	1.747	0.011	0.580	0.004
94	2	2	0	7.651	1.805	0.012	0.611	0.013
94	2	2	1	5.158	1.065	0.011	0.918	0.021
94	2	2	1	6.281	1.047	0.012	1.001	0.098
94	2	2	3	5.502	0.983	0.010	0.717	0.011
94	2	2	3	4.349	1.127	0.009	0.621	0.088
94	2	2	5	6.716	0.841	0.008	0.063	0.084

Table A-11. Cont.

Year	Fer	Trt	Day	Lac	Ace	Pro	Etoh	But
94	2	2	5	4.257	0.110	0.010	0.151	0.075
94	2	2	7	1.030	0.223	0.006	0.222	0.035
94	2	2	7	0.994	0.234	0.007	0.241	0.027
94	2	3	0	7.818	1.694	0.002	1.747	0.008
94	2	3	0	6.802	1.885	0.011	0.748	0.008
94	2	3	1	6.638	0.544	0.016	0.571	0.086
94	2	3	1	6.361	0.717	0.010	1.912	0.094
94	2	3	3	0.917	0.156	0.005	0.103	0.008
94	2	3	3	0.835	0.171	0.007	0.258	0.037
94	2	3	5	0.190	0.097	0.004	0.127	0
94	2	3	5	0.385	0.094	0.004	0.151	0
94	2	3	7	0.376	0.104	0.006	0.122	0
94	2	3	7	0.385	0.126	0.006	0.113	0
94	2	4	0	8.745	1.902	0.003	0.399	0.011
94	2	4	0	7.214	1.837	0.004	0.482	0.012
94	2	4	1	8.152	0.572	0.006	1.161	0.019
94	2	4	1	6.649	0.639	0.009	1.464	0.099
94	2	4	3	1.541	0.647	0.008	0.485	0.085
94	2	4	3	1.630	0.619	0.007	0.423	0.026
94	2	4	5	0.462	0.094	0.004	0.265	0.078
94	2	4	5	0.406	0.228	0.004	0.111	0.009
94	2	4	7	0.552	0.135	0.005	0.118	0
94	2	4	7	0.214	0.389	0.291	0.005	0.091
93	1	1	0	7.658	2.096	0.012	1.511	0.008
93	1	1	0	9.916	2.323	0.012	1.171	0.009
93	1	1	1	5.186	2.594	0.034	0.065	0.060
93	1	1	1	4.940	0.975	0.070	0.145	0.067
93	1	1	3	0.562	1.682	0.017	0.265	0.064
93	1	1	3	0.052	0.836	0.137	0.080	0.066
93	1	1	5	0.028	0.844	0	0.288	0.019
93	1	1	5	0.035	0.348	0	0.371	0.043
93	1	1	7	0.016	0.309	0.050	0.425	0.049
93	1	1	7	0.067	0.293	0	0.357	0.098
93	1	2	0	7.600	1.788	0.012	1.207	0.008
93	1	2	0	8.568	1.878	0.012	1.611	0.009
93	1	2	1	7.889	1.953	0.124	0.100	0.057
93	1	2	1	4.233	2.148	0.068	0.171	0.047
93	1	2	3	0.225	1.033	0.105	0.214	0.016
93	1	2	3	0.312	1.350	0.065	0.244	0.089
93	1	2	5	0.116	0.092	0.115	0.302	0.012
93	1	2	5	0.019	0.091	0.004	0.241	0.015
93	1	2	7	0.014	0.071	0	0.431	0.044
93	1	2	7	0.114	0.043	0	0.382	0
93	1	3	0	8.203	1.778	0.012	1.645	0.009
93	1	3	0	7.045	1.980	0.011	1.237	0.016
93	1	3	1	3.290	0.860	0	0.224	0.064
93	1	3	1	2.858	0.760	0	0.122	0.057
93	1	3	3	2.306	2.022	0.051	0.408	0.043
93	1	3	3	0.259	1.445	0.114	0.414	0.069
93	1	3	5	0.002	0	0	0.526	0
93	1	3	5	0.047	0	0.013	0.491	0
93	1	3	7	0.053	0	0.009	0.350	0.092
93	1	3	7	0.023	0	0.031	1.840	0
93	1	4	0	8.183	1.833	0.012	1.361	0.008
93	1	4	0	7.837	1.312	0.012	1.401	0.009
93	1	4	1	5.160	0.342	0	0.653	0.024
93	1	4	1	6.851	0.800	0	1.023	0.058
93	1	4	3	0.861	2.480	0.058	0.283	0.051

Table A-11. Cont.

Year	Fer	Trt	Day	Lac	Ace	Pro	Etoh	But
93	1	4	3	0.286	1.002	0.050	0.187	0.022
93	1	4	5	0.006	0.020	0.036	0	0.053
93	1	4	5	0.030	0	0.024	0.020	0.012
93	1	4	7	0.014	0	0.036	0	0.020
93	1	4	7	0.023	0	0	0.006	0.023
93	2	1	0	5.480	2.152	0.015	1.074	0.013
93	2	1	0	6.388	2.050	0.016	1.038	0.008
93	2	1	1	8.932	2.892	0.045	1.522	0.070
93	2	1	1	8.884	2.534	0.020	1.687	0.064
93	2	1	3	7.038	1.519	0.041	0.568	0.056
93	2	1	3	3.605	0.218	0.068	0.468	0.090
93	2	1	5	1.883	0.094	0.031	0.495	0.010
93	2	1	5	1.230	0.074	0	0.286	0.069
93	2	1	7	0.057	0.058	0.011	0.269	0.015
93	2	1	7	0.561	0.062	0.040	0.234	0.014
93	2	2	0	6.383	1.850	0.015	1.103	0.013
93	2	2	0	5.606	2.072	0.016	1.203	0.008
93	2	2	1	7.783	3.271	0.043	1.457	0.004
93	2	2	1	5.622	2.682	0.024	1.199	0.018
93	2	2	3	6.562	2.678	0.057	1.217	0.006
93	2	2	3	4.504	0.662	0.070	0.304	0.068
93	2	2	5	3.218	0.974	0.105	0.400	0.061
93	2	2	5	2.357	0.230	0.011	0.092	0
93	2	2	7	1.230	0.924	0.090	1.080	0.035
93	2	2	7	1.115	0.914	0.016	0	0
93	2	3	0	6.223	1.845	0.015	1.003	0.013
93	2	3	0	7.721	1.663	0.016	1.045	0.008
93	2	3	1	8.663	1.966	0.112	1.123	0
93	2	3	1	8.360	1.880	0.141	1.143	0
93	2	3	3	4.620	2.123	0.072	0.641	0
93	2	3	3	4.630	2.095	0.100	0.988	0
93	2	3	5	3.297	0.967	0.103	0.146	0
93	2	3	5	2.921	0.523	0.133	0.625	0
93	2	3	7	2.975	0.030	0.121	0.696	0
93	2	3	7	1.097	0.325	0.082	0.640	0
93	2	4	0	7.201	1.618	0.015	1.048	0.013
93	2	4	0	6.828	1.918	0.016	1.190	0.008
93	2	4	1	4.450	1.975	0.092	1.466	0.014
93	2	4	1	6.601	1.873	0.144	1.381	0.085
93	2	4	3	3.180	1.070	0.139	0.973	0.019
93	2	4	3	3.414	1.663	0.068	0.515	0.085
93	2	4	5	2.384	0.019	0.040	0	0.033
93	2	4	5	1.729	0.370	0.069	0.141	0.043
93	2	4	7	2.186	0.368	0.075	0.163	0.015
93	2	4	7	1.694	0.087	0.088	0 0	.032

Abbreviations:
Fer = Fermentation length; 1 = 40 d, 2 = 100 d
Trt = treatment; 1 = control, 2 = enzyme, 3 = inoculant, 4 = E+I
D = day of aerobic exposure
Lac = lactic acid; Ace = acetic acid; Pro = propionic acid; Etoh = ethanol; But = butyric acid

Table A - 12. Data used for analysis of water soluble carbohydrate contents (g/100 g DM) in forage sorghum exposed to air in a tempearate environment (Chapter 3)

Year	Fer	Trt	Day	Glucose	Xylose	Galac.	Ara	Fructose
94	1	1	0	2.430	0.305	0.248	0.092	0.276
94	1	1	0	1.957	0.375	0.337	0.089	0.187
94	1	1	1	0.674	0.139	0.184	0.021	0.269
94	1	1	1	0.329	0.129	0.171	0.010	0.070
94	1	1	3	0.578	0.069	0.091	0.087	0.089
94	1	1	3	0.626	0.096	0.127	0.019	0.132
94	1	1	5	0.629	0	0	0.101	0.268
94	1	1	5	0.473	0	0	0.061	0.317
94	1	1	7	0.731	0	0	0.045	0.333
94	1	1	7	0.592	0	0	0.083	0.540
94	1	2	0	1.910	0.199	0.154	0.071	0.241
94	1	2	0	1.659	0.561	0.397	0.078	0.166
94	1	2	1	1.469	0.323	0.428	0.165	0.915
94	1	2	1	0.754	0.173	0.23	0.142	1.010
94	1	2	3	0.736	0.073	0.097	0.010	0.806
94	1	2	3	0.744	0.103	0.136	0.094	0.266
94	1	2	5	0.642	0	0	0.078	0.472
94	1	2	5	0.524	0	0	0	0.456
94	1	2	7	0.543	0	0	0.125	0.452
94	1	2	7	0	0	0	0	0.251
94	1	3	0	1.148	0.737	0.309	0.088	1.898
94	1	3	0	1.215	0.135	0.222	0.084	1.650
94	1	3	1	0.711	0.189	0.250	0.084	0.573
94	1	3	1	0.694	0.075	0.099	0	0.410
94	1	3	3	0.653	0	0	0	0.346
94	1	3	3	0.804	0	0	0	0.275
94	1	3	5	0.581	0	0	0	0.358
94	1	3	5	0.622	0	0	0	0.359
94	1	3	7	0.593	0	0	0	0.165
94	1	3	7	0.564	0.022	0.029	0.081	0.093
94	1	4	0	1.687	0.351	0.340	0.175	2.400
94	1	4	0	1.342	0.243	0.322	0.104	1.338
94	1	4	1	0.734	0.127	0.168	0.374	0.65
94	1	4	1	0.167	0.101	0.133	0.197	0.634
94	1	4	3	0.727	0	0	0	0.135
94	1	4	3	0.790	0	0	0.116	0.160
94	1	4	5	0.625	0	0	0.101	0.314
94	1	4	5	0.648	0.037	0.049	0	0.324
94	1	4	7	0.723	0	0	0	0.227
94	1	4	7	0	0.012	0.016	0	0.276
94	2	1	0	0.561	0.607	0.156	0.130	0.533
94	2	1	0	1.609	0.775	0.369	0.182	0.457
94	2	1	1	0.688	0.167	0.222	0.050	0.326
94	2	1	1	2.386	0.159	0.210	0.080	0.166
94	2	1	3	0.601	0.124	0.164	0.062	0.483
94	2	1	3	0.562	0.053	0.071	0.032	0.300
94	2	1	5	1.129	0	0	0	0.209
94	2	1	5	0.412	0.071	0.094	0	0.151
94	2	1	7	0.891	0.131	0.174	0.073	0.136
94	2	1	7	0.898	0	0	0.054	0.121
94	2	2	0	1.083	0.796	0.243	0.190	0.537
94	2	2	0	1.016	0.504	0.230	0.097	0.379
94	2	2	1	1.178	0.373	0.494	0.183	0.617
94	2	2	1	1.443	0.22	0.291	0.059	0.662
94	2	2	3	0.890	0.243	0.322	0.090	0.466
94	2	2	3	0.652	0.114	0.152	0.146	0.515
94	2	2	5	0.656	0.119	0.157	0.109	0.169
94	2	2	5	0.746	0.082	0.108	0.061	0.108
94	2	2	7	1.406	0	0	0	0.274
94	2	2	7	1.390	0.170	0.225	0.139	0.156
94	2	3	0	1.517	0.644	0.236	0.142	1.907
94	2	3	0	0.737	0.697	0.136	0.187	0.929
94	2	3	1	0.893	0.127	0.169	0.172	1.221
94	2	3	1	2.086	0.212	0.281	0.193	2.269
94	2	3	3	1.129	0.150	0.198	0.197	0.183

Table A - 12. Cont.

Year	Fer	Trt	Day	Glucose	Xylose	Galac.	Ara	Fructose
94	2	3	3	0.785	0	0	0.128	0.237
94	2	3	5	0.778	0	0	0	0.086
94	2	3	5	1.044	0.063	0.083	0.127	0.103
94	2	3	7	0.793	0.067	0.089	0.085	0.062
94	2	3	7	0	0	0	0.016	0.043
94	2	4	0	0.853	0.893	0.219	0.225	1.043
94	2	4	0	1.229	0.395	0.202	1.754	1.104
94	2	4	1	1.325	0.167	0.222	0.282	1.283
94	2	4	1	1.023	0.268	0.355	0.362	1.378
94	2	4	3	0	0.084	0.112	0.292	0.337
94	2	4	3	0	0.043	0.057	0.252	0.356
94	2	4	5	1.013	0.054	0.072	0.291	0.256
94	2	4	5	0.643	0.039	0.052	0.043	0.072
94	2	4	7	0.866	0	0	0.025	0.072
94	2	4	7	0	0.046	0.061	0	0.099
93	1	1	0	1.145	0.213	0.063	0.090	0.396
93	1	1	0	1.017	0.343	0.085	0.033	0.452
93	1	1	1	0.050	0.030	0.040	0	0.571
93	1	1	1	0.038	0	0	0.034	0.141
93	1	1	3	0.052	0	0	0.026	0.024
93	1	1	3	0.037	0	0	0	0
93	1	1	5	0.019	0.003	0.005	0	0
93	1	1	5	0.031	0	0	0	0.018
93	1	1	7	0.043	0	0	0	0.021
93	1	1	7	0.041	0	0	0	0.028
93	1	2	0	1.071	0.279	0.097	0.124	0.357
93	1	2	0	1.087	0.316	0.094	0.023	0.307
93	1	2	1	0.122	0.062	0.082	0.037	0.198
93	1	2	1	0.070	0.025	0.033	0.034	0.216
93	1	2	3	0.032	0	0	0	0
93	1	2	3	0.059	0	0	0.015	0
93	1	2	5	0	0	0	0.029	0
93	1	2	5	0.021	0.007	0.010	0	0.017
93	1	2	7	0.052	0	0	0	0
93	1	2	7	0.037	0	0	0	0
93	1	3	0	1.061	0.102	0.070	0.075	1.285
93	1	3	0	1.050	0.279	0.054	0.064	0.657
93	1	3	1	0.172	0.015	0.019	0	0.058
93	1	3	1	0	0	0	0	0.080
93	1	3	3	0	0	0	0	0.053
93	1	3	3	0	0	0	0	0
93	1	3	5	0	0	0	0.370	0
93	1	3	5	0	0	0	0	0.086
93	1	3	7	0	0	0	0	0
93	1	3	7	0	0	0	0	0
93	1	4	0	1.193	0.294	0.108	0.087	1.097
93	1	4	0	1.118	0.334	0.111	0.122	0.451
93	1	4	1	0	0	0	0	0.348
93	1	4	1	0	0	0	0	0.161
93	1	4	3	0	0	0	0	0.213
93	1	4	3	0	0	0	0	0
93	1	4	5	0	0.152	0.202	0	0
93	1	4	5	0	0	0	0	0.163
93	1	4	7	0	0	0	0	0
93	1	4	7	0	0	0	0	0
93	2	1	0	1.069	0.503	0.065	0.125	0.559
93	2	1	0	1.081	0.526	0.058	0.108	0.203
93	2	1	1	0.049	0	0	0.074	0.079
93	2	1	1	0.046	0	0	0.100	0.099
93	2	1	3	0.037	0	0	0.098	0.041
93	2	1	3	0.037	0	0	0	0.125
93	2	1	5	0	0	0	0.043	0.032
93	2	1	5	0.041	0.027	0.036	0	0
93	2	1	7	0	0	0	0	0
93	2	1	7	0	0	0	0	0
93	2	2	0	1.037	0.720	0.045	0.091	0.399

Table A - 12. Cont.

Year	Fer	Trt	Day	Glucose	Xylose	Galac.	Ara	Fructose
93	2	2	0	1.056	0.402	0.054	0.094	0.480
93	2	2	1	0	0	0	0.141	0.564
93	2	2	1	0	0	0	0.049	0.122
93	2	2	3	0.057	0.031	0.042	0.024	0.093
93	2	2	3	0.024	0.030	0.040	0.102	0.135
93	2	2	5	0.071	0	0	0.066	0.040
93	2	2	5	0.011	0	0	0.039	0.077
93	2	2	7	0	0.017	0.023	0.058	0.184
93	2	2	7	0	0.017	0.023	0.058	0.090
93	2	3	0	1.064	0.683	0.056	0.105	0.735
93	2	3	0	1.043	0.612	0.072	0.104	1.298
93	2	3	1	0	0.037	0.049	0.070	0.105
93	2	3	1	0	0	0	0.062	0.053
93	2	3	3	0	0	0	0.122	0
93	2	3	3	0	0	0	0.067	0
93	2	3	5	0	0	0	0	
93	2	3	5	0	0	0	0.048	0
93	2	3	7	0	0	0	0.044	0.055
93	2	3	7	0	0	0	0.07	0.068
93	2	4	0	1.056	0.501	0.057	0.071	0.518
93	2	4	0	1.071	0.603	0.059	0.091	1.109
93	2	4	1	0	0	0	0.051	0.333
93	2	4	1	0.025	0	0	0.156	0.157
93	2	4	3	0.018	0	0	0.086	0.115
93	2	4	3	0	0	0	0.065	0.043
93	2	4	5	0	0	0	0.005	0
93	2	4	5	0.009	0	0	0	0
93	2	4	7	0	0	0	0.102	0
93	2	4	7	0	0	0	0.019	0.020

Abbreviations:
Y =Year; Fer = length of fermentation, 1 = 40 d, 2 = 100d; Trt = treatment, 1 = control, 2 = enzyme, 3 = inoculant, 4 = E + I; Galac = galactose; Ara = arabinose

Table A- 13. Data used for analysis of fermentation end-products in forage sorghum silage exposed to air in a tropical environment (Chapter 3)

Year	Fer	Trt	Day	Lac	Ace	Pro	Etoh	But
94	1	1	0	4.109	0.822	0.029	0.453	0.050
94	1	1	0	5.593	0.761	0.036	0.529	0.027
94	1	1	1	3.881	0.566	0.003	1.132	0.112
94	1	1	1	2.841	0.471	0.002	0.453	0.106
94	1	1	3	2.949	0.452	0.003	0.816	0.123
94	1	1	3	2.434	0.305	0.002	0.632	0.106
94	1	1	5	2.501	0.318	0.003	0.624	0.078
94	1	1	5	2.262	0.206	0.007	0.365	0.096
94	1	1	7	1.294	0.431	0.000	0.439	0.118
94	1	1	7	1.189	0.730	0.006	1.298	0.125
94	1	2	0	5.944	0.561	0.021	0.431	0.059
94	1	2	0	4.038	0.350	0.000	0.431	0.035
94	1	2	1	3.544	0.472	0.005	0.526	0.133
94	1	2	1	3.010	0.379	0.002	0.397	0.095
94	1	2	3	2.596	0.377	0.001	0.556	0.111
94	1	2	3	1.209	0.290	0.002	0.466	0.116
94	1	2	5	2.739	0.393	0.001	0.565	0.136
94	1	2	5	2.363	0.237	0.004	0.372	0.061
94	1	2	7	1.381	0.623	0.005	0.727	0.018
94	1	2	7	1.394	0.328	0.009	0.511	0.126
94	1	3	0	5.180	0.835	0.255	0.406	0.024
94	1	3	0	4.823	0.701	0.000	0.327	0.096
94	1	3	1	3.130	0.630	0.002	0.392	0.046
94	1	3	1	3.032	0.513	0.004	1.001	0.053
94	1	3	3	2.961	0.487	0.001	0.612	0.138
94	1	3	3	2.013	0.456	0.002	0.439	0.034
94	1	3	5	2.475	0.220	0.002	0.951	0.046
94	1	3	5	2.548	0.368	0.002	0.643	0.058
94	1	3	7	1.521	0.136	0.002	0.564	0.027
94	1	3	7	1.261	0.193	0.003	0.594	0.052
94	1	4	0	3.421	1.354	0.000	0.235	0.001
94	1	4	0	3.834	0.881	0.093	0.346	0.065
94	1	4	1	3.579	0.959	0.001	0.677	0.059
94	1	4	1	2.268	0.308	0.001	1.914	0.040
94	1	4	3	2.146	0.767	0.002	0.866	0.059
94	1	4	3	3.311	0.125	0.002	0.755	0.031
94	1	4	5	1.215	0.222	0.002	0.755	0.069
94	1	4	5	2.114	0.177	0.002	0.354	0.039
94	1	4	7	2.240	0.437	0.004	1.679	0.134
94	1	4	7	1.652	0.241	0.001	1.157	0.061
94	2	1	0	2.902	0.714	0.020	0.202	0.053
94	2	1	0	2.588	0.803	0.040	0.283	0.076
94	2	1	1	1.460	0.866	0.004	0.418	0.122
94	2	1	1	1.044	0.555	0.004	0.661	0.071
94	2	1	3	2.899	0.433	0.003	0.407	0.097
94	2	1	3	1.242	0.468	0.004	0.448	0.080
94	2	1	5	1.794	0.530	0.006	0.345	0.015
94	2	1	5	1.643	0.493	0.005	0.350	0.060
94	2	1	7	1.424	0.489	0.004	0.405	0.065
94	2	1	7	0.964	0.458	0.008	0.322	0.028
94	2	2	0	2.791	0.775	0.024	0.227	0.010
94	2	2	0	1.875	0.733	0.020	0.396	0.092
94	2	2	1	1.827	0.402	0.004	0.421	0.063
94	2	2	1	1.918	0.564	0.004	0.728	0.070
94	2	2	3	2.706	0.491	0.002	0.345	0.064
94	2	2	3	2.945	0.621	0.002	0.324	0.190
94	2	2	5	2.562	0.313	0.002	0.268	0.102

Table A- 13. Cont.

Year	Fer	Trt	Day	Lac	Ace	Pro	Etoh	But
94	2	2	5	2.052	0.497	0.002	0.232	0.084
94	2	2	7	1.183	0.295	0.002	0.237	0.084
94	2	2	7	1.279	0.370	0.003	0.251	0.060
94	2	3	0	3.287	0.819	0.039	0.296	0.019
94	2	3	0	5.102	0.795	0.020	0.331	0.014
94	2	3	1	2.589	0.364	0.002	0.759	0.119
94	2	3	1	3.949	0.623	0.002	0.442	0.065
94	2	3	3	1.707	0.293	0.002	0.202	0.111
94	2	3	3	1.728	0.449	0.001	0.198	0.115
94	2	3	5	1.548	0.242	0.005	0.124	0.060
94	2	3	5	1.400	0.162	0.001	0.032	0.056
94	2	3	7	1.439	0.259	0.003	0.045	0.037
94	2	3	7	0.938	0.276	0.002	0.052	0.136
94	2	4	0	4.263	0.782	0.021	0.401	0.065
94	2	4	0	4.224	0.793	0.020	0.328	0.036
94	2	4	1	2.634	0.620	0.002	0.692	0.049
94	2	4	1	2.896	0.413	0.003	0.714	0.070
94	2	4	3	1.702	0.147	0.001	0.234	0.041
94	2	4	3	1.568	0.356	0.001	0.196	0.057
94	2	4	5	1.767	0.175	0.004	0.145	0.098
94	2	4	5	1.870	0.247	0.002	0.143	0.144
94	2	4	7	0.709	0.190	0.001	0.041	0.100
94	2	4	7	0.972	0.446	0.001	0.045	0.067
93	1	1	0	1.484	0.352	0.084	0.937	0.107
93	1	1	0	1.390	0.298	0.000	0.090	0.132
93	1	1	1	0.860	0.098	0.003	1.290	0.099
93	1	1	1	0.908	0.080	0.002	0.400	0.117
93	1	1	3	0.361	0.290	0.001	0.530	0.073
93	1	1	3	0.306	0.463	0.001	0.211	0.066
93	1	1	5	0.255	0.019	0	0.234	0.015
93	1	1	5	0.243	0.018	0	0.222	0.015
93	1	1	7	0.121	0.018	0	0.190	0.036
93	1	1	7	0.120	0.019	0	0.193	0.012
93	1	2	0	1.702	0.342	0.060	0.459	0.060
93	1	2	0	2.407	0.452	0.056	1.090	0.066
93	1	2	1	1.281	0.031	0.002	1.285	0.057
93	1	2	1	1.847	0.042	0.001	1.213	0.089
93	1	2	3	1.006	0.267	0	0.139	0.039
93	1	2	3	1.336	0.223	0.000	0.136	0.064
93	1	2	5	0.777	0.117	0.003	0.077	0.095
93	1	2	5	0.938	0.186	0.002	0.016	0.066
93	1	2	7	0.247	0.135	0.000	0.014	0.052
93	1	2	7	0.045	0.070	0.000	0.012	0.013
93	1	3	0	1.979	0.488	0.027	0.521	0.077
93	1	3	0	1.318	0.634	0.060	0.490	0.086
93	1	3	1	0.882	0.043	0	0.846	0.094
93	1	3	1	0.462	0.063	0	0.989	0.020
93	1	3	3	1.283	0.289	0.002	0.745	0.038
93	1	3	3	1.030	0.299	0.002	0.692	0.051
93	1	3	5	0.683	0.133	0.001	0.164	0.013
93	1	3	5	2.224	0.103	0.002	0.142	0.017
93	1	3	7	0.129	0.281	0.001	0.012	0.032
93	1	3	7	0.154	0.110	0.001	0.008	0.008
93	1	4	0	1.011	0.453	0.060	0.366	0.072
93	1	4	0	1.367	0.380	0.032	0.618	0.050
93	1	4	1	0.784	0.425	0.016	0.987	0.090
93	1	4	1	0.647	0.216	0.026	1.941	0.086
93	1	4	3	0.962	0.122	0.006	0.784	0.018

Table A- 13. Cont.

Year	Fer	Trt	Day	Lac	Ace	Pro	Etoh	But
93	1	4	3	1.149	0.224	0.002	0.432	0.073
93	1	4	5	0.299	0.164	0.001	0.231	0.061
93	1	4	5	0.135	0.186	0.002	0.340	0.082
93	1	4	7	0.127	0.232	0.002	0.311	0.073
93	1	4	7	0.141	0.115	0.002	0.172	0.086
93	2	1	0	1.767	0.724	0.039	0.463	0.047
93	2	1	0	1.165	0.459	0.060	0.470	0.039
93	2	1	1	1.472	1.484	0.004	1.069	0.170
93	2	1	1	1.083	0.967	0.004	0.826	0.109
93	2	1	3	1.022	1.510	0.005	0.056	0.087
93	2	1	3	0.925	1.254	0.001	0.134	0.125
93	2	1	5	0.619	0.466	0.001	0.161	0.111
93	2	1	5	0.820	0.767	0.002	0.209	0.143
93	2	1	7	0.114	0.670	0.001	0.054	0.107
93	2	1	7	0.112	0.365	0.001	0.045	0.057
93	2	2	0	1.850	0.269	0.088	0.313	0.047
93	2	2	0	1.466	0.733	0.080	0.969	0.057
93	2	2	1	2.862	1.580	0.002	1.076	0.088
93	2	2	1	0.113	1.322	0.002	0.990	0.097
93	2	2	3	1.397	1.627	0.001	0.766	0.089
93	2	2	3	1.121	1.129	0.002	0.939	0.073
93	2	2	5	0.534	1.080	0.002	0.061	0.028
93	2	2	5	0.423	1.379	0.003	0.047	0.039
93	2	2	7	0.121	0.704	0.001	0.100	0.030
93	2	2	7	0.112	0.265	0.001	0.039	0.016
93	2	3	0	1.206	0.409	0.044	0.903	0.037
93	2	3	0	1.592	0.721	0.071	0.558	0.028
93	2	3	1	0.770	1.352	0.002	0.622	0.029
93	2	3	1	1.474	1.195	0.005	0.958	0.067
93	2	3	3	1.139	1.360	0.001	0.243	0.153
93	2	3	3	0.686	1.456	0.002	0.054	0.087
93	2	3	5	0.417	0.648	0.002	0.024	0.106
93	2	3	5	0.216	0.902	0.004	0.015	0.115
93	2	3	7	0.106	0.822	0.001	0.011	0.060
93	2	3	7	0.102	0.904	0.002	0.058	0.088
93	2	4	0	1.581	0.485	0.019	0.652	0.050
93	2	4	0	1.915	0.641	0.045	0.735	0.020
93	2	4	1	0.251	0.353	0.001	1.365	0.150
93	2	4	1	1.209	2.241	0.002	0.596	0.087
93	2	4	3	0.835	1.506	0.003	0.090	0.080
93	2	4	3	0.465	1.281	0.003	0.079	0.040
93	2	4	5	0.323	0.969	0.003	0.040	0.077
93	2	4	5	0.125	0.603	0.002	0.061	0.101
93	2	4	7	0.316	0.231	0.002	0.049	0.163
93	2	4	7	0.247	1.092	0.002	0.070	0.087

Abbreviations:
Fer = Fermentation length, 1 = 40 d, 2 = 100 d; Trt = treatment, 1 = control, 2 = enzyme, 3 = inoculant, 4 = E+I; D = day of aerobic exposure; Lac = lactic acid; Ace = acetic acid; Pro = propionic acid, Etoh = ethanol, But = butyric acid.

Table a-aa. Data used for analysis of water soluble carbohydrate contents in aerobic stability studies from Chapter 3 (Tropical Environment)

Y	Fer	Trt	Day	Glucose	Xylose	Galactose	Arab	Fructose
94	1	1	0	0.5022	0.3022	0.2182	0.106	0.2824
94	1	1	0	0.4962	0.2415	0.1582	0.094	0.3366
94	1	1	1	0.4539	0.1443	0.1598	0.084	0.248
94	1	1	1	0.5462	0.1379	0.1527	0.081	0.1743
94	1	1	3	0.2137	0.1196	0.1324	0.0699	0.2743
94	1	1	3	0.3472	0.1168	0.1294	0.1121	0.157
94	1	1	5	0.2877	0.1343	0.1487	0.1103	0.1544
94	1	1	5	0.214	0.0947	0.1049	0.0873	0.1222
94	1	1	7	0.599	0.1159	0.1283	0.144	0.2017
94	1	1	7	0.1462	0	0	0	0
94	1	2	0	0.2747	0.2195	0.1857	0.1381	0.1932
94	1	2	0	0.4957	0.1878	0.2104	0.1125	0.2465
94	1	2	1	0.4392	0.2494	0.2162	0.0746	0.2445
94	1	2	1	0.3828	0.1491	0.1651	0.1305	0.1828
94	1	2	3	0.5085	0.0966	0.107	0.1509	0.2113
94	1	2	3	0.5388	0.1419	0.1572	0.1518	0.2126
94	1	2	5	0.3507	0.0922	0.1021	0.1496	0.2096
94	1	2	5	0.1987	0.254	0.1454	0	0
94	1	2	7	0.2347	0.1466	0.1623	0.206	0.2884
94	1	2	7	0.1073	0.175	0.1938	0.1778	0.249
94	1	3	0	0.419	0.1177	0.1228	0.058	0.2329
94	1	3	0	0.392	0.1038	0.1332	0.068	0.1689
94	1	3	1	0.6445	0.1307	0.1447	0.1631	0.2284
94	1	3	1	0.2223	0.1317	0.1458	0.0427	0.2399
94	1	3	3	0.5277	0.098	0.1086	0.1452	0.2033
94	1	3	3	0.3004	0.1292	0.1431	0.147	0.2059
94	1	3	5	0.1632	0.1096	0.1214	0.2409	0.3374
94	1	3	5	0.2589	0.1139	0.1262	0.1964	0.275
94	1	3	7	0.4319	0.0761	0.0843	0.1406	0.1969
94	1	3	7	0.2878	0.0896	0.0993	0.1596	0.2235
94	1	4	0	0.4111	0.1496	0.1251	0.1348	0.4148
94	1	4	0	0.1175	0.1682	0.1576	0.1129	0.2087
94	1	4	1	0.5805	0.1227	0.1359	0.1583	0.2217
94	1	4	1	0.6241	0.1971	0.2182	0.4452	0.3234
94	1	4	3	0.6323	0.1802	0.1996	0.2488	0.3484
94	1	4	3	0.4635	0.1154	0.1278	0.1824	0.2554
94	1	4	5	0.3509	0.1357	0.1502	0.1419	0.2987
94	1	4	5	0.0526	0.0754	0.0835	0.0792	0.111
94	1	4	7	0.6286	0.1678	0.1858	0.3545	0.1964
94	1	4	7	0.5151	0.1251	0.1386	0.228	0.1193
94	2	1	0	0.2896	0.1704	0.086	0.0427	0.1012
94	2	1	0	0.4211	0.2432	0.2264	0.098	0.0718
94	2	1	1	0.222	0.0544	0.0603	0.1113	0.1559
94	2	1	1	0.2853	0.4005	0.4435	0.1949	0.0729
94	2	1	3	0.3677	0.1102	0.122	0.1085	0.1519
94	2	1	3	0.1788	0.3453	0.3824	0.1552	0.1173
94	2	1	5	0	0	0	0	0
94	2	1	5	0.428	0.2635	0.2918	0.1656	0.2319
94	2	1	7	0.2103	0.2937	0.3252	0.1495	0.1093
94	2	1	7	0.3495	0.1374	0.1522	0.0139	0.0194
94	2	2	0	0.2121	0.1628	0.1275	0.0862	0.0768
94	2	2	0	0.3327	0.1979	0.1486	0.1441	0.0686
94	2	2	1	0.3595	0.2792	0.3092	0.1272	0.0781
94	2	2	1	0.2953	0.2563	0.2838	0.2456	0.0439
94	2	2	3	0.4905	0.1741	0.1928	0.0674	0.0944
94	2	2	3	0.0633	0.1723	0.1908	0.0277	0.0387
94	2	2	5	0.2865	0.2913	0.3226	0	0
94	2	2	5	0.1487	0	0	0	0
94	2	2	7	0.2491	0.0994	0.11	0.0816	0.1142
94	2	2	7	0.1902	0.3245	0.3594	0	0
94	2	3	0	0.3761	0.1038	0.0919	0.0884	0.3964
94	2	3	0	0.2575	0.1537	0.1258	0.1209	0.1084
94	2	3	1	0.1494	0.1183	0.131	0.1155	0.1617
94	2	3	1	0.3866	0.1269	0.1405	0.1246	0.1745

Table a-aa. Cont.

Y	Fer	Trt	Day	Glucose	Xylose	Galactose	Ara	Fructose
94	2	3	3	0.2532	0.1731	0.1917	0.2339	0.1276
94	2	3	3	0.422	0.1708	0.1892	0.0938	0.1314
94	2	3	5	0.2279	0.0919	0.1017	0.0419	0.0586
94	2	3	5	0.3538	0.1422	0.1574	0.0352	0.0493
94	2	3	7	0.212	0	0	0.0933	0.1307
94	2	3	7	0.1087	0.1098	0.1216	0	0
94	2	4	0	0.2904	0.3121	0.1866	0.1750	0.2200
94	2	4	0	0.1489	0.2048	0.1594	0.1580	0.2088
94	2	4	1	0.1137	0.279	0.309	0.1031	0.1444
94	2	4	1	0.2474	0.3306	0.3661	0.1633	0.2287
94	2	4	3	0.1744	0.1339	0.1482	0.0883	0.1236
94	2	4	3	0	0.2868	0.0928	0	0
94	2	4	5	0	0.3429	0.9334	0	0
94	2	4	5	0.4236	0.0725	0.0803	0.0353	0.0494
94	2	4	7	0.0954	0	0	0.0404	0.0566
94	2	4	7	0.2211	0.0925	0.1024	0.0279	0.0391
93	1	1	0	0.1229	0.0990	0.0567	0.0171	0.0175
93	1	1	0	0.1064	0.0723	0.0307	0.0207	0.0784
93	1	1	1	0	0	0	0.0158	0.0222
93	1	1	1	0	0	0	0	0
93	1	1	3	0	0	0	0.0725	0.1016
93	1	1	3	0.0685	0	0	0	0
93	1	1	5	0.0992	0	0	0	0
93	1	1	5	0.0669	0	0	0	0
93	1	1	7	0.0224	0	0	0	0
93	1	1	7	0.0748	0	0	0	0
93	1	2	0	0.1735	0.1066	0.1795	0.1442	0.0760
93	1	2	0	0.1145	0.2032	0.0699	0.1198	0.1151
93	1	2	1	0.0103	0.2843	0.3148	0.1534	0.0948
93	1	2	1	0.0373	0	0	0	0
93	1	2	3	0.0385	0	0	0.0801	0.1122
93	1	2	3	0.0504	0	0	0.0161	0.0225
93	1	2	5	0.0461	0.0125	0.0139	0	0
93	1	2	5	0	0	0	0	0
93	1	2	7	0	0.0332	0.0368	0.0188	0.0263
93	1	2	7	0.0059	0.0517	0.0573	0.015	0.021
93	1	3	0	0.0826	0.1282	0.0585	0.0813	0.2665
93	1	3	0	0.0902	0.1109	0.0901	0.0342	0.2537
93	1	3	1	0	0	0	0.034	0.0476
93	1	3	1	0	0	0	0	0
93	1	3	3	0.0543	0	0	0	0
93	1	3	3	0	0.0353	0.0391	0.0484	0.0678
93	1	3	5	0.0102	0.0391	0.0433	0.074	0.1037
93	1	3	5	0.0329	0	0	0.0295	0.0413
93	1	3	7	0.0336	0	0	0	0
93	1	3	7	0.0548	0	0	0	0
93	1	4	0	0.0487	0.1663	0.0671	0.0980	0.1302
93	1	4	0	0.0309	0.1221	0.0782	0.0617	0.1651
93	1	4	1	0.0231	0	0	0	0
93	1	4	1	0.038	0	0	0	0
93	1	4	3	0	0	0	0	0
93	1	4	3	0.0593	0.06	0.0665	0.0479	0.067
93	1	4	5	0.0097	0.0408	0.0452	0.0371	0.052
93	1	4	5	0.0099	0.0172	0.019	0	0
93	1	4	7	0.0859	0.0197	0.0218	0	0
93	1	4	7	0.0096	0.0197	0.0218	0.027	0.0379
93	2	1	0	0.0552	0.034	0.0125	0.1443	0.0199
93	2	1	0	0.0335	0.0729	0.0432	0.1884	0.0175
93	2	1	1	0.0213	0.0377	0.0418	0.1761	0.5266
93	2	1	1	0.0338	0.0385	0.0426	0.0623	0.0873
93	2	1	3	0.0553	0.0278	0.0308	0.0202	0.0283
93	2	1	3	0.0793	0.0419	0.0464	0.0246	0.0344
93	2	1	5	0.0804	0.0593	0.0657	0.0188	0.0263
93	2	1	5	0.5193	0.0737	0.0816	0.0173	0.0242

Table a-aa. Cont.

Y	Fer	Trt	Day	Glucose	Xylose	Galactose	Ara	Fructose
93	2	1	7	0	0.0567	0.0628	0.0589	0.1303
93	2	2	0	0.0808	0.0392	0.0639	0.1342	0.1083
93	2	2	1	0.0881	0.1571	0.174	0.0742	0.1039
93	2	2	1	0.0143	0.0741	0.0821	0.4201	0.5882
93	2	2	3	0.0158	0.0178	0.0197	0.0197	0.0277
93	2	2	3	0.0424	0.0278	0.0308	0.0301	0.0421
93	2	2	5	0.0978	0.0363	0.0401	0.0182	0.0254
93	2	2	5	0.0741	0.0479	0.053	0.0159	0.0223
93	2	2	7	0.0016	0.0363	0.0402	0.0126	0.0177
93	2	2	7	0.0019	0.0316	0.035	0.0114	0.016
93	2	3	0	0.0267	0.0325	0.0306	0.1279	0.0627
93	2	3	0	0.0686	0.2193	0.0306	0.1283	0.1014
93	2	3	1	0.0687	0.0466	0.0516	0.09	0.126
93	2	3	1	0.0459	0.1434	0.1588	0.135	0.189
93	2	3	3	0.0989	0.02	0.0221	0.186	0.2605
93	2	3	3	0.0995	0.0266	0.0295	0.0877	0.1228
93	2	3	5	0.0324	0.0374	0.0415	0.0173	0.0243
93	2	3	5	0.0301	0.0373	0.0413	0.0161	0.0225
93	2	3	7	0.0856	0	0	0.0208	0.0291
93	2	3	7	0.0332	0.0719	0.135	0.0226	0.0317
93	2	4	0	0.0456	0.1411	0.0437	0.1384	0.0518
93	2	4	0	0.0234	0.0511	0.1513	0.0260	0.034
93	2	4	1	0.0124	0.0972	0.1077	0.3444	0.4823
93	2	4	1	0.0200	0.0552	0.0611	0.1099	0.1539
93	2	4	3	0.0624	0.0188	0.0208	0.1747	0.2446
93	2	4	3	0.0794	0.0371	0.0411	0	0
93	2	4	5	0.0544	0.0313	0.0347	0.0134	0.0187
93	2	4	5	0.0163	0.043	0.0476	0	0
93	2	4	7	0.0166	0	0	0	0
93	2	4	7	0.0077	0	0	0.0219	0.0307

Abbreviatins:
Y = year, Fer = length of fermentation; 1 = 40 d; 2 = 100 d; trt = treatment; 1 = control, 2 = enzyme, 3 = inoculant, 4 = E+I; Arab = arabinose

Table A-15. Data used for analysis of organic acid contents (g/100 g DM) of forage sorghum ensiled at two stages of maturity in a tropical environment (Chapter 4)

Mat	Trt	Day	Citric	Malic	Oxal	Succinic
90	1	0	0.875	4.277	1.076	4.416
90	1	0	0.742	4.918	1.150	2.212
90	1	0	0.968	4.491	1.147	2.006
90	1	1	0.265	2.805	1.275	3.513
90	1	1	0.321	3.697	1.301	3.109
90	1	1	0.285	2.407	1.122	2.537
90	1	3	0.28	2.98	1.171	3.236
90	1	3	0.188	3.663	1.135	1.833
90	1	3	0.279	1.353	1.741	2.006
90	1	7	0.204	2.755	1.471	2.880
90	1	7	0.412	2.315	0.254	2.217
90	1	7	0.336	3.007	1.224	3.359
90	1	21	0.509	3.753	1.047	3.138
90	1	21	0.287	2.922	1.074	2.630
90	1	21	0.379	2.225	1.163	1.867
90	1	100	0.125	1.033	0.914	1.078
90	1	100	0.108	1.167	0.479	0.831
90	1	100	0.173	1.369	0.527	1.198
90	2	0	0.798	4.700	1.678	3.431
90	2	0	0.612	4.773	1.085	3.724
90	2	0	0.744	5.612	1.160	2.844
90	2	1	0.359	2.838	1.831	4.289
90	2	1	0.785	2.163	1.486	4.419
90	2	1	0.407	2.386	0.424	2.824
90	2	3	0.237	2.091	1.138	3.275
90	2	3	0.169	3.988	1.111	2.560
90	2	3	0.403	1.937	1.148	2.910
90	2	7	0.393	1.849	1.580	3.761
90	2	7	0.507	2.282	1.701	3.366
90	2	7	0.354	2.101	1.072	2.097
90	2	21	0.456	1.102	1.074	2.509
90	2	21	0.387	2.414	1.250	1.005
90	2	21	0.308	1.052	0.755	1.858
90	2	100	0.347	1.344	1.065	1.045
90	2	100	0.184	1.396	0.529	1.134
90	2	100	0.237	2.047	0.507	1.251
90	3	0	0.511	4.560	1.19	3.607
90	3	0	0.802	4.345	1.380	3.553
90	3	0	0.371	3.998	1.123	2.685
90	3	1	0.163	2.548	1.366	3.322
90	3	1	0.824	3.611	1.235	2.474
90	3	1	0.797	2.393	1.010	3.258
90	3	3	0.799	2.592	1.410	2.484
90	3	3	0.728	2.724	0.995	2.536
90	3	3	0.730	2.583	1.266	2.488
90	3	7	0.638	3.685	1.123	2.740
90	3	7	0.773	3.081	1.224	2.043
90	3	7	0.249	2.829	1.169	0.930
90	3	21	0.177	2.247	1.225	1.734
90	3	21	0.304	2.708	1.068	1.932
90	3	21	0.181	1.795	0.983	1.433
90	3	100	0.124	1.753	0.943	1.316
90	3	100	0.177	1.998	0.852	1.919
90	3	100	0.169	1.934	0.987	1.459
90	4	0	0.720	5.001	1.034	3.565
90	4	0	0.306	4.567	1.311	2.966
90	4	0	0.987	4.221	1.355	4.856
90	4	1	0.497	3.080	1.58	2.282
90	4	1	0.336	2.889	1.318	2.658
90	4	1	0.306	2.143	1.57	2.445
90	4	3	0.283	3.035	1.691	2.567
90	4	3	0.639	2.603	0.948	3.821
90	4	3	0.121	3.839	1.353	1.879
90	4	7	0.736	3.515	1.108	1.923

Table A-15. Cont.

Mat	Trt	Day	Citric	Malic	Oxal	Succinic
90	4	7	0.469	3.104	1.705	2.187
90	4	7	0.516	2.858	0.947	1.984
90	4	21	0.314	3.934	1.296	1.873
90	4	21	0.312	3.430	0.984	1.858
90	4	21	0.110	2.453	1.436	1.253
90	4	100	0.137	1.949	0.914	1.870
90	4	100	0.104	1.006	0.768	1.656
90	4	100	0.104	2.329	0.905	0.996
110	1	0	2.424	4.810	0.141	3.114
110	1	0	1.640	5.134	0.148	4.833
110	1	0	1.198	4.863	0.147	2.739
110	1	1	1.297	4.917	0.237	2.764
110	1	1	1.947	4.763	0.235	2.688
110	1	1	0.973	4.892	0.247	1.723
110	1	3	1.656	4.876	0.336	1.534
110	1	3	1.108	4.151	0.257	1.199
110	1	3	1.077	4.905	0.297	1.174
110	1	7	1.210	4.867	0.342	1.015
110	1	7	0.816	4.600	0.292	1.828
110	1	7	0.844	3.984	0.260	1.955
110	1	21	0.836	4.144	0.193	1.757
110	1	21	0.745	3.559	0.194	1.222
110	1	21	0.673	4.407	0.272	1.061
110	1	100	0.790	4.293	0.400	1.319
110	1	100	0.37	4.333	0.094	1.489
110	1	100	0.996	3.031	0.128	1.064
110	2	0	1.370	4.953	0.145	2.224
110	2	0	1.582	4.770	0.157	4.726
110	2	0	1.363	4.951	0.187	4.962
110	2	1	1.834	4.980	0.05	4.925
110	2	1	1.220	4.041	0.169	3.543
110	2	1	1.88	5.121	0.097	1.931
110	2	3	1.47	4.735	0.357	1.799
110	2	3	0.968	4.824	0.373	1.392
110	2	3	1.48	4.755	0.321	1.213
110	2	7	1.079	4.562	0.526	1.406
110	2	7	0.886	4.762	0.253	1.255
110	2	7	0.710	4.764	0.228	1.114
110	2	21	0.365	4.529	0.228	1.690
110	2	21	0.158	4.436	0.452	1.590
110	2	21	0.629	4.328	0.232	0.908
110	2	100	1.056	2.127	0.104	1.450
110	2	100	0.400	3.360	0.056	1.631
110	2	100	0.724	3.711	0.298	1.611
110	3	0	1.431	5.018	0.171	4.090
110	3	0	1.387	4.814	0.128	3.764
110	3	0	1.053	4.680	0.145	4.074
110	3	1	1.167	4.766	0.112	3.865
110	3	1	1.825	4.930	0.123	3.484
110	3	1	0.989	5.556	0.135	2.641
110	3	3	1.032	5.220	0.281	1.995
110	3	3	0.856	4.269	0.219	1.902
110	3	3	1.390	5.126	0.346	1.327
110	3	7	1.102	3.442	0.316	1.780
110	3	7	1.649	4.701	0.189	1.232
110	3	7	1.496	4.013	0.252	0.941
110	3	21	0.900	3.330	0.328	1.347
110	3	21	1.218	3.881	0.39	1.768
110	3	21	1.155	4.142	0.199	1.248
110	3	100	0.681	4.29	0.119	0.855
110	3	100	0.863	3.342	0.144	0.933
110	3	100	0.868	3.344	0.144	0.933
110	4	0	0.630	4.868	0.115	3.154
110	4	0	1.911	5.048	0.126	2.563
110	4	0	0.893	3.308	0.108	3.454

Table A-15. Cont.

Mat	Trt	Day	Citric	Malic	Oxal	Succinic
110	4	1	1.688	4.714	0.120	4.206
110	4	1	1.554	5.437	0.194	3.950
110	4	1	1.554	5.473	0.194	3.950
110	4	3	0.707	4.721	0.079	1.057
110	4	3	1.003	4.547	0.098	1.421
110	4	3	1.467	5.051	0.053	1.028
110	4	7	1.083	4.664	0.239	1.768
110	4	7	1.146	4.183	0.213	1.475
90	4	7	1.009	4.256	0.087	1.201
90	4	21	0.838	4.909	0.253	1.429
90	4	21	1.409	3.573	0.241	1.409
90	4	21	0.681	3.891	0.272	1.555
90	4	100	0.918	3.859	0.223	0.894
90	4	100	0.890	3.730	0.211	0.814
90	4	100	1.093	2.150	0.219	0.778

Abbreviations:
Mat = maturity (days); Trt = treatment, 1 = control, 2 = enzyme, 3 = inoculant, 4 = E + I; Oxal = Oxaloacetic acid

Table A-16. Data used for analysis of pH and microbial succession in forage sorghum ensiled at two stages of maturity in a tropical environment (Chapter 4)

Mat	Trt	D	pH	LAB	Ent	YM	LAY
90	1	0	5.67	2.3	4.15	3.68	2.69
90	1	0	5.76	3.07	4.03	2.84	2.3
90	1	0	5.89	3.41	6.49	3.34	3.29
90	1	1	5.24	7.73	7.89	5.51	5.23
90	1	1	4.74	7.72	7.99	6.06	4.33
90	1	1	4.82	8.17	7.55	5.66	5.21
90	1	3	4.74	8.35	6.52	6.68	6.25
90	1	3	4.78	8.01	6.68	6.95	6.04
90	1	3	4.53	8.42	6.19	7.39	5.45
90	1	7	4.7	7.16	7.84	8	7.25
90	1	7	4.72	7.18	7.57	7.03	6.48
90	1	7	4.6	7.4	7.41	7.53	7.32
90	1	21	4.58	6.35	2.3	6.95	5.1
90	1	21	4.53	7.61	6.6	6.57	6.96
90	1	21	4.38	6.54	6.6	5.89	5.91
90	1	100	4.09	5.54	3	5.4	5.29
90	1	100	4.19	6.24	4.65	4.3	4.6
90	1	100	4.16	6.36	4.62	5.32	4.85
90	2	0	5.65	2.9	4.33	2.77	2.9
90	2	0	5.35	3.98	5.25	3.38	3.49
90	2	0	6.1	4.16	5.84	3.7	3.62
90	2	1	4.86	7.43	8.05	5.15	4.45
90	2	1	4.87	8.27	7.72	5.12	5.16
90	2	1	4.73	8.22	7.38	5.77	5.58
90	2	3	4.7	8.02	6.91	6.68	6.25
90	2	3	4.75	8.31	6.42	6.95	6.04
90	2	3	4.72	8.45	6.09	7.39	6.45
90	2	7	4.7	8.03	6.22	7.98	6.34
90	2	7	4.8	8.07	6.17	7.96	7.9
90	2	7	4.74	8.26	6.29	7.74	7.29
90	2	21	4.5	6.72	3.71	6.13	5.82
90	2	21	4.65	5.92	1	5.55	5.15
90	2	21	4.6	6.77	6.72	6.31	6.79
90	2	100	4.2	6.05	3.62	4.84	4.69
90	2	100	4.12	6.12	5.1	4.77	4.47
90	2	100	4.19	5.82	2.47	4.6	4.6
90	3	0	5.23	5.37	4.98	3.59	2.69
90	3	0	5.24	5.14	6.87	3.55	2.69
90	3	0	5.87	5.27	6.08	3.59	3.04
90	3	1	4.62	8.83	8.85	5.14	5.1
90	3	1	4.57	9.12	8.94	5.7	5.52
90	3	1	4.56	9.07	9.09	5.9	5.14
90	3	3	4.35	9.16	5.71	6.03	5.97
90	3	3	4.4	9.19	6.6	6.78	6.42
90	3	3	4.28	8.75	6.59	6.8	6.75
90	3	7	4.15	7.85	5.29	7.59	7.3
90	3	7	4.25	8.41	4.25	7.69	7.32
90	3	7	4.4	8.55	3.19	6.36	6.23
90	3	21	4.25	6.77	5.84	6.25	6.02
90	3	21	4.35	6.47	3.3	6.1	5.97
90	3	21	4.35	6.36	4.09	5.89	5.66
90	3	100	4.2	5.92	4.08	5.04	4
90	3	100	4.08	4.88	3	4.95	4.3
90	3	100	4.1	6.02	3.38	4.77	4.3
90	4	0	5.43	4.45	5.31	2	2.69
90	4	0	5.32	5.2	6.02	2.77	2.6
90	4	0	5.88	5.36	6.36	2.47	3.49
90	4	1	4.52	8.85	7.53	5.34	4.49
90	4	1	4.7	8.94	7.44	5.86	5.04
90	4	1	4.64	9.09	7.09	5.38	5.15
90	4	3	4.35	8.94	6.02	7.07	6.03
90	4	3	4.22	8.71	5.94	6.9	6.71
90	4	3	4.29	9.13	6.41	7	6.59
90	4	7	4.25	8.09	5.96	7.1	7.24
90	4	7	4.25	8.01	5.6	7.26	7

Table A-16. Cont.

Mat	Trt	D	pH	LAB	Ent	YM	LAY
90	4	7	4.25	7.52	6	7.46	7.41
90	4	21	4.15	6.62	6	6.22	6.02
90	4	21	4.35	6.54	3.4	6.07	5.47
90	4	21	4.35	6.63	3	5.71	5.04
90	4	100	4.07	6	4.2	5.26	4.47
90	4	100	3.96	5.65	4.45	4.8	4.77
90	4	100	4.1	4.95	3.92	4.47	4.8
110	1	0	5.48	3.22	6.59	4.19	3.5
110	1	0	5.47	2.98	6.53	4.95	3.37
110	1	0	5.45	3.15	6.46	5.15	3.3
110	1	1	4.88	8.15	7.15	6.5	6.65
110	1	1	5.01	8.36	7.2	5.96	6.25
110	1	1	4.87	8.43	7.14	6.21	6.43
110	1	3	4.36	9.86	5.65	7.49	6.79
110	1	3	4.3	8.77	6.67	7.16	6.6
110	1	3	4.3	8.85	5.85	6.83	6.2
110	1	7	4.3	8.13	5.95	6.83	6.87
110	1	7	4.26	7.94	5.49	6.95	6.83
110	1	7	4.21	8.14	4.46	6.15	6.67
110	1	21	4.36	6.66	3.83	5.62	5.29
110	1	21	4.3	7.14	3.83	5.97	5.96
110	1	21	4.38	7.35	4.18	6.1	6.27
110	1	100	4.19	6.15	3.35	5.66	5.56
110	1	100	4.14	6.48	3.35	5.97	5.84
110	1	100	4.14	6.48	3.35	5.97	5.84
110	2	0	5.42	3.55	6.74	4.45	3.19
110	2	0	5.43	3.9	6.56	4.34	3.45
110	2	0	5.41	3.54	6.31	3.78	3.31
110	2	1	4.94	8.12	7.14	6.04	3.07
110	2	1	4.93	8.25	7.24	5.66	4.93
110	2	1	4.98	8.4	7.3	5.79	4.98
110	2	3	4.24	9.08	5.3	7.14	7.33
110	2	3	4.25	9.11	5.22	7.16	6.64
110	2	3	4.26	9.08	4.39	7.27	7
110	2	7	4.2	8.38	5.7	7.28	7.3
110	2	7	4.23	8.27	6.03	6.98	7.09
110	2	7	4.24	8.39	5.59	7.27	7.08
110	2	21	4.27	6.48	4	5.55	5.58
110	2	21	4.23	7.87	4.23	6.37	6.5
110	2	21	4.24	7.07	3.62	6.53	6.39
110	2	100	4.11	6.5	3.5	5.63	5.37
110	2	100	4.09	6.43	3.52	5.86	5.68
110	2	100	4.1	6.68	3.25	5.81	5.59
110	3	0	5.51	4.91	6.57	4.54	3.36
110	3	0	5.5	5.05	6.57	4.12	3.47
110	3	0	5.49	4.92	6.65	3.89	3.31
110	3	1	4.59	9.12	7.13	6.72	6.45
110	3	1	4.49	8.85	7.14	6.52	6.28
110	3	1	4.5	9.02	7.13	6.02	6.11
110	3	3	4.09	9.65	4.12	7.2	7.01
110	3	3	4.1	9.19	3.72	6.86	6.93
110	3	3	4.12	9	4.71	7.23	6.96
110	3	7	4.1	8.72	5.73	6.97	6.95
110	3	7	4.12	8.59	5.7	6.97	6.99
110	3	7	4.1	8.32	5.28	6.81	6.28
110	3	21	4.14	7.32	3.82	6.66	6.33
110	3	21	4.1	6.94	3.89	6.6	6.39
110	3	21	4.1	7.14	3.59	6.42	6.35
110	3	100	4.14	7.12	3.47	5.93	5.8
110	3	100	4.12	6.47	3.5	5.96	5.8
110	3	100	4.12	5.94	2.98	5.48	5.22
110	4	0	5.48	5.15	6.73	4.76	3.34
110	4	0	5.38	5.09	6.5	5.08	3.48
110	4	0	5.47	5.13	6.37	5.3	3.32
110	4	1	4.44	9.21	7.14	7.06	7

Table A-16. Cont.

Mat	Trt	D	pH	LAB	Ent	YM	LAY
110	4	1	4.45	8.94	7.1	6.78	6.84
110	4	1	4.49	9.03	7.07	6.89	6.91
110	4	3	4.11	9.2	4.92	7.01	7.2
110	4	3	4.07	9.09	5.77	6.45	6.13
110	4	3	4.1	9.95	3.19	7.23	6.64
110	4	7	4.07	8.03	4	7.15	7.18
110	4	7	4.06	8.46	5.53	6.88	7.12
110	4	7	4.09	8.29	5.09	7.37	6.95
110	4	21	4.14	5.95	3.07	5.69	5.17
110	4	21	4.14	6.89	3.27	6.32	6.16
110	4	21	4.1	7.12	3.32	6.23	6.3
110	4	100	4.14	6.55	3.59	5.52	5.48
110	4	100	4.06	6.45	3.41	5.9	5.69
110	4	100	4.18	6.79	3.31	5.98	5.69

Abbreviations:
Mat = maturity (days); Trt = treatment, 1 = control, 2 = enzyme, 3 = inoculant, 4 = E+I; LAB = Lactic acid-producing bacteria, Ent = Enterobacteriaceae, YM = yeasts and molds, LAY = lactate assimilating yeast.

Table A-17. Data used for analysis of fermentation end-products (g/100 g DM) in forage sorghum ensiled at two stages of maturity in a tropical environment (Chapter 4)

M	T	D	Ace	Lac	Pro	Ethanol	But
1	1	0	0.083	0.146	0	0.061	0
1	1	0	0.027	0.053	0	0.057	0
1	1	0	0.126	0.027	0	0.132	0
1	1	1	0.128	1.348	0.005	0.480	0.020
1	1	1	0.256	1.268	0.000	0.510	0.030
1	1	1	0.230	1.298	0	0.466	0.030
1	1	3	0.917	5.550	0.007	0.516	0.086
1	1	3	0.606	6.809	0.003	0.659	0.033
1	1	3	0.656	3.485	0.006	0.546	0.077
1	1	7	0.795	5.890	0.000	0.665	0.050
1	1	7	0.642	8.991	0.006	0.856	0.048
1	1	7	0.850	6.596	0.008	0.934	0.073
1	1	21	0.614	5.830	0.039	0.627	0.052
1	1	21	0.907	6.850	0.041	0.704	0.018
1	1	21	0.599	5.401	0.042	0.413	0.071
1	1	100	0.714	2.902	0.020	0.202	0.053
1	1	100	0.803	2.585	0.040	0.283	0.076
1	1	100	0.732	2.125	0	0.103	0.011
1	2	0	0.037	0.077	0	0.096	0
1	2	0	0.082	0.099	0	0.029	0
1	2	0	0.089	0.092	0	0.099	0
1	2	1	0.223	1.081	0	0.540	0.024
1	2	1	0.259	1.249	0.005	0.627	0.031
1	2	1	0.172	1.242	0	0.478	0.067
1	2	3	0.609	4.877	0.003	0.715	0.071
1	2	3	1.141	6.614	0.009	0.623	0.077
1	2	3	0.795	6.717	0.003	0.831	0.049
1	2	7	1.175	6.54	0.004	1.089	0.003
1	2	7	0.886	7.374	0.006	0.884	0.053
1	2	7	0.534	6.926	0.007	0.835	0.052
1	2	21	0.722	5.541	0.038	0.651	0.097
1	2	21	0.767	6.411	0.038	0.638	0.031
1	2	21	0.686	5.945	0.036	0.740	0.042
1	2	100	0.775	2.791	0.024	0.227	0.000
1	2	100	0.733	1.875	0.020	0.391	0.092
1	2	100	0.874	2.514	0.040	0.126	0.079
1	3	0	0.106	0.092	0	0.081	0
1	3	0	0.082	0.023	0	0.154	0
1	3	0	0.117	0.158	0.001	0.022	0
1	3	1	0.288	2.281	0.000	0.148	0.029
1	3	1	0.406	1.289	0.000	0.191	0.056
1	3	1	0.312	1.237	0.005	0.962	0.052
1	3	3	0.556	9.318	0.005	0.594	0.085
1	3	3	0.573	8.720	0.007	0.715	0.060
1	3	3	0.792	8.692	0.007	0.749	0.078
1	3	7	0.705	8.730	0.006	0.811	0.091
1	3	7	0.756	7.874	0.005	0.817	0.037
1	3	7	0.786	5.850	0	0.83	0.099
1	3	21	0.726	5.617	0	0.436	0.080
1	3	21	0.469	7.282	0.061	0.588	0.047
1	3	21	0.628	6.201	0	0.595	0.038
1	3	100	0.319	3.287	0.039	0.296	0.019
1	3	100	0.795	5.102	0.020	0.331	0.014
1	3	100	0.769	3.460	0	0.250	0.071
1	4	0	0.023	0.158	0	0.055	0
1	4	0	0.105	0.149	0	0.139	0
1	4	0	0.127	0.041	0.007	0.072	0
1	4	1	0.205	2.255	0.000	0.936	0.060
1	4	1	0.215	1.329	0.009	0.009	0.050
1	4	1	0.342	1.519	0	0.402	0.034
1	4	3	0.774	9.463	0.002	0.822	0.007
1	4	3	0.759	8.517	0.004	0.669	0.008
1	4	3	0.354	8.16	1 0.007	0.553	0.097
1	4	7	0.557	7.642	0.009	0.639	0.098

Table A-17. Cont.

M	T	D	Ace	Lac	Pro	Ethanol	But
1	4	7	0.447	7.877	0	0.859	0.057
1	4	7	0.844	6.509	0.007	0.571	0.015
1	4	21	0.672	6.923	0.028	0.666	0.064
1	4	21	0.776	6.269	0.060	0.694	0.097
1	4	21	0.755	4.335	0	0.090	0.089
1	4	100	0.782	4.263	0.021	0.401	0.065
1	4	100	0.793	4.224	0.02	0.328	0.036
1	4	100	0.637	3.274	0	0.149	0.032
2	1	0	0.040	0.073	0	0.033	0
2	1	0	0.012	0.072	0	0.114	0
2	1	0	0.073	0.104	0	0.341	0
2	1	1	0.291	0.160	0.006	0.269	0.067
2	1	1	0.378	0.213	0.008	0.321	0.078
2	1	1	0.261	0.232	0.009	0.413	0.044
2	1	3	0.440	5.271	0.001	1.465	0.012
2	1	3	0.491	6.422	0.004	1.440	0.012
2	1	3	0.572	6.374	0.001	1.558	0.012
2	1	7	0.281	3.814	0.008	1.099	0.053
2	1	7	0.327	3.484	0	1.400	0.070
2	1	7	0.451	4.170	0.000	2.009	0.012
2	1	21	0.345	4.947	0	1.260	0.082
2	1	21	0.333	4.954	0	1.349	0.080
2	1	21	0.341	4.835	0.033	1.626	0.077
2	1	100	0.149	3.057	0.022	0.622	0.092
2	1	100	0.243	3.431	0.032	0.955	0.054
2	1	100	0.213	3.299	0.030	0.853	0.093
2	2	0	0.073	0.105	0	0.061	0
2	2	0	0.031	0.092	0	0.155	0
2	2	0	0.056	0.056	0	0.020	0
2	2	1	0.290	0.407	0.006	0.399	0.040
2	2	1	0.256	0.321	0.005	0.454	0.027
2	2	1	0.121	0.953	0.005	0.586	0.038
2	2	3	0.573	5.886	0.004	1.285	0.088
2	2	3	0.448	5.818	0.003	1.663	0.070
2	2	3	0.253	6.486	0.003	1.518	0.086
2	2	7	0.328	4.178	0.009	2.904	0.001
2	2	7	0.224	3.323	0	1.181	0.066
2	2	7	0.245	4.857	0	1.668	0.086
2	2	21	0.246	4.400	0.016	1.757	0.082
2	2	21	0.275	3.732	0.021	2.180	0.061
2	2	21	0.149	4.011	0	1.636	0.041
2	2	100	0.366	3.348	0.035	0.727	0.030
2	2	100	0.236	3.716	0.026	0.727	0.022
2	2	100	0.255	3.779	0.023	0.752	0.049
2	3	0	0.049	0.081	0	0.053	0.000
2	3	0	0.032	0.064	0	0.040	0.005
2	3	0	0.042	0.026	0.006	0.140	0.002
2	3	1	0.231	1.679	0.004	0.446	0.032
2	3	1	0.231	1.691	0.008	0.984	0.042
2	3	1	0.216	1.750	0.006	0.804	0.028
2	3	3	0.659	5.954	0.003	1.067	0.043
2	3	3	0.540	4.705	0.004	1.158	0.026
2	3	3	0.569	4.841	0.003	1.995	0.049
2	3	7	0.300	5.386	0	3.149	0.054
2	3	7	0.200	4.883	0	1.006	0.045
2	3	7	0.357	5.615	0	1.270	0.039
2	3	21	0.317	3.751	0	1.130	0.022
2	3	21	0.234	3.661	0.024	1.914	0.049
2	3	21	0.377	4.554	0.008	1.797	0.049
2	3	100	0.202	2.719	0.015	0.312	0.017
2	3	100	0.217	3.044	0.020	0.275	0.051
2	3	100	0.263	3.434	0.069	0.956	0.051
2	4	0	0.042	0.044	0	0	0.001
2	4	0	0.050	0.278	0	0.087	0.005
2	4	0	0.087	0.305	0	0.088	0.006
2	4	1	0.217	1.675	0.004	0.419	0.004

Table A-17 Cont.

M	T	D	Ace	Lac	Pro	Ethanol	But
2	4	1	0.257	2.246	0.008	0.605	0.064
2	4	1	0.326	2.591	0.006	0.942	0.066
2	4	3	0.428	5.296	0	1.256	0.048
2	4	3	0.550	6.046	0.005	1.256	0.057
2	4	3	0.591	5.674	0.002	1.319	0.047
2	4	7	0.300	4.716	0	1.777	0.077
2	4	7	0.451	4.828	0	2.507	0.050
2	4	7	0.334	5.023	0	2.316	0.073
2	4	21	0.375	4.778	0	2.176	0.057
2	4	21	0.240	3.825	0.032	2.049	0.064
2	4	21	0.234	4.118	0.056	2.742	0.055
2	4	100	0.243	3.489	0.047	0.258	0.060
2	4	100	0.260	3.355	0.012	0.445	0.070
2	4	100	0.279	3.592	0.046	0.589	0.068

Abbreviations:

M = stage of maturity (1 = 90 d; 2 = 110 d), T = treatment (1, control; 2, enzyme, 3, inoculant, 4 E+I), Ace = acetic acid, Lac = lactic acid, Pro = propionic acid, But = butyric acid

Table A-18. Data used for analysis of water soluble carbohydrate contents (g/100 g DM) in forage sorghum ensiled at 110 d in a tropical environment. (Chapter 4)

Trt	Day	Glucose	Xylose	Gal.	Arab.	Fructose
1	0	7.8261	0	0.08695	0	4.0159
1	0	6.0558	0.08718	0.09311	0	3.7341
1	0	7.6661	0.08853	0.1035	0	3.5784
1	1	1.0613	0.11393	0.70957	0	1.3909
1	1	2.0933	0.12298	0.43655	0	1.1712
1	1	0.8459	0.07888	0	0	1.29
1	3	0.4969	0.13959	0	0	0.6536
1	3	0.3582	0.04005	0	0	0.5954
1	3	0.5136	0.15404	0	0.0368	0.5404
1	7	0.6029	0.09022	0.83233	0	0.8222
1	7	0.209	0.13351	0	0	0.5304
1	7	0.4111	0.15249	0	0	0.6916
1	21	0.9877	0.21583	1.06147	0	0.2308
1	21	0.6322	0.26912	2.69327	0.1672	0.0446
1	21	1.1636	0.24791	1.35543	0	0.4243
1	100	0.9394	0.25088	1.44644	0.1717	0.6985
1	100	1.0462	0.3742	0	0.1652	0.7971
1	100	0.9743	0.30471	0.75067	0.1704	0.7157
2	0	6.1417	0.08266	0.13144	0	3.3322
2	0	7.9579	0.12068	0.18033	0	3.6954
2	0	7.6108	0.0751	0.12715	0	3.4077
2	1	1.191	0.07942	0.53746	0	0.9598
2	1	0.6861	0.10798	1.13358	0	1.6856
2	1	0.8609	0.14148	0	0	2.367
2	3	0.615	0.07179	0	0	0.7287
2	3	0.5872	0.10609	0.9068	0	0.6315
2	3	0.4928	0.10123	0.77242	0	0.5739
2	7	0.4935	0.1167	1.4578	0.0548	0.8982
2	7	0.4428	0.1063	1.52004	0.0546	0.6837
2	7	0.7362	0.1194	0	0	0.5604
2	21	0.2926	0.07219	1.50041	0.0512	0.549
2	21	0.604	0.09995	1.11424	0.0756	0.7973
2	21	0.4918	0.08408	1.14652	0.0622	0.6746
2	100	1.3139	0.34158	2.16859	0.2076	0.249
2	100	0.2713	0.35914	2.4512	0.2332	0.576
2	100	0.9302	0.32132	1.92781	0.1428	0.302
3	0	7.9415	0.0599	0.0711	0	3.1258
3	0	7.8935	0.04025	0.03363	0	3.1321
3	0	6.058	0.02418	0	0	3.8616
3	1	0.6981	0.09171	0	0	1.9768
3	1	0.7027	0.13446	1.32984	0	1.1043
3	1	0.6989	0.1402	0	0	1.3897
3	3	0.4515	0.13209	0	0	1.9556
3	3	0.5166	0.14486	0	0	0.9651
3	3	0.5003	0.18038	0	0.1055	1.117
3	7	0.5978	0.1951	0	0.0989	0.9746
3	7	0.423	0.12669	0	0	0.8124
3	7	0.5918	0.14438	1.67299	0.0602	1.0666
3	21	0.4146	0.12068	1.36911	0.0682	0.7348
3	21	0.7153	0.12831	1.6349	0.0616	0.6387
3	21	0.44	0.06942	0.67637	0	0.7854
3	100	0.4655	0.1949	2.05811	0.1072	0.2218
3	100	0.4771	0.19476	1.82924	0.1207	0.2656
3	100	0.4165	0.14661	0	0.1359	0.4906
4	0	6.314	0	0.1134	0	5.8289
4	0	7.8375	0.07442	0.11135	0	4.5156
4	0	7.7864	0.05747	0.07703	0	1.1799
4	1	0.895	0.08745	0.94905	0	2.0904

Table A-18. Cont.

Trt	Day	Glucose	Xylose	Gal.	Arab.	Fructose
4	1	0.9031	0.07253	0.36374	0	1.2148
4	1	0.651	0.11291	1.36638	0.0788	1.4664
4	3	0.252	0.04579	0	0	1.5516
4	3	0.3144	0.09839	0	0	1.484
4	3	0.5664	0.0938	0.88732	0.0619	1.5858
4	7	0.9247	0.09704	0.50869	0.1041	1.3533
4	7	0.8458	0.09644	0.35011	0.0784	0.9655
4	7	0.4869	0.11467	1.40683	0.0879	0.8023
4	21	1.0521	0.10569	0.48079	0.1079	0.967
4	21	0.8016	0.12676	0.75109	0.0988	1.1764
4	21	0.2073	0.0961	0.8332	0.1137	0.9337
4	100	0.4317	0.15012	1.72003	0.1314	0.1849
4	100	0.54	0.17538	1.4679	0.1524	0.1395
4	100	0.3364	0.18889	1.49125	0.1644	0.1448

Abbreviations:
Day = Day of ensiling
Trt = treatment , 1 = control, 2 = enzyme, 3 = inoculant, 4 = E+I; Gal = galactose, Arab = arabinose

Table A-19. Data used for analysis of structural carbohydrate content (g/100 g DM) in forgae sorghum ensiled at two stages of maturity in a tropical environment (Chapter 4)

Maturity	Trt	Day	NDF	ADF	Hemi	Cellulose
90	1	0	66.573	40.136	26.438	32.148
90	1	0	65.369	39.992	26.377	31.671
90	1	0	67.344	41.387	25.457	34.547
90	2	0	64.533	38.054	26.479	28.980
90	2	0	64.894	38.774	26.121	32.030
90	2	0	65.716	38.838	26.878	31.857
90	3	0	63.187	37.333	25.855	29.774
90	3	0	65.045	38.882	26.164	31.438
90	3	0	66.867	38.875	27.993	31.711
90	4	0	66.934	39.958	26.977	32.977
90	4	0	66.140	40.958	25.183	33.512
90	4	0	67.553	42.268	25.286	36.410
90	1	100	64.762	42.119	23.644	34.406
90	1	100	57.851	43.435	24.416	33.355
90	1	100	63.088	40.817	24.271	34.183
90	2	100	61.792	39.248	22.545	32.950
90	2	100	62.834	38.946	23.889	32.411
90	2	100	63.731	39.733	23.998	33.520
90	3	100	64.981	40.256	24.725	33.918
90	3	100	64.066	39.386	24.681	31.718
90	3	100	63.704	39.269	24.435	31.466
90	4	100	63.294	39.053	24.241	33.265
90	4	100	62.668	38.243	24.426	32.378
90	4	100	63.768	38.778	24.990	32.029
110	1	0	64.47	35.95	28.52	31.22
110	1	0	67.39	38.62	28.77	38.62
110	1	0	66.54	37.85	28.69	37.85
110	2	0	71.09	42.22	28.89	42.2
110	2	0	69.81	41.05	28.76	41.05
110	2	0	66.66	38.34	28.32	38.34
110	3	0	66.74	38.36	28.37	38.36
110	3	0	66.14	37.74	28.41	37.74
110	3	0	69.07	38.20	30.88	38.2
110	4	0	69.24	38.04	31.2	38.04
110	4	0	66.77	37.92	28.85	37.92
110	4	0	72.43	45.29	27.14	45.29
110	1	100	64.2	39.46	24.75	39.46
110	1	100	69.62	44.21	25.41	44.21
110	1	100	64.72	39.82	24.9	39.82
110	2	100	61.82	38.07	23.75	38.07
110	2	100	60.1	35.96	24.14	35.96
110	2	100	65.54	43.48	22.06	43.48
110	3	100	64.1	42.82	21.28	42.82
110	3	100	62.15	37.43	24.72	37.43
110	3	100	65.91	40.52	25.39	30.52
110	4	100	61.79	37.22	24.57	37.22
110	4	100	62.46	34.77	27.69	34.77
110	4	100	63.15	33.96	29.19	33.96

Abbreviations:
Trt = treatment; 1 = control, 2 = enzyme, 3 = inoculant, 4 = E +I,
Day = day of ensiling
Hemi = hemicellulose

Table A-20. Data used for analysis of pH and temperature in forage sorghum ensiled at two stages of maturity and exposed to air in a tropical environment. (Chapter 4)

Trt	Day	pH	Tem
1	0	4.19	30
1	0	4.14	28.888
1	1	.	29.444
1	1	.	28.888
1	2	.	28.888
1	2	.	30
1	3	4.3	31.111
1	3	4.25	30.555
1	4	.	30
1	4	.	28.888
1	5	.	30
1	5	.	29.444
1	6	.	28.888
1	6	.	28.888
1	7	4.45	28.888
1	7	4.4	28.888
2	0	4.11	29.444
2	0	4.09	29.444
2	1	.	28.888
2	1	.	30
2	2	.	31.111
2	2	.	30
2	3	4.3	32.222
2	3	4.25	31.111
2	4	.	31.111
2	4	.	30
2	5	.	29.444
2	5	.	29.444
2	6	.	28.888
2	6	.	28.888
2	7	4.45	29.444
2	7	4.65	28.888
3	0	4.14	28.888
3	0	4.12	28.888
3	1	.	31.111
3	1	.	34.444
3	2	.	35.555
3	2	.	34.444
3	3	5.7	35.555
3	3	6.2	33.333
3	4	.	31.111
3	4	.	32.222
3	5	.	31.111
3	5	.	31.111
3	6	.	30
3	6	.	31.111
3	7	6	30
3	7	6	28.888
4	0	4.14	29.444
4	0	4.06	29.444
4	1	.	30.555
4	1	.	31.111
4	2	.	36.666
4	2	.	34.444
4	3	5.6	35.555
4	3	5.95	34.444
4	4	.	34.444
4	4	.	34.444
4	5	.	33.333
4	5	.	32.222
4	6	.	31.111
4	6	.	30.555

Table A-20. Cont.

Trt	Day	pH	Tem
4	7	5.9	31.111
4	7	6.05	30

Abbreviations:
Trt = treatment, 1 = control, 2 = enzyme, 3 = inoculant, 4 = E+I
(.) No determined
Tem = temperature

Table A-21. Data used for analysis of fermentation end-products in forage sorghum ensiled at two stages of maturity and exposed to air in a tropical environment (Chapter 4)

M	T	D	Lac	Ace	Prop	Ethanol	But
90	1	0	2.902	0.714	0.020	0.020	0.053
90	1	0	2.588	0.803	0.040	0.283	0.076
90	1	3	2.291	0.429	0.003	0.225	0.030
90	1	3	1.432	0.077	0	0.064	0.019
90	1	7	0.948	0.292	0.006	0.590	0.015
90	1	7	0.971	0.299	0	0.568	0.021
90	2	0	2.791	0.775	0.024	0.227	0.000
90	2	0	1.875	0.733	0.020	0.396	0.092
90	2	3	2.617	0.602	0.006	0.861	0.049
90	2	3	2.265	0.597	0.001	0.525	0.054
90	2	7	1.611	0.604	0	0.560	0.044
90	2	7	1.407	0.258	0.002	0.365	0.031
90	3	0	3.287	0.319	0.039	0.296	0.019
90	3	0	5.102	0.795	0.020	0.331	0.014
90	3	3	0.664	0.403	0.010	0.524	0.043
90	3	3	0.907	0.281	0.009	0.516	0.031
90	3	7	0.260	0.301	0	0.437	0.045
90	3	7	0.115	0.282	0	0.172	0.041
90	4	0	4.263	0.782	0.021	0.401	0.065
90	4	0	4.224	0.793	0.020	0.328	0.036
90	4	3	0.553	0.374	0.010	0.430	0.189
90	4	3	0.603	0.430	0.008	0.331	0.121
90	4	7	0.056	0.337	0.005	0.362	0.170
90	4	7	1.375	0.284	0.001	1.157	0.061
110	1	0	3.057	0.149	0.022	0.622	0.092
110	1	0	3.431	0.243	0.032	0.955	0.054
110	1	3	2.140	0.086	0.001	0.168	0.094
110	1	3	2.048	0.165	0.001	0.251	0.034
110	1	7	1.143	0.072	0.001	0.164	0.048
110	1	7	2.267	0.165	0.001	0.239	0.067
110	2	0	3.348	0.366	0.035	0.727	0.030
110	2	0	3.716	0.236	0.026	0.727	0.022
110	2	3	1.872	0.069	0.002	0.215	0.061
110	2	3	2.202	0.095	0.001	0.083	0.107
110	2	7	2.503	0.144	0.001	0.185	0.025
110	2	7	0.325	0.033	0.003	0.156	0.028
110	3	0	2.719	0.202	0.015	0.312	0.017
110	3	0	3.044	0.217	0.020	0.275	0.051
110	3	3	0.487	0.136	0.002	0.422	0.048
110	3	3	0.092	0.163	0.004	0.090	0.041
110	3	7	3.121	0.122	0.002	0.141	0.063
110	3	7	0.076	0.102	0.002	0.184	0.065
110	4	0	3.489	0.243	0.047	0.258	0.060
110	4	0	3.555	0.260	0.012	0.445	0.070
110	4	3	2.921	0.114	0.001	0.072	0.087
110	4	3	0.207	0.147	0.001	0.068	0.027
110	4	7	0.321	0.044	0.007	0.134	0.082
110	4	7	0.064	0.105	0.001	0.141	0.060

Abbreviations:
M =stage of maturity, T = treatment, D = day of aerobic exposure, Lac = lactic acid, Ace =acetic acid, Pro = propionic, But = buturyc acid.

Table A-22. Data used for analysis of water soluble carbohydrate contents (g/100 g DM) in forage sorghum ensiled at two stages of maturity and exposed to air in a tropical environment. (Chapter 4)

M	T	D	Glucose	Xylose	Galactose	Arabinose	Fructose
90	1	0	0.2896	0.1704	0.086	0.0427	0.1012
90	1	0	0.4211	0.2432	0.2264	0.098	0.0718
90	1	3	0.6677	0.1102	0.122	0.1085	0.1519
90	1	3	0.8788	0.3453	0.3824	0.1552	0.2173
90	1	7	0.7103	0.2937	0.3252	0.1495	0.2093
90	1	7	0.5495	0.1374	0.1522	0.0139	0.0194
90	2	0	0.2121	0.1628	0.1275	0.0862	0.0768
90	2	0	0.3327	0.1979	0.1486	0.1441	0.0686
90	2	3	0.5905	0.1741	0.1928	0.0674	0.0944
90	2	3	0.0633	0.1723	0.1908	0.0277	0.0387
90	2	7	0.6491	0.0994	0.11	0.0816	0.1142
90	2	7	0.1902	0.3245	0.3594	0	0
90	3	0	0.3761	0.1038	0.0919	0.0884	0.3964
90	3	0	0.2575	0.1537	0.1258	0.1209	0.1084
90	3	3	0.2532	0.1731	0.1917	0.2339	0.3276
90	3	3	0.222	0.1708	0.1892	0.0938	0.1314
90	3	7	0.112	0	0	0.0933	0.1307
90	3	7	0.2087	0.1098	0.1216	0	0
90	4	0	0.2904	0.3121	0.1866	0.1750	0.2200
90	4	0	0.1489	0.2048	0.1594	0.1580	0.2088
90	4	3	0.6744	0.1339	0.1482	0.0883	0.1236
90	4	3	0	0.2868	0.0928	0	0
90	4	7	0.2954	0.0100	0	0.0404	0.0566
90	4	7	0.1211	0.0925	0.1024	0.0279	0.0391
110	1	0	0.9391	0.2508	0.000	0.167	0.7971
110	1	0	0.9743	0.3700	0.7506	0.1704	0.7157
110	1	3	0.5561	0.0652	0.0722	0.1009	0.3433
110	1	3	0.5844	0.035	0.0388	0.1057	0.3465
110	1	7	0.5403	0	0	0.1032	0.2122
110	1	7	0.6236	0.0536	0.0594	0.1326	0.3139
110	2	0	1.3139	0.3415	1.9270	0.2076	0.249
110	2	0	0.2713	0.3591	2.1680	0.2332	0.576
110	2	3	0.8219	0.0706	0.0782	0.1411	0.178
110	2	3	0.7521	0.0808	0.0894	0.1367	0.1055
110	2	7	0.8018	0	0	0.1859	0.0658
110	2	7	0.6557	0	0	0.0747	0
110	3	0	0.465	0.1949	1.058	0.1072	0.2218
110	3	0	0.4771	0.1947	1.229	0.1207	0.2650
110	3	3	0.4634	0	0	0	0.1008
110	3	3	0.4491	0	0	0.0758	0.0475
110	3	7	0.5113	0	0	0	0
110	3	7	0.7726	0.075	0.0831	0.1149	0.106
110	4	0	0.4317	0.1502	1.020	0.1524	0.1395
110	4	0	0.5400	0.1754	1.467	0.1644	0.1448
110	4	3	0.487	0	0	0.0232	0.0133
110	4	3	0.4784	0	0	0	0
110	4	7	0.5066	0	0	0.0561	0
110	4	7	0.5653	0.0631	0.0698	0.1124	0.0869

Abbreviations:
M = stage of maturity, T = treatment, d = day of aerobic exposure

Table A-23. Data used for analysis of pH and lactic acid-producing bacterial populations in Johnson grass ensiled at two regrowth periods. (Chapter 5).

R	Trt	D	pH	LAB
1	1	0	5.85	4.25
1	1	0	5.66	3.73
1	1	0	5.66	5
1	1	1	4.72	6.82
1	1	1	4.79	7.25
1	1	1	4.97	7.61
1	1	3	5.03	7.58
1	1	3	4.77	7.51
1	1	3	5.1	7.81
1	1	7	5.17	7.06
1	1	7	4.79	7.13
1	1	7	4.8	6.93
1	1	14	4.86	7.14
1	1	14	4.91	7.05
1	1	14	4.85	6.91
1	1	21	4.57	7.12
1	1	21	4.71	6.94
1	1	21	4.7	7.28
1	1	100	4.81	7.02
1	1	100	4.92	6.69
1	1	100	4.73	7.36
1	2	0	5.7	4.65
1	2	0	5.63	5.66
1	2	0	5.69	5.15
1	2	1	4.83	8.73
1	2	1	4.52	9.09
1	2	1	4.46	9.01
1	2	3	4.42	8.33
1	2	3	4.45	8.35
1	2	3	4.47	8.59
1	2	7	4.41	7.44
1	2	7	4.4	7.43
1	2	7	4.29	7.49
1	2	14	4.65	7.07
1	2	14	4.68	7.21
1	2	14	4.71	7.01
1	2	21	4.75	7.49
1	2	21	4.66	7.43
1	2	21	4.66	7.19
1	2	100	3.85	6.57
1	2	100	4.8	6.58
1	2	100	4.78	6.69
2	1	0	5.67	3.82
2	1	0	5.97	3.38
2	1	0	5.9	4.66
2	1	1	5.22	7.04
2	1	1	5.57	6.91
2	1	1	5.54	6.82
2	1	3	5.6	7.03
2	1	3	5.66	7.11
2	1	3	5.49	7.07
2	1	7	5.46	7.09
2	1	7	5.15	8.08
2	1	7	5.41	7.81
2	1	14	5.28	7.31
2	1	14	5.25	6.94
2	1	14	5.24	7.31

Table A-23. Cont.

R	Trt	D	pH	LAB
2	1	21	5.72	7.21
2	1	21	4.71	7.41
2	1	21	4.82	7.6
2	1	100	5.03	6.53
2	1	100	4.97	6.38
2	1	100	4.93	6.36
2	2	0	5.88	5.44
2	2	0	5.85	5.21
2	2	0	5.84	5.49
2	2	1	4.55	8.64
2	2	1	4.33	8.66
2	2	1	4.71	8.76
2	2	3	4.64	7.47
2	2	3	4.48	7.53
2	2	3	5	7.43
2	2	7	4.76	7.97
2	2	7	4.4	7.98
2	2	7	4.42	7.9
2	2	14	4.43	6.1
2	2	14	4.36	5.9
2	2	14	4.42	6.26
2	2	21	4.14	6.71
2	2	21	3.93	5.64
2	2	21	3.98	6.36
2	2	100	5.06	6.42
2	2	100	5.15	6.42
2	2	100	4.99	6.22

Abbreviations:
R = regrowth period, 1 = 45 d, 2 = 110 d; Trt = treatment, 1 = control, 2 = E+I,
LAB = lactic acid-producing bacteria

Table A-24. Data used for analysis of fermentation end-products (g/ 100 g DM) in Johnson grass ensiled at two regrowth periods (Chapter 5).

R	T	D	Lac	Ace	Etoh	But
45	1	0	0	0.01509	0	0
45	1	0	0	0	0	0
45	1	0	0	0	0.00572	0
45	1	1	0.613	0.04267	0	0.004
45	1	1	0.9223	0.18481	0.02726	0.0608
45	1	1	0.3118	0.19531	0.06762	0.0641
45	1	3	0.1499	0	0	0.0058
45	1	3	0.3537	0.12772	0.01838	0
45	1	3	0.2383	0	0.07088	0
45	1	7	0.1951	0.77733	0.12153	0.5051
45	1	7	0.2421	0	0.03043	0.202
45	1	7	0	0	0.04643	0
45	1	14	0.1045	0.82274	0.07765	0.9339
45	1	14	0.0564	0.51161	0.05514	0.5099
45	1	14	0.2745	0.54122	0.04571	0.613
45	1	21	0.0728	0.70212	0.04748	0.6906
45	1	21	0	0	0.03535	0
45	1	21	0.0622	0.66344	0.06718	0.1095
45	1	100	0.0519	0.60176	0.0656	0.08
45	1	100	0.0543	0.63829	0.06507	0.1727
45	1	100	0.0482	0.61056	0.05056	0
45	2	0	0	0.01649	0	0
45	2	0	0	0	0	0
45	2	0	0	0	0	0
45	2	1	0.3803	0.12979	0.06437	0
45	2	1	0.9992	0.12291	0.04027	0.0174
45	2	1	0	0	0	0
45	2	3	1.7788	0.27067	0.05012	0.045
45	2	3	1.4212	0.27481	0.05795	0.0408
45	2	3	1.4809	0.25085	0.04916	0.0361
45	2	7	0.7916	0.28494	0.05786	0.0459
45	2	7	0.7668	0.27533	0.06067	0.0453
45	2	7	0.8073	0.29826	0.03983	0.0341
45	2	14	0.7649	0.32362	0.04924	0.0223
45	2	14	0.7676	0.30491	0.05812	0.02
45	2	14	0.968	0.34403	0.06876	0.0427
45	2	21	0.7265	0.49882	0.35174	0.0462
45	2	21	0.6421	0.52174	0.11396	0.031
45	2	21	0.6802	0.46946	0.04291	0.0427
45	2	100	0.2704	0.54171	0.03509	0.0299
45	2	100	0.3072	0.48758	0.03764	0.0227
45	2	100	0.2846	0.48351	0.04019	0.0208
110	1	0	0	0	0	0
110	1	0	0	0.1014	0	0
110	1	0	0.3896	0.0336	0	0
110	1	1	0.6888	0.2383	0.04664	0
110	1	1	0.5022	0.2021	0.12588	0
110	1	1	0.4012	0.0872	0.08768	0.0609
110	1	3	0.0251	0.0433	0.02156	0.0223
110	1	3	0.3045	0.1346	0.17712	0.1227
110	1	3	0.3279	0.0279	0.19684	0.0501
110	1	7	0.206	0.0448	0.15547	0.0305
110	1	7	0.2391	0.0734	0.03828	0
110	1	7	0.2623	0.0819	0.09286	0
110	1	14	0.902	0.1467	0.09529	0.175
110	1	14	0.8231	0.1552	0.08367	0.1414
110	1	14	0.821	0.1504	0.09136	0.1427
110	1	21	0.271	0.0746	0.07213	0.0232
110	1	21	0.2386	0.0381	0.05299	0.0221
110	1	21	0.2259	0.0473	0.06511	0.0216
110	1	100	0.157	0.0366	0.0224	0.0114
110	1	100	0.1331	0.0162	0.02775	0.0223
110	1	100	0.1086	0.0249	0.02608	0.0213

Table A-24. Cont.

R	T	D	Lac	Ace	Etoh	But
110	2	0	0.0243	0	0	0
110	2	0	0.0177	0.0187	0	0
110	2	0	0.0395	0.0154	0	0
110	2	1	0.3919	0.092	0	0
110	2	1	0.9783	0.1	0.01897	0
110	2	1	0.8589	0.1028	0.05985	0.0515
110	2	3	1.1425	0.1657	0.08133	0.0808
110	2	3	1.4149	0.1261	0.07105	0.0722
110	2	3	1.1598	0.1295	0.07105	0.0678
110	2	7	0.353	0	0	0.0112
110	2	7	0.8939	0.1179	0.07982	0.0325
110	2	7	1.357	0.1645	0.07046	0.0432
110	2	14	1.3928	0.1163	0.04313	0.0431
110	2	14	0.9681	0.089	0.07347	0.0321
110	2	14	1.1379	0.0666	0.05792	0.0269
110	2	21	0.6915	0.0686	0.03318	0.0066
110	2	21	0.6374	0.0675	0.04656	0
110	2	21	0.4089	0.0433	0.0489	0.0193
110	2	100	0.2079	0.0374	0.03143	0.0141
110	2	100	0.157	0.0549	0.03912	0.0131
110	2	100	0.0967	0.0243	0.05274	0.0135

Abbreviations:
R = regrowth period, T = treatment (1 = control; 2 = enxyme plus inoculant), D = day of ensiling, Lac = lactic acid, Ace = acetic acid, Etoh = ethanol, But = butiryc acid.

Table A-25. Data used for analysis of water soluble carbohydrates in Johnson grass ensiled at two regrowth periods in a tropical environment. (Chapter 5)

R	T	D	Glucose	Xylose	Arabinose	Fructose
45	1	0	0.679	0.85489	0.03705	0.01044
45	1	0	0.7453	0.92328	0.05238	0.02057
45	1	0	0.7865	0.72812	0	0.03936
45	1	1	0.213	0.66915	0	0
45	1	1	0.1116	0.31378	0	0.03593
45	1	1	0.0226	0.31378	0	0.06634
45	1	3	0.0826	0.09773	0	0.10182
45	1	3	0.0159	0	0	0.08691
45	1	3	0.0728	0.09997	0	0.03518
45	1	7	0.674	0	0	0.09123
45	1	7	0.1974	0	0	0.04085
45	1	7	0	0.10886	0	0.02668
45	1	14	0.1324	0.14459	0	0.09287
45	1	14	0.1776	0.14371	0	0.05531
45	1	14	0.5199	0.15396	0	0.01953
45	1	21	0.203	0	0	0.03682
45	1	21	0	0	0.12221	0.09347
45	1	21	0.0234	0	0	0.02967
45	1	100	0.0468	0	0.1006	0.01923
45	1	100	0.0395	0.00439	0.04855	0.03131
45	1	100	0.0212	0.01211	0.05089	0.0161
45	2	0	0.8388	0.9566	0	0.14595
45	2	0	0.6925	0.87359	0	0.05024
45	2	0	0.5914	0.7564	0	0
45	2	1	0.1335	0.16597	0	0.05859
45	2	1	0.1276	0.14791	0	0.04636
45	2	1	0.1578	0.15796	0	0.089
45	2	3	0.1248	0.14176	0	0.11434
45	2	3	0.1202	0.1523	0	0.04472
45	2	3	0.152	0	0	0.06351
45	2	7	0	0.12282	0	0
45	2	7	0.1293	0.15191	0	0.08005
45	2	7	0.0144	0	0	0.08527
45	2	14	0.135	0.15699	0	0.11852
45	2	14	0.1706	0.18325	0	0.06679
45	2	14	0	0.13824	0	0.06917
45	2	21	0.1604	0.16265	0	0.11911
45	2	21	0.1968	0.14781	0	0.08512
45	2	21	0.0818	0.12155	0.04855	0.04785
45	2	100	0.0876	0.14215	0.05451	0.04293
45	2	100	0.0892	0.12887	0.07314	0.03339
45	2	100	0.0846	0.10778	0.05823	0.04651
110	1	0	0.086	0.09837	0	0.17899
110	1	0	0.1897	0.1151	0	0.07976
110	1	0	0.190	0.1024	0	0.07529
110	1	1	0.1229	0	0	0.09477
110	1	1	0.1048	0.11359	0	0.09061
110	1	1	0.0807	0.08042	0	0.11848
110	1	3	0.1005	0.10376	0.07032	0.07968
110	1	3	0	0.10386	0	0.04342
110	1	3	0.1093	0.08395	0	0.04404
110	1	7	0.0695	0.07401	0.04728	0.04542
110	1	7	0.0434	0	0	0.0214
110	1	7	0.0211	0	0.05058	0.02695
110	1	14	0.0097	0	0.06938	0.04304
110	1	14	0	0.14425	0	0.07344
110	1	14	0.0931	0.12766	0	0.02217
110	1	21	0	0.17011	0.04569	0.06998
110	1	21	0	0.08803	0	0.0398
110	1	21	0.0085	0	0	0.04542
110	1	100	0.0063	0	0	0.04219
110	1	100	0.0071	0	0	0.03526
110	1	100	0.0073	0	0	0.04712
110	2	0	0.212	0	0	0.19855

Table A-25. Cont.

R	T	D	Glucose	Xylose	Arabinose	Fructose
110	2	o	0.1831	0	0	0.07421
110	2	0	0.031	0.11803	0.06344	0.07999
110	2	1	0.0725	0.08506	0	0.06759
110	2	1	0.0816	0.10043	0	0.08345
110	2	1	0.0273	0.09373	0	0.09192
110	2	3	0.0961	0.13043	0	0.05581
110	2	3	0	0.06398	0	0.08884
110	2	3	0.0976	0.07835	0	0.05112
110	2	7	0.0156	0.09811	0	0.03534
110	2	7	0.0296	0.07149	0	0.06582
110	2	7	0.1479	0.16487	0	0.08399
110	2	14	0.0793	0.12499	0	0.07114
110	2	14	0.1042	0.08072	0	0.07368
110	2	14	0.1113	0.15418	0.07301	0.08291
110	2	21	0.0806	0.09816	0	0.06475
110	2	21	0.0851	0.10013	0	0.0689
110	2	21	0.1681	0.15831	0.07961	0.08345
110	2	100	0.0854	0.18013	0.1171	0.16398
110	2	100	0.0877	0.13131	0.10507	0.14714
110	2	100	0.0876	0.14234	0.08996	0.14088

Abbreviations;
R = regrowth period, T = treatment (1 = control, 2 = enzyme plus inoculant), D = day of ensiling

Table A-26. Data used for analysis of structural carbohydrates in Johnson grass ensiled at two regrowth periods in a tropical environment. (Chapter 5)

R	Trt	D	NDF	Hemi.	ADF	Cellulose
45	1	0	70.110	32.909	37.20091	33.137
45	1	0	68.626	31.262	37.36405	32.976
45	1	0	68.812	29.818	38.99383	32.517
45	2	0	66.415	27.823	38.59208	33.479
45	2	0	66.713	28.669	38.04413	33.691
45	2	0	68.281	28.621	39.65911	34.608
45	1	100	65.065	24.514	40.55104	32.214
45	1	100	68.101	25.192	42.90930	36.241
45	1	100	67.023	24.464	42.55888	36.170
45	2	100	66.124	26.190	39.93418	32.752
45	2	100	66.700	25.629	41.07142	35.125
45	2	100	66.058	26.089	39.96859	29.367
110	1	0	72.055	33.625	38.42934	32.788
110	1	0	70.675	29.361	41.31316	35.263
110	1	0	70.552	31.049	39.50252	33.305
110	2	0	69.595	27.616	41.97883	35.578
110	2	0	68.575	27.089	41.48601	35.167
110	2	0	69.967	27.245	42.72113	33.996
110	1	100	70.676	27.015	43.66095	31.073
110	1	100	69.609	25.374	44.23488	34.843
110	1	100	71.425	26.400	45.02492	37.427
110	2	100	62.408	23.154	39.25438	28.490
110	2	100	76.998	28.920	48.07809	37.787
110	2	100	70.422	27.121	43.30083	35.915

Abbreviations:
R = regrowth period (days), Trt = treatment, 1 = control, 2 = E+I; D = day of ensiling; Hemi. = hemicellulose

Table A-27. Data used for statistics analysis in Chapter 6.

FS	Eap	Rep	NDFD	ADFD	HCD	CELD
J45	0	1	6.004	0.381	6.386	.5012
J45	0	2	7.553	0.086	7.465	1.234
J45	0	3	6.179	0.438	6.618	.2479
J45	1	1	8.374	0.783	7.590	1.224
J45	1	2	5.805	4.433	6.430	3.971
J45	1	3	7.861	5.795	6.060	6.836
J45	4	1	7.580	4.871	4.452	5.504
J45	4	2	7.216	4.487	6.729	2.607
J45	4	3	11.76	3.174	8.593	4.525
J45	8	1	11.59	3.574	8.018	4.495
J45	8	2	16.52	6.791	9.732	7.795
J45	8	3	17.18	6.998	10.19	8.022
J110	0	1	3.623	0.441	4.064	.0003
J110	O	2	4.082	0.038	4.121	.0004
J110	0	3	4.263	0.459	4.714	.1173
J110	1	1	4.309	0.965	5.275	.3116
J110	1	2	4.721	0.280	5.002	1.074
J110	1	3	4.779	0.719	5.498	.3666
J110	4	1	8.440	0.443	7.997	1.363
J110	4	2	5.435	1.557	7.010	.1325
J110	4	3	6.764	0.710	7.474	.3769
J110	8	1	11.12	1.929	9.191	2.360
J110	8	2	8.375	0.242	8.132	.6610
J110	8	3	7.993	0.245	8.238	.6444
FS90	0	1	3.833	0.872	4.706	.0005
FS90	0	2	3.679	0.305	4.977	.0004
FS90	0	3	3.757	0.232	4.989	.0003
FS90	1	1	7.499	1.118	6.381	.7459
FS90	1	2	6.729	0.494	6.235	.5112
FS90	1	3	7.172	0.817	6.354	.6860
FS90	4	1	9.127	2.191	6.936	2.197
FS90	4	2	8.075	1.988	6.086	1.476
FS90	4	3	7.395	2.085	5.309	1.941
FS90	8	1	10.76	3.438	7.330	3.401
FS90	8	2	11.78	4.518	7.264	4.304
FS90	8	3	10.20	3.761	6.447	3.867

Abbreviations:
FS = forage Specie; J45 - Johnson grass harvested at 45 d of regrowth, J110 - johnson grass harvested at 110 d of regrowth, FS90 - forage sorghum harvested at 90 d of growth.
Ear = enzyme application rate. Multiples of rate recommended by manufacturer
Rep = Replicate
NDFD = NDF disappearance
ADFD = ADF disappearance
HCD = hemicellulose disappearance
CELD = cellulose disappearance

Table A-28. Data used for analysis in chapter 7 (Exp. 1)

Enzyme application rate	Forage preparation	NDF-Disappearance [a]
0	D	6.64
0	D	5.17
0	D	6.91
.5	D	8.45
.5	D	7.69
.5	D	7.08
1	D	9.10
1	D	10.39
1	D	10.85
4	D	11.66
4	D	11.06
4	F	10.29
0	F	3.27
0	F	4.50
0	F	2.73
.5	F	3.55
.5	F	5.30
.5	F	4.08
1	F	4.18
1	F	5.50
1	F	4.50
4	F	4.70
4	F	4.83
4	F	8.19

Abbreviations:
D = Dry
F = Freeze-dried
a = g/100 g DM

Table A-29 Data used for analysis in Chapter 7 (Exp. 2)

pH	Enzyme Aplication Rate [a]	NDFD[b]
3.5	0	3.601
3.5	0	4.202
3.5	0	3.018
3.5	1	8.843
3.5	1	8.914
3.5	1	9.486
3.5	2	9.349
3.5	2	10.38
3.5	2	11.24
3.5	4	10.11
3.5	4	10.54
3.5	4	10.70
3.5	8	11.60
3.5	8	13.26
3.5	8	9.572
4.5	0	6.735
4.5	0	5.386
4.5	0	8.433
4.5	1	10.94
4.5	1	10.78
4.5	1	13.34
4.5	2	11.16
4.5	2	12.52
4.5	2	11.51
4.5	4	10.23
4.5	4	5.407
4.5	4	11.53
4.5	8	10.74
4.5	8	11.13
4.5	8	10.93
5.5	0	6.726
5.5	0	6.181
5.5	0	6.350
5.5	1	11.64
5.5	1	10.70
5.5	1	12.31
5.5	2	11.92
5.5	2	11.13
5.5	2	10.49
5.5	4	11.54
5.5	4	11.45
5.5	4	11.70
5.5	8	11.30
5.5	8	11.35
5.5	8	10.94

NDFD = NDF disappearance
a = multiples of rate recommended by manufacturer
b = NDF disappearance (g/100 g DM)

Table A-30. Data used for analysis in Chapter 7 (Exp. 3)

Ratio	Enzyme Aplication rate (g/ton DM)	NDFD
2:1	0	4.772
2:1	0	3.728
2:1	0	5.741
2:1	2	7.633
2:1	2	6.711
2:1	2	5.818
2:1	5	6.720
2:1	5	6.275
2:1	5	6.072
2:1	10	7.084
2:1	10	6.285
2:1	10	6.670
4:1	0	4.728
4:1	0	3.728
4:1	0	5.741
4:1	2	6.265
4:1	2	9.688
4:1	2	6.757
4:1	5	6.344
4:1	5	6.401
4:1	5	6.686
4:1	10	5.885
4:1	10	5.845
4:1	10	7.134
6:1	0	4.772
6:1	0	3.728
6:1	0	5.741
6:1	2	6.526
6:1	2	8.167
6:1	2	5.467
6:1	5	6.808
6:1	5	8.529
6:1	5	7.234
6:1	10	5.761
6:1	10	7.133
6:1	10	6.875

Abbreviation:
NDFD = Neutral detergent fiber disappearance

Table A-31 Data used for analysis in Chapter 7 (Exp. 4)

Enzyme Aplication Rate	NDFD[a]
0	6.035
0	6.916
0	5.818
.08	6.720
.08	6.275
.08	6.072
.16	7.084
.16	6.285
.16	6.670

a = NDF disappearance (g/100 gDM)

Table A-32 Data used for analysis in Chapter 7 (Exp. 5)

Enzyme Application Rate	NDFD[a]
0	6.035
0	6.006
0	6.315
.25	13.06
.25	12.56
.25	13.60
.50	12.34
.50	13.40
.50	13.19

a = NDF disappearance (g/100 g DM)

Table A-33. Data used for analysis of enzyme comparison in Chapter 7

Enzyme Preparation	NDFD*
Viscozyme	2.213
Viscozyme	2.284
Viscozyme	2.856
Viscozyme	2.719
Viscozyme	3.758
Viscozyme	4.615
Viscozyme	3.482
Viscozyme	3.912
Viscozyme	4.074
Viscozyme	4.971
Viscozyme	6.632
Viscozyme	2.942
Viscozyme	4.312
Viscozyme	4.157
Viscozyme	6.712
Viscozyme	4.538
Viscozyme	5.891
Viscozyme	4.888
Viscozyme	3.608
Viscozyme	4.907
Viscozyme	4.114
Viscozyme	4.502
Viscozyme	4.304
Viscozyme	5.018
Viscozyme	4.070
Viscozyme	5.685
Viscozyme	5.293
Viscozyme	4.500
Viscozyme	3.863
Viscozyme	4.913
Viscozyme	4.829
Viscozyme	5.077
Viscozyme	4.913
Viscozyme	4.829
Viscozyme	5.077
Viscozyme	4.674
Viscozyme	4.722
Viscozyme	4.314
Biocellulase A: Bioxylanase A	2.893
Biocellulase A: Bioxylanase A	1.971
Biocellulase A: Bioxylanase A	1.078
Biocellulase A: Bioxylanase A	1.980
Biocellulase A: Bioxylanase A	1.535
Biocellulase A: Bioxylanase A	1.332
Biocellulase A: Bioxylanase A	2.344
Biocellulase A: Bioxylanase A	1.545

Table A-33. Cont.

Enzyme Preparation	NDFD
Biocellulase A: Bioxylanase A	1.930
Biocellulase A: Bioxylanase A	1.525
Biocellulase A: Bioxylanase A	4.948
Biocellulase A: Bioxylanase A	2.017
Biocellulase A: Bioxylanase A	1.604
Biocellulase A: Bioxylanase A	1.661
Biocellulase A: Bioxylanase A	1.946
Biocellulase A: Bioxylanase A	1.145
Biocellulase A: Bioxylanase A	1.105
Biocellulase A: Bioxylanase A	2.394
Biocellulase A: Bioxylanase A	1.786
Biocellulase A: Bioxylanase A	3.427
Biocellulase A: Bioxylanase A	0.727
Biocellulase A: Bioxylanase A	2.068
Biocellulase A: Bioxylanase A	3.789
Biocellulase A: Bioxylanase A	2.494
Biocellulase A: Bioxylanase A	1.021
Biocellulase A: Bioxylanase A	2.393
Biocellulase A: Bioxylanase A	2.135
Biocellulase A: Bioxylanase A	0.032
Biocellulase A: Bioxylanase A	1.001
Ecogran	0.470
Ecogran	0.025
Ecogran	0.834
Ecogran	0.035
Ecogran	0.420
Ecogran	0.616
Cellulose G	7.956
Cellulose G	6.455
Cellulose G	7.498
Cellulose G	6.230
Cellulose G	7.035
Cellulose G	7.083

a = NDF disappearance

Table A- 34. Data used for analysis of fermentation-endproducts (g/100 g DM) and pH in forage sorghum ensiled with commercial enzyme mixtures (Chapter 8)

Enz	Day	Ace	Lac	Pro	Etoh	But	pH
1	0	0.148	0.336	0.002	0.075	0.006	5.3
1	0	0.385	1.084	0.002	0.183	0.008	5.1
1	0	0.234	0.916	0.003	0.135	0.006	5.3
2	0	0.182	0.608	0.002	0.377	0.000	5.3
2	0	0.266	0.540	0.001	0.173	0.000	5.4
2	0	0.180	0.672	0.001	0.150	0.000	5.4
3	0	0.147	0.727	0.003	0.069	0	5.4
3	0	0.124	0.530	0.002	0.059	0.007	5.1
3	0	0.113	0.409	0.007	0.071	0	5.2
4	0	0.084	0.475	0.001	0.048	0.004	5.2
4	0	0.116	0.673	0.002	0.071	0	5.3
4	0	0.128	0.423	0.002	1.184	0.004	5.4
5	0	0.236	0.909	0.003	0.094	0	5.2
5	0	0.140	0.607	0.001	0.054	0.005	5.1
5	0	0.194	0.612	0.002	0.056	0.007	5.2
6	0	0.127	0.498	0.007	0.065	0.004	5.2
6	0	0.213	0.835	0.003	0.078	0.004	5.3
6	0	0.144	0.274	0.003	0.069	0.009	5.2
1	40	1.632	8.745	0.004	0.374	0.015	3.5
1	40	1.592	7.940	0.005	0.985	0.009	3.5
1	40	1.601	8.120	0.006	0.854	0.014	3.6
2	40	1.788	7.600	0.002	1.207	0.008	3.55
2	40	1.678	8.568	0.002	1.161	0.009	3.6
2	40	1.588	7.364	0.001	1.253	0.016	3.55
3	40	1.378	8.612	0.004	0.644	0.047	3.5
3	40	1.788	6.122	0.005	0.852	0.025	3.6
3	40	1.643	5.395	0.006	0.829	0	3.5
4	40	1.583	9.986	0.005	1.701	0.005	3.6
4	40	1.823	9.908	0.004	0.656	0.020	3.5
4	40	1.362	8.734	0.003	0.831	0.003	3.5
5	40	1.298	8.380	0.003	0.686	0.002	3.6
5	40	1.714	8.610	0.004	0.561	0.008	3.5
5	40	1.782	8.012	0.000	0.448	0.005	3.55
6	40	1.577	7.371	0.002	0.752	0.002	3.5
6	40	1.658	5.529	0.005	0.842	0.004	3.6
6	40	1.324	8.084	0.006	0.932	0.002	3.6
1	100	1.328	2.935	0	0.910	0.001	3.70
1	100	1.809	7.864	0.003	0.349	0.015	3.6
1	100	1.931	8.062	0.004	0.573	0.004	3.75
2	100	1.850	6.383	0.005	1.103	0.013	3.7
2	100	1.672	5.606	0.006	1.003	0.008	3.65
2	100	1.817	6.033	0.005	0.841	0.006	3.65
3	100	1.581	8.394	0.005	0.698	0.031	3.65
3	100	1.281	7.457	0.002	0.435	0.050	3.5
3	100	1.387	7.614	0.007	0.597	0.021	3.5
4	100	1.268	7.198	0.002	0.475	0.007	3.5
4	100	1.402	6.733	0.006	0.736	0.007	3.5
4	100	1.329	8.860	0.005	1.678	0.009	3.45
5	100	1.284	7.979	0.000	0.603	0.007	3.6
5	100	1.239	7.763	0.008	0.949	0.010	3.65
5	100	1.135	6.322	0.007	0.545	0.018	3.5
6	100	1.416	3.218	0.004	0.484	0.006	3.65
6	100	1.998	6.889	0.003	0.505	0.003	3.5
6	100	1.165	7.305	0.005	0.808	0.015	3.55

Abbreviations:
Enz = enzyme, 1 = no enzyme, 2 = Viscozyme, 3 = Ecogram (1X), 4 = Ecogram (2X), 5 = Cellulase G (1X), 6 = Cellulase G (2X); Lac = lactic acid, Ace = acetic acid, Pro = propionic acid, Etoh = ethanol, But = butyric acid.

Table A-36. Data used for analysis of stuctural carbohydrate contents (g/100 g DM) in forage sorghum ensiled with commercial enzyme mixtures (Chapter 8)

Enz	Day	NDF	Hemi	ADF	Cellulose
1	0	63.71355	25.1569	38.5567	33.09339
1	0	61.59406	24.536	37.058	32.66263
1	0	59.62215	23.2821	36.3401	31.74662
2	0	62.24739	23.2056	39.0418	33.3101
2	0	64.34587	24.986	39.3599	35.20494
2	0	62.54359	24.61	37.9336	34.48339
3	0	60.91975	25.1398	35.78	30.09919
3	0	59.33509	24.5084	34.8267	30.38719
3	0	54.61952	22.7422	31.8773	28.56648
4	0	55.61244	23.1323	32.4801	29.3705
4	0	57.34848	25.7386	31.6098	27.97348
4	0	56.66374	23.5119	33.1519	27.65584
5	0	54.23005	21.381	32.8491	28.7814
5	0	57.63625	22.0803	35.556	31.52326
5	0	58.25021	22.2569	35.9933	31.29676
6	0	54.79036	20.9258	33.8645	29.40618
6	0	59.56851	27.7766	32.7919	28.62603
6	0	54.24958	22.559	31.6905	27.95239
1	40	57.2093	21.8605	35.3488	31.48635
1	40	57.95132	22.4341	35.5172	31.17647
1	40	59.09423	22.5712	36.523	32.46896
2	40	54.50322	20.5861	33.9171	28.29521
2	40	59.45205	22.5245	36.9276	33.42466
2	40	54.35619	21.9734	32.3828	29.37369
3	40	59.57517	25.1605	34.4146	29.47472
3	40	57.4352	21.2819	36.1533	32.61395
3	40	55.3245	19.4403	35.8842	31.92472
4	40	58.76216	21.5915	37.1706	33.35102
4	40	57.90787	21.6891	36.2188	32.09213
4	40	56.7865	18.9249	37.8616	34.16732
5	40	57.11593	22.4694	34.6466	30.00943
5	40	58.89083	22.7652	36.1257	31.39422
5	40	58.52761	22.0859	36.4417	32.49489
6	40	55.78122	20.2695	35.5118	31.40961
6	40	58.02034	22.0462	35.9742	31.67058
6	40	58.2268	32.125	26.1018	20.49027
1	100	60.72096	24.1128	36.6081	32.31229
1	100	59.08732	23.2912	35.7961	32.27973
1	100	58.80542	22.8448	35.9606	30.97291
2	100	56.49698	25.9707	30.5263	27.52373
2	100	55.87409	19.1681	36.706	33.37081
2	100	56.62935	23.1585	33.4708	28.87448
3	100	55.33005	20.8473	34.4828	29.3399
3	100	59.16929	22.1683	37.001	32.62496
3	100	59.86408	24.7751	35.089	30.61169
4	100	58.22087	22.2011	36.0198	31.11576
4	100	57.45397	22.9262	34.5278	30.40982
4	100	56.59145	22.7237	33.8678	29.37451
5	100	59.58195	24.1649	35.4171	31.09982
5	100	58.82006	23.0451	35.775	31.88119
5	100	58.12562	23.3101	34.8156	30.46859
6	100	55.89058	21.9466	33.944	29.71077
6	100	56.34359	21.4522	33.8914	29.62819
6	100	58.21505	22.6521	35.5629	30.1575

Abbreviations:
Enz =enzyme, 1 = no enzyme, 2 = Viscozyme, 3 = Ecogram (1X), 4 = Ecogram (2X), 5 = Cellulase G (1X), 6 = Cellulase G (2X), Hemi. = hemicellulose

APPENDIX - B

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