The Ensilability of Selected Tropical Grasses

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Abstract

The aims of this thesis were to assess and improve the ensiling of *Pennisetum purpureum* (Schumach.), *Brachiaria mutica* (Forssk.) and *Panicum maximum* (Jacq.) as an option to conserve forage from tropical grasses. Experiments compared the influence of molasses and herbage wilting on the ensiling process. Results showed that silage quality of both direct cut and wilted herbage without molasses was generally poor and of high pH. Wilting reduced dry matter loss, however, it also decreased fermentation rates. The inclusion of molasses improved silage quality in both direct cut and wilted herbage by lowering the pH and increasing lactic acid production. Further, grasses were low in water soluble carbohydrates and had a high buffering capacity which contributed to their difficulty to ensile without a carbohydrate addition. Increasing molasses rates often showed a negative quadratic effect on silage quality, indicating that an optimal rate of molasses may exist.

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Chapter 1. Introduction

Problem and Justification

According to Hao (2006) most Hawaii livestock and dairy operations are dependent on imported feeds to sustain their operation. As costs of operating a farm or livestock operation continually rise, including energy and importing costs, dairies must become more feed sustainable in order to survive. The utilization of local, grass-based forages is becoming a viable alternative. Both remaining dairies in Hawaii are currently adopting management practices to produce forage grass on-site.

When growing forages on a commercial scale, harvested forage is often in surplus and must be conserved. The main goal of conservation is to preserve high nutritive forage with minimal losses and a good hygienic quality. Storage of forage is also of considerable strategic importance to ensure a constant and consistent supply of feed for dairy animals (C. Lee, 2005, personal communication). Forage not properly stored will decay and be lost (Woolford, 1984). The two most popular methods of forage preservation are ensiling and haymaking (Buxton et al., 2003). The haymaking process dries the forage to a stage where microbial or chemical reactions cease, whereas ensiling preserves the crop at high moisture through a fermentation process (Raymond et al., 1986). All conservation methods have advantages and disadvantages and must be adapted to the climate conditions and technical solutions. Conservation of forage as hay is highly dependent on weather conditions, which may cause very high losses (Buxton et

al., 2003), and is thought to not be the best choice for a wet tropical climate. Ensiling is likely the better alternative. It allows for grasses to be harvested and conserved in a much shorter time, therefore reducing the chance of loss due to short, intense rainfall typical of tropical climates. Certain tropical grasses have been selected for a high potential for use as dairy forage in Hawaii because they produce high yields of quality forage (Valencia-Gica, 2007), although their ability to be conserved needs to be studied.

There are problems associated with the ensiling of grass herbage. In order to produce quality silage suitable bacteria, moisture content, pH, and sufficient amounts of carbohydrates must be present. Certain microorganisms may produce silage with high DM loss as carbohydrates are converted to CO_2 gas (Buxton et al., 2003). Ensiling of freshly cut herbage may produce high amounts of toxic effluent and hinder proper bacteria growth (McDonald, 1981). Wilting of cut grass may overcome these problems although it may stimulate the growth of yeasts and molds in some circumstances if fermentation is delayed or air leaks into the silo (Lattemae, 1997).

Thesis Objectives

Research is needed to provide information for conserving tropical grass forage for later feed-out. The nutritional value of the grass forage needs to be preserved to ensure the production of high amounts of quality milk. As the climate in Hawaii often restricts the possibility of hay-making, ensiling may be easier to adapt to the optimal harvesting time.

The objectives of this thesis were to study:

1. The ensilability of selected tropical forage grasses, including their water soluble carbohydrate content and buffering capacity.
2. The effect of wilting and silage additives on silage quality.

Chapter 2. Review of Literature

General Characteristics of Selected Tropical Forage Grasses

Three tropical grasses were selected for this study. Two grasses, *Brachiaria mutica* and *Pennisetum purpureum,* were selected based on previous research by Valencia-Gica (2007) who showed that these grasses were high yielding and of good nutritional quality. A third grass, *Panicum maximum* was selected as it is the main forage grass currently being grown at the two remaining dairies on Hawaii.

Pennisetum purpureum (Schumach.), also called "Bana Grass," "Napier Grass" or "Elephant Grass," is a robust perennial forming large broad clumps with erect stems up to 6 m (Bogdan, 1977). *P. purpureum* occurs naturally throughout tropical Africa and has been introduced to practically all tropical and subtropical countries (Parsons, 1972). It grows best at high temperatures and can tolerate considerable periods of drought. *P. purpureum* will not tolerate prolonged flooded and waterlogged conditions. *P. purpureum* is valued for high yields, good palatability and nutritional quality, especially at 3 to 4 wk harvest intervals (Bogdan, 1977). Valencia-Gica (2007) reported dry matter yields of 51 Mg ha^{-1} y^{-1} with an N application rate of approximately 1200 kg N ha^{-1} y^{-1}.

Brachiaria mutica (Forsk.) or "California Grass," native to West Africa (Parsons, 1972) is cultivated on a large scale in South and Central America, Australia, Fiji, the Philippines, humid West Africa and Puerto Rico. *B. mutica* is a perennial with flowering stems 1-2 m high ascending from long, hollow, and highly noded prostrate shoots freely

rooting at the nodes and forming dense cover (Bogdan, 1977). It produces very long trailing stems up to 2.5 m which easily spread into unoccupied areas. The species frequently forms colonies on stream banks and lowlands and can withstand waterlogging and long-term flooding (Holm et al., 1977). *B. mutica* persists in areas with rainfall as low as 900 mm y^{-1} but does not tolerate extended dry conditions. Low cutting of *B. mutica* swards is favored, and cutting 1 to 7 cm above the ground level can result in some 20% higher yields of herbage than cutting at 15 to 20 cm (Bogdan, 1977). Fertilizing especially with N, is needed for high yield and quality herbage. Valencia-Gica (2007) produced yields of nearly 60 Mg ha^{-1} y^{-1} with the application of approximately 1200 kg N ha^{-1} y^{-1}.

Panicum maximum (Jacq.) or "Guinea Grass" is native to tropical Africa and is an important fodder grass in many tropical areas (Parsons, 1972). A tufted perennial 0.5 to 4.5 m high, stems are mostly erect but can be ascending, glabrous or hairy, stout to slender, with 3 to 15 nodes. *P. maximum* performs best in humid areas with over 1000 mm precipitation y^{-1} but will tolerate drought. *P. maximum* does not tolerate heavy clays or prolonged flooding and waterlogged conditions (Bogdan, 1977). *P. maximum* responds best to a cutting height of 15 to 20 cm above ground. Cutting at shorter heights may retard growth. *P. maximum* should be cut frequently to ensure high palatability (Bogdan, 1977). Fertilizing, especially with N, is needed for high yield and quality herbage. Yields upwards of 45 Mg ha^{-1} y^{-1} have been reported (Crowder et al., 1970) with the application of approximately 900 kg N ha^{-1} y^{-1}.

Forage Quality

The quality of forage is based on how well animals consume it and how efficiently the nutrients in the forage are converted into animal products (Ball et al., 2001). Dry matter (DM), crude protein (CP), neutral-detergent fiber (NDF), and calcium, phosphorus and potassium content are just some parameters that indicate forage quality.

Dry matter represents the substance in the forage; protein, fats, fiber, etc., except water. Total forage yield as well as the price of forage is based on the DM weight. Forage quality is compared based on a DM basis to eliminate the dilution effect of moisture. Thus the ability to quantify the moisture content in forage is important to ensure correct livestock feeding rates. Moisture content is also important when preserving forage. Hay must be dried to a certain moisture content, approximately 85% DM (Raymond et al., 1986), to ensure efficient preservation. Silage can be too wet to ensile and may need to be wilted first, to ensure proper fermentation (Buxton et al., 2003).

Protein is a key nutrient and is essential to the health and productivity of livestock. Forage protein deficiencies often exist and protein supplements are a common addition in ration preparation (Ball et al., 2001). Protein content of forage is typically expressed as crude protein which is a conventional term for all nitrogenous substances: mineral N, amide N, amino acids, and proteins; and its determination is based on that of total N. Proteins contain about 16% nitrogen so the crude protein is

determined by multiplying the nitrogen figure by 100/16 or 6.25. Crude protein contents usually range from 3 to 20 per cent, or even more in young plants. Crude protein contents decrease as grasses mature and this decrease is even more accelerated for tropical grasses (Bogdan, 1977). Arthington and Brown (2005) looked at forage quality of four tropical forages; Bahia grass (*Paspalum notatum*), Limpo grass (*Hemarthria altissima*), Bermuda grass (*Cynodon dactylon*) and Star grass (*Cynodon spp.*) harvested at 4 and 10 weeks. Crude protein decreased on the average by 38% from week 4 to week 10 in the study. Van Man and Wiktorsson (2003) studied *P. purpureum* and two varieties of Guinea grass (*Panicum maximum* cv. 280 and *Panicum maximum* cv. I.429) at 4 cutting frequencies (4, 6, 8, and 10 weeks) and confirmed that CP decreased significantly as grasses matured. In the animal, CP is utilized for the growth and the replacement of cells and tissue, and for the formation of milk and is of particular value for young growing animals and for lactating cows (Bogdan, 1977). Crude protein of feed should be at least 13.0 per cent DM for a lactating heifer (NRC, 2001).

The CP content of silage is generally similar to that of the forage from which it was made, however, during ensiling plant proteases break down a portion of the protein in the plant tissue increasing the amount of non-protein nitrogen and volatile nitrogen in silage (Woolford, 1984). The optimum pH for plant protease function is between 5.5 and 6.0, so a rapidly falling pH in the silo will decrease proteolysis (McDonald, 1981). Further, wilting the herbage prior to ensiling has been shown to reduce the amount of protein degradation and increase nitrogen retention in the animal (Marsh, 1979).

After protein, fiber may be the most important constituent in forage quality. Fiber content in forage affects the amount of forage that can be consumed and how well the forage is digested. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) are the two main fiber measures for forage quality. NDF represents the structural or cell wall material, including cellulose, hemicellulose, lignin, silica and cutin. NDF is mostly indigestible and is dependent on microorganisms in the rumen for digestion (Robinson, 1999). The NDF content relates to how much forage an animal can consume. Livestock tend to eat more forage if it has a lower NDF content. The result is more weight gain or milk yield per day (Rayburn, 1997). Acid detergent fiber is NDF without the hemicellulose (Robinson, 1999), because the acid digestion further removes hemicellulose. Acid detergent fiber is the best indicator of energy and fiber requirement for healthy rumen fermentation. Energy estimates in forage are calculated from the ADF. Low ADF concentrations mean higher energy value and digestibility although total ADF content should be greater than 19 g kg^{-1} of DM for a dairy cow; concentrations less may depress milk butterfat (Rayburn, 1997). Studies by Van Man and Wiktorsson (2003) and Arlington and Brown (2005) showed an increase in NDF and ADF as the tropical grasses matured.

Minerals, especially P, Ca, K and Na, are required to maintain healthy and productive lactating cows and mineral deficiencies can lead to a variety of ailments reducing milk yields (McDowell, 1992). The recommended P, Ca, K, and Na concentrations in the feed consumed by a 600 kg cow producing 25 kg milk day^{-1} are

0.32, 0.62, 0.24 and 1.0 g kg^{-1} of DM, respectively (NRC, 2001). Tropical grasses frequently contain adequate levels of Ca and K (Bogdan, 1977).

Forage Conservation

After a crop is harvested the plant dies and begins to decompose; sugars oxidize and plant proteins break down (Raymond et al., 1986). Proper forage conservation practices should stop these destructive processes quickly and completely to preserve as much yield and feeding value of the crop as possible. Two main methods used to preserve forage are haymaking and ensiling.

Haymaking

The process of haymaking is to preserve forage by drying, so that microbial and enzymatic activity is minimal. The moisture content needed to safely store a crop depends on its composition and age at harvest. Mature grasses contain fewer sugars that can oxidize, and can be safely stored with higher levels of moisture (Hopkins, 2000). According to Raymond et al. (1986) mature grasses are safe to store when moisture content has been reduced to between 15 and 18 g kg^{-1} of plant material, whereas young leafy grass needs to be dried to below 12 g kg^{-1} of plant material.

Traditionally haymaking involves cutting the grass in the field and allowing it to dry (Raymond et al., 1986), although this has many disadvantages especially in a tropical environment. Field-cured hay can suffer significant damage from rain, microbial respiration and requires additional machine operations (Buxton et al., 2003). In

addition, tropical humid environments may make field drying to an acceptable moisture level difficult (Raymond et al., 1986). Often conditioning of the herbage either through breaking or crimping the stems or the use of drying agents is used to shorten drying times.

Barn hay-drying systems have many advantages over traditional methods because they reduce field exposure time and ensure proper drying for hay production. Disadvantages are that they require considerable start-up costs as well as continuous power and fuel costs to operate (Raymond et al., 1986).

Ensiling

Whereas haymaking preservation is based on drying the crop, the principles of silage preservation are based on the natural processes of fermentation. The ensiling process begins as an aerobic phase immediately following the closing of the silo and O_2 trapped in the forage slowly diminishes. This phase is confined to only a few hours assuming the forage is sealed properly (McDonald, 1981).

Once the last trace of O_2 has been depleted in the silo, the main fermentation phase begins (Buxton et al., 2003). The epiphytic lactic acid bacteria (LAB) are the essential microorganism for spontaneous silage fermentation. Although early in the fermentation process other anaerobic microorganisms such as enterobacteria, clostridia, and yeasts compete with the LAB (Buxton et al., 2003), as fermentation increases LAB become the dominant microbial population. The speed of this change is

directly correlated to the rate of pH decline and lactic acid production (Merry and Davies, 1999). The production of silo gases, effluent and the shrinking of silage mass are all external indications of the fermentation process. The process continues from anywhere from 7d to more than 30d (Buxton et al., 2003).

As fermentation intensity decreases, the ensiling process moves to the stable phase. Provided the silo remains sealed, little occurs in this phase. Only acid-tolerant enzymes are active, resulting in a slow acid hydrolysis of structural and storage carbohydrates (Buxton et al., 2003). This provides an important supply of water-soluble carbohydrates (WSC) beneficial to both the forage consumer and the ensilage process (Buxton et al., 2003). Finally, the LAB population greatly decreases as they become inhibited by their own production of acidity. In theory, the stable phase can last indefinitely, although in practice this is usually not longer than 1 year or the next harvest.

When silage reaches the feed-out period, O_2 again has free access to the forage. In the presence of O_2, aerobic microorganisms, particularly yeasts, start to multiply and deterioration of the forage can occur. This is especially common, if an excess of WSC's are still present in the silage after fermentation. Forage deterioration can be as much as 4% to 6% DM day^{-1} where O_2 is present (Buxton et al., 2003). Because O_2 only comes in contact with approximately the first 1m of a silo face, a feed-out at this rate should minimize losses (Buxton et al., 2003).

While the aim of silage is to preserve the forage, there will be losses in forage as a result of the silage process. Although major loss is due to air infiltration during storage, if the seal is imperfect, and at feed out, other sources of losses may accur.

Fermentation is a biochemical process where a substrate, usually simple sugars, is converted into other products. Over 20 different reactions depending on substrate and microorganism take place during fermentation (Buxton et al., 2003). Some of these reactions may cause no DM loss as the sugar is merely converted to another product. On the other hand, some fermentation reactions cause a net DM loss, usually because sugars are converted to CO_2. Substrates available for fermentation are known as water soluble carbohydrates and mainly include glucose, fructose and sucrose.

The most common and desirable silage fermentation is a homolactic reaction, where glucose is simply converted into lactic acid in the presence of homofermentative lactic acid bacteria. The reaction is:

$$\mathbf{1mol\ C_6H_{12}O_6 \rightarrow 2\ mol\ C_3H_6O_3} \qquad \textbf{Equation2.1}$$

Another type of fermentation that is less desirable is a heterolactic reaction, where glucose is converted into lactic acid, ethanol and CO_2 in the presence of heterofermentative lactic acid bacteria. The reaction is:

$$\mathbf{1\ mol\ C_6H_{12}O_6 \rightarrow 1\ mol\ C_3H_6O_3 + 1\ mol\ C_2H_5OH + 1mol\ CO_2} \qquad \textbf{Equation2.2}$$

In equation 2.2, the production of CO_2 is in a gaseous form and is completely lost. The DM loss is 24.4% of the initial glucose. This fermentation is less desirable than the homolactic fermentation because of the loss of DM to gas.

Another fermentation which is even less desirable is the clostridial fermentation with butyric acid being produced. The reaction is:

$$\mathbf{1\ mol\ C_6H_{12}O_6 \rightarrow 1\ mol\ C_4H_8O_2 + 2\ mol\ CO_2 + 2\ mol\ H_2} \qquad \textbf{Equation2.3}$$

In equation 2.3, both CO_2 and H_2 are in gaseous form and lost to the environment, representing 51% of the initial glucose.

The substrates available for fermentation, or water soluble carbohydrates (WSC) range between 5 and 15% of the total DM. If all fermentation were heterofermentative roughly a 2 to 4% loss of DM would be expected from the conversion of glucose to CO_2, whereas if all fermentation was clostridial roughly a 3 to 7.5% loss of DM would be expected. Because numerous fermentation reactions are taking place maximum fermentation losses are usually in the order of 4% (McGechan, 1990), depending on the amount of WSC present. The more the fermentation is homolactic the less the DM loss.

Silage effluent or leachate is another source of forage quality loss (Buxton et al., 2003). Silage effluent is undesirable to both forage quality and the environment. Effluent contains valuable nutrients and can represent as much as 10% DM loss under very wet conditions. In addition effluent has very high biochemical oxygen demand (BOD), in the range of 40,000 to 90,000 mg O_2 L^{-1} (McDonald, 1981). Such high BOD

coupled with low pH makes silage effluent a very concentrated pollutant that can rapidly deplete O_2 in streams and lakes. The problem is one that can be avoided by wilting the grass prior to ensiling. When the silage moisture is below 70% in horizontal silos and 60% in tower silos, there is practically no effluent production (Pitt and Parlange, 1987). The problems of effluent, therefore, only exist in high moisture silages. Another environmental problem with the silage process is the plastic consumption. Although large bunker silos use approximately 0.3 kg of plastic Mg^{-1} DM, individually wrapped baylage uses approximately 3.6 kg of plastic Mg^{-1} DM (Buxton et al., 2003), a 12-fold difference.

Wilting the forage prior to ensiling has been one way of trying to increase the quality of the ensiling process (Buxton et al., 2003). Wilting to 30% DM should limit clostridia populations and enhance the possibilities of achieving a lactic acid dominant fermentation (McDonald, 1981). The main effect of wilting to the ensiling process is the delay of bacterial multiplication of any kind by decreasing the availability of intercellular plant juices (McDonald, 1981). Due to this microbial inhibition, stable wilted silage may be achieved at a pH of approximately 5.0 (March, 1979), whereas stable direct cut silage needs to be below pH 4.2 to ensure stability (Catchpoole and Henzell, 1971). Wilting, as discussed earlier, may also improve protein quality by decreasing the amount of protein breakdown in the silo. Wilting of forage before ensiling is common practice in regions of North America and Europe where the crop can be wilted for 24 to 48 hours in the field with little risk of rain damage (Buxton et al., 2003). In wet tropical environments it may

be difficult to leave a crop in the field for 24 or more hours and not expect rain damage. Another disadvantage to wilting forage is the possibility of air infiltration in the silo. Air infiltration of high DM material may be increased by decreased compaction which may result in a bad fermentation. This is particularly a problem with C_4 tropical grasses due to their structural rigidity (Jarrige et al., 1982).

The higher temperature as well as the loss of nutrients can adversely affect the nutritional quality of wilted silage (McDonald, 1981). Due to the Maillard reaction, large insoluble polymers of carbohydrate and N containing compounds form rendering these nutrients unavailable to the animal (Weiss et al., 1986). Forages with >45% DM are particularly prone to heating because air infiltration and less fermentation occurs (Weiss et al., 1986).

Laboratory Ensiling

Large commercial-scale silos are the ultimate relevant research unit for providing information applicable to silage research (O'Kiely and Wilson 1991). However, to evaluate numerous experimental variables and their interactions, scaled down laboratory or micro-silos are necessary. The use of micro-silos allow for many replications of treatments in order to determine the ensilabilty of a grass with a degree of confidence. Micro-silos by nature will have a larger surface-to-volume ratio than a large field silo, so the entire micro-silo usually serves as a sample. A large field silo can be opened and closed in order to take samples with limited alteration to the silage inside, whereas, opening a micro-silo may change moisture, temperature and oxygen

tension (Parker, 1980). The entire contents of laboratory silos can be weighed, processed, and analyzed accurately to test the effects of "treatments." This can only be done under the assumption that the fermentation process is reasonably similar to that taking place in field-scale silos. A survey of studies directly comparing fermentation in field-scale and small-scale silos resulted in the conclusion that forage in both silo types did undergo a similar fermentation (Meiske et al., 1975). O'Kiely and Wilson (1991) noted that interactions between laboratory silos and farm-scale silos could cause serious problems when treatments causing different fermentation patterns were being compared. Despite this potential difficulty, O'Kiely and Wilson (1991) concluded that laboratory silos were an accurate and reliable experimentation unit. Results from any type of laboratory silo should be field-tested using commercial silos before being applied at the producer level.

Forage Ensilability

Successfully producing quality silage requires suitable bacteria, moisture and carbohydrates. Lactic acid bacteria are the main microorganisms responsible for successful silage. Members of the lactic acid bacteria (LAB) group are unified by the following properties: all are gram-positive, microaerophilic, asporogenous, usually non-motile and ferment sugars (Buxton et al., 2003). Further division may be made on the type of fermentation; whether it is homofermentive, in which case sugars are fermented entirely to lactic acid (equation 2.1), or heterofermentive where products in addition to lactic acid are formed from sugars, principally CO_2 (equation 2.2).

A substrate, usually WSC, which include fructose, glucose, sucrose and fructosans (Woolford, 1984) must be available in sufficient quantities in order for the LAB's to ferment and preserve the feed. Many factors can influence the amount of these sugars including species, stage of maturity, leaf-to-stem ratio, time of day, light intensity, and temperature (Woolford, 1984).

Organic acids and their salts can exert considerable influence on the efficiency of the ensiling process by virtue of their ability to buffer against pH change. Their concentration affects the relative ensilability of a crop in terms of the amount of acid that will be necessary to achieve stability (Woolford, 1984). The buffering action of the organic acids in forage crops is strongest over the pH range of conventional fermented silage, specifically 6.0 to 4.0. The intensity of this action is referred to as buffering capacity and is defined as the quantity of lactic acid required to lower the pH of 1 g of herbage to pH 4.0 (Woolford, 1984). During ensiling there is a rapid and usually complete dissimilation of organic acids, particularly of citric and malic acids. Although this may sound desirable, as the major buffering constituents are removed, they are replaced by fermentation acids with stronger buffering properties and there is a loss of DM in the form of CO_2. The net result is usually a two- to four-fold increase in buffering capacity during ensiling (McDonald and Henderson, 1962; Woolford, 1984). Silage prepared from wilted material is expected to have a lower buffering capacity than direct-cut material because during wilting (aerobic conditions) the organic acids will not be transformed into stronger acids but respired as CO_2 and water (Woolford, 1984).

Because organic acids buffer over the normal range of pH silage, increased amounts of fermentable sugars must be present in order to overcome this buffering. For example, it has been calculated that 30 to 50 g lactic acid kg^{-1} DM is the minimum necessary to achieve a pH of 4.0 in silage. However, in practice a similar end point is reached by a much larger amount of acid, usually around 100 g kg^{-1} DM. Another way to look at this, Smith (1962) showed that only 1.32 g hexose kg^{-1} DM would give preservation, assuming no buffering action by the plant tissues. However, with buffering properties of the herbage the consensus of opinion is that 60 to 80 g of sugar kg^{-1} DM is a realistic range which would favor preservation (Woolford, 1984).

Ensiling of Selected Tropical Grasses

Literature available on the three tropical forage grasses of interest (*P. purpureum*, *B. mutica*, and *P. maximum*) and their preservation or ensilability varies immensely with species.

The majority of available literature on ensiling tropical grasses is with *P. purpureum.* Woodward (1990) confirmed ensiling was preferred over haymaking for preservation of *P. purpureum.* They concluded hay from *P. purpureum* was difficult to make because of problems associated with solar drying of the thick stems while, *P. purpureum* can be successfully preserved through ensiling and that fresh chopped materials contain adequate concentrations of water soluble carbohydrates. Michelena and Molina (1990) looked at the effect of wilting on *P. purpureum* ensiling. They showed that wilting resulted in higher N content, higher pH, higher lactic acid content and lower

volatile fatty acids content than silage from freshly cut forage. It was concluded that ensiling *P. purpureum* at about 30% DM produced high quality silage. Rodriguez et al. (1989) concluded that further drying to 40% DM improved fermentation, nutritive value and dry yield of ensilage.

Ibrahim et al. (1989) attempted to ensile wet *P. maximum*. They concluded that pH remained too high because of insufficient lactic acid, probably because of lack of WSC. Shao et al. (2005) reported similar unsuccessful results of *P. maximum* silage due to extended phases of respiration and aerobic microorganism activity, causing a high loss of carbohydrates. Niimi et al. (2006) showed that by lightly wilting *P. maximum*, it slightly lowered the pH while significantly increasing the lactic acid of the silage. Loures et al. (2005) showed wilting *P. maximum* greatly reduced effluent production.

There is very little literature on the ensilability of *B. mutica*. Bogdan (1977) reported ensiling of *B. mutica* gave relatively poor results, as it resulted in pH >5.0, large amounts of butyric acid, and considerable losses of DM In contrary, Paterson (1945) reported successfully ensilaging *B. mutica* with DM losses of 10 percent. Ellis (1989) in her M.S. thesis reported successful ensiling of B. mutica with a pH of 4.6 and 4.8 for direct cut and wilted silage, respectively. *B. mutica* is usually present in waterlogged soils which makes machine mowing difficult and may be why little work has been done on ensilability.

Knowledge Gaps

While much research has been done regarding ensiling of forage grasses, few studies considered tropical grasses or took place in a wet tropical environment. Related studies on ensiling tropical forage have all been done elsewhere (Florida, Australia, Puerto Rico and Brazil) and many are not in English. For example, the large majority of *P. purpureum* silage research has been done in Brazil and is only available in Portuguese. In addition, studies focusing on the effect of DM on ensiling often are conflicting and conclude different DM contents for optimal fermentation.

Ensilability studies, particularly on *B. mutica*, vary greatly and are contradictory. Given the range of the results, it is difficult to use this information in developing practical recommendations for dairy operators or feed growers in Hawai'i. Therefore, there is a need to conduct a study on the specific grasses or cultivars that are relevant to Hawai'i dairies or feed growers. This information could then be used to aid local dairies or feed growers in forage preservation, resulting in a more sustainable and economical industry.

Knowledge gaps on the ensiling of tropical grasses exist and need to be considered when a local producer needs to conserve a large harvest. Knowledge gaps include the advantages and disadvantages of wilting which may increase the LAB population, although decrease the overall fermentation intensity. Another gap is the WSC content of the grasses grown in Hawaii and if the WSC content is ample to ensure proper fermentation. Tropical grasses often contain insufficient WSC (Yunus et al., 2001)

resulting in low silage quality. If grasses contain insufficient substrate for fermentation, silage additives need to be considered in order to ensure efficient and reliable fermentation.

Chapter 3. Ensilability of Selected Tropical Grasses, the Effect of Wilting and Molasses Additions

Abstract

Tropical grasses are known for high yields and good forage quality during the wet season. After harvesting, these grasses need to be conserved to allow for later feed-out. In a series of two experiments, silage made from *Pennisetum purpureum* (Schumach.), *Brachiaria mutica* (Forssk.) and *Panicum maximum* (Jacq.) were studied to determine fermentative characteristics. In Experiment 1, direct cut and wilted grasses were ensiled in 56cm^3 micro-silos. Wilting the grass significantly reduced dry matter (DM) loss in the silo. Wilting significantly raised the silage pH of *B. mutica* from 5.3 to 6.65 and *P. maximum* from 4.6 to 6.5, and significantly decreased total silage acid production. For *P. purpureum* silage*,* however, wilting increased total silage acid production although the silage pH remained higher (5.65) than direct cut (4.45). In Experiment 2, three rates of molasses (0, 40, and 100 L tonne^{-1} fresh material) were added to both direct cut and wilted grass and ensiled in 56cm^3 micro-silos. Results showed that molasses significantly reduced silage pH to less than 4.25 and increased lactic acid content to above 5% DM in both wilted and direct cut *B. mutica* and *P. purpureum*.

Introduction

When growing forages on a commercial scale, harvested forage is often in surplus and must be conserved. The goal of forage conservation is to preserve feed with minimal nutritional losses and a good hygienic quality to allow for later feed-out. Storage of forage is of considerable importance to ensure a constant and consistent supply of feed for dairy animals (C. Lee, 2005, personal communication). Forage not properly stored will decay and be lost (Woolford, 1984).

The preservation of forage through silage is based on the production of organic acids through fermentation reactions with an accompanied reduction in pH. Silage technology is more flexible and less weather dependent than preservation by making hay (Lattemae, 1997). In a wet, tropical environment unexpected precipitation may severely damage a field-drying crop resulting in dry matter (DM) and nutritional losses.

Pennisetum purpureum (Schumach.), *Brachiaria mutica* (Forssk.) and *Panicum maximum* (Jacq.) are widely planted in the tropical and subtropical regions of the world (Bogdan, 1977). These grasses are known for high yields with moderate to good forage quality (Crowder et al., 1970; Valencia-Gica, 2007), especially during the wet season and/or if adequately fertilized with nitrogen (N). Grass yields will often exceed dairy animal requirements during the rainy season while yields may be inadequate during the dry season. Ensiling the excess grass allows animals to be fed a consistent quality feed throughout the year which may increase animal production.

Often tropical grasses contain high amounts of water (<20% DM) and when ensiled directly may produce effluent or leachate. This effluent is a source of forage loss (Buxton et al., 2003), both of valuable nutrients and biomass. In addition, effluent has a very high biochemical oxygen demand, in the range of 40,000 to 90,000 mg O_2 L^{-1} (McDonald, 1981), making it a highly toxic pollutant especially if released near local waterways. Wilting of the grass to approximately 30% DM prior to ensilage will eliminate the effluent risks (Buxton et al., 2003), while also restricting unwanted clostridial growth (Lattemae, 1997).

The low level of water soluble carbohydrates (WSC) is often a cause of poor silage from tropical grasses (McDonald et al., 1995; Yunus et al., 2001). Low WSC levels often result in silages with high pH and low lactic acid contents (Catchpoole and Henzell, 1971), resulting in poorly preserved feed. Molasses is often added to silage as a sugar additive and is well known to increase fermentation, feeding quality and preservation (Bolsen et al., 1996; Lattemae, 1997).

Because crop factors such as moisture and WSC concentration affect the fermentation process (Kung and Shaver, 2001), it should be possible to improve fermentation by manipulating these factors prior to ensiling. In this study, three tropical grasses (*P. purpureum*, *B. mutica*, and *P. maximum*), were ensiled; and the effects of wilting and molasses additions on silage quality were measured. It was hypothesized that:

1. Wilting of forage prior to ensiling will decrease effluent and DM loss (Kung and Shaver, 2001) in the silo, therefore increasing silage quality.
2. Adding molasses to the forage prior to ensiling will increase fermentation (van Niekerk et al., 2007) in the silo, resulting in a lower silage pH and increased stability.

Materials and Methods

Grass Species and Harvesting

Pennisetum purpureum (cv HA 5690), *Brachiaria mutica*, and *Panicum maximum* were field grown (Figure 3.1) in separate plots established in January 2008 at University of Hawai'i at Manoa, Honolulu, HI, USA (21° 18' N/157° 48' W). Plots of *P. purpureum* and *B. mutica* were fertilized with 16-16-16 NPK fertilizer after every cutting and irrigated every day. The *P. maximum* plot was not irrigated or fertilized because of its difficult accessibility. Grasses from the plots was harvested at six weeks by sickle to 15 cm above the ground for *P. purpureum* and *P. maximum* and 5 cm above the ground for *B. mutica* in April 2008 for Experiment 1, and the regrowth was similarly harvested in May 2008 for Experiment 2.

Forage Quality Analysis

The harvested plant materials were collected in large black plastic bags and three grab samples from each bag were composited together and oven dried at 70° C for dry matter (DM) determination and ground in a Thomas Wiley mill to pass through a

1-mm screen. Dried and ground plant material was then analyzed by near infrared reflectance spectroscopy (Foss NIRSystems Model 6500 with Win ISI II v1.5) for water soluble carbohydrates, net energy of lactation (NEL), neutral detergent fiber (NDF) and crude protein (CP) by Dairy One (DHI Forage Testing Lab, Ithaca, NY).

Experiment 1 Ensiling

Immediately after harvest, *P. purpureum*, *B. mutica*, and *P. maximum* were chopped to 2 to 8mm lengths using hand shears. Treatments were either immediate ensiling of the grass after harvest (direct cut) or wilting of grass for 24h indoors at 23° C under fluorescent lighting prior to ensiling. Grasses were ensiled in 56 cm^3 plastic tube (Fisher Scientific Cat. No. 06-443-20) "silos" with screw cap tops. Examples of chop length and silos are shown in Figure 3.2. Grasses were compacted by hand into tubes and weighed in order to calculate fresh matter (FM) and DM weight and density. Plastic tops were then screwed on and plastic laboratory film was wrapped around tubes to ensure anaerobic conditions. Grasses were ensiled for 28 to 35d at room temperature (25° C), for the reason that that silo fermentation is usually complete within 30d (Buxton et al., 2003). Some variation in ensiling time was because silos were sent to the laboratory for analyses which were often delayed until the following week. Because of the size of the silos, the entire silo served as a single sample.

Silage Quality Measurements

There were five silos or samples for each treatment; three samples were used to calculate DM loss after ensiling and the remaining two were used to analyze silage fermentation. Dry matter loss was determined by the difference of DM weight in the silo on day 0 and at time of opening.

The two remaining tubes were analyzed for pH, lactic acid, acetic acid, propionic acid, butyric acid, crude protein and the amount of N as ammonia (Amm-N%) as part of a fermentation report performed by Dairy One (DHI Forage Testing Lab, Ithaca, NY). Silage pH was determined using a 15g wet sample placed into 250-ml beaker with 200ml deionized water. The mixture was then stirred, and allowed to stabilize five minutes. The pH was then determined using a Thermo Orion Posi-pH Symphony Electrode and Thermo Orion 410A meter. Silage samples were then filtered through a disposable syringe filter [Millipore Millex 5.0 mm PVDF (Durapore) membrane, Millipore Corp., Billerica, MA] and the extract was analyzed for acids stated above by gas chromatography. To determine acetic, butyric and propionic acid concentrations an aliquot of extract mixed in a 1:1 ratio with 0.06M Oxalic acid containing 100ppm Trimethylacetic acid was injected into a Perkin Elmer Autosystem XL Gas Chrmatograph containing a Supelco packed column with the following specifications: 2m x 2mm Tightspec ID, 4% Carbowax 20M on 80/120 B-DA. For lactic acid an aliquot of extract analyzed for L-Lactate using a YSI 2700 SELECT Biochemistry Analyzer equipped with an L-Lactate membrane. Total Lactic acid is determined by multiplying the concentration of L-Lactate by 2.0. Crude protein was measured as total N by the Kjeldahl method and

multiplied by 6.25. The amount of N as ammonia was quantified by titration, after extraction with water followed by alkaline distillation.

Data Analysis

The data were analyzed statistically using a t-test on the means of direct cut and wilted with the null-hypothesis that the means were equal. The null hypothesis was rejected if p-values were ≤0.05. All statistical analyses were done using SAS (2007) software.

Experiment 2 Ensiling

Immediately after harvest, *P. purpureum*, *B. mutica*, and *P. maximum* were chopped into about 2 to 8mm lengths using hand shearers. Treatments were either immediate ensiling of grass (direct cut) after being mixed with molasses (DM 78%) at a rate of 0 , 40 and 100 L tonne^{-1} FM, or wilting of grass for 24h indoors at 23°C followed by an addition of molasses at a rate of 0 , 40 and 100 L tonne^{-1} FM prior to ensilage. Molasses was heated before application to reduce viscosity, and thoroughly mixed in by hand. The molasses used was considered food-grade and contained approximately 427g WSC, 179g other carbohydrates, 220g H_20, 8.5g Ca, 2.5g K and <1g of Cu, Fe, Mg, Mn, P, Se, Na, Zn, niacin, folate and pantothenic acid kg^{-1} molasses.

Experiment 2 Silage Quality Measurements

Grasses were measured for DM loss and chemically analyzed as in Experiment 1.

Experiment 2 Data Analysis

The general linear model (GLM) procedure of SAS (2007) was used to test the statistical differences between the rates of molasses. Planned treatment comparisons were conducted to test if a linear and/or quadratic effect were significant with increasing molasses additions.

Bana Grass (*P. purpureum*)

California Grass (*B. mutica*)

Guinea Grass (*P. maximum*)

Figure 3. 1 Selected tropical grasses for ensilability study.

"Silo" tubes

Close-up of "silos"

Chopped silage

Figure 3. 2 Examples of "silos" and silage used in experiment.

Results and Discussion

Experiment 1

The purpose of Experiment 1 was to determine the ensilability of the three tropical grasses as measured by the fermentative characteristics and to investigate if wilting the grass prior to ensiling improved the ensilability. In order to determine the ensilability of a material a criterion must be defined. Catchpoole and Henzell (1971), who studied ensiling of tropical grasses, defined quality silage as having a pH value <4.2, a butyric acid concentration of <0.2% of DM and a lactic acid concentration between 3% and 13% of DM.

Forage Analysis

In general, the forage quality of the grasses ensiled was similar with the exception that *P. maximum* was consistently of lowest quality (Table 3.1). *P. maximum* is not necessarily a lower quality forage grass (Bogdan, 1977), but in this case it was grown under conditions with moisture and nutrient stress. Major quality differences were net energy for lactation (NEL) ranging from 0.55 to 1.18 mcal kg^{-1}, crude protein (CP) ranging from 155 to 222 g kg^{-1} DM and total water soluble carbohydrates (WSC) ranging from 57 to 92 g kg^{-1} DM. The WSC is important to the silage process because it is the substrate used by the lactic acid bacteria (LAB) to produce lactic acid (McDonald, 1981).

Table 3. 1 Forage analysis of grass material used for ensiling.

Plant Material for Ensilage	CP	WSC	NEL	NDF
	% DM	% DM	Mcal kg^{-1}	% DM
Pennisetum purpureum (Exp. 1)	18.0	9.2	1.16	60.2
Pennisetum purpureum(Exp. 2)	16.2	9.1	1.30	59.8
Brachiaria mutica(Exp. 1)	22.2	5.7	1.18	59.9
Brachiaria mutica (Exp. 2)	19.6	6.5	1.23	62.8
Panicum maximum (Exp. 1)	15.5	7.0	0.55	64.1
Panicum maximum direct cut(Exp. 2)	12.8	7.4	0.50	68.4
Pacicum maximum wilted (Exp. 2)	12.2	8.9	0.53	65.8

CP: Crude protein, WSC: Water soluble carbohydrates, NEL: Net energy for lactation, NDF: Neutral detergent fiber

All analyses done using near infrared spectroscopy

Table 3. 2 Effects of wilting on the dry matter, crude protein and ammonia content of silage.

Plant Material	Treatment	Initial DM%	Final DM% (SD)	Silage Density kg m^{-3} (SD)	DM % Loss (SD)	CP (SD) % DM	Amm-N % of Total N
Pennisetum purpureum	Direct Cut	15.64	14.36 (1.4)	129.3 (5.9)	12.81 (8.52)	19.5 (0)	17.5 (3.5)
	Wilted	28.69	27.56 (0.7)	238.4 (9.3)	2.47 (0.89)	19.3 (0.28)	12.0 (1.4)
Brachiaria mutica	Direct Cut	18.15	15.36 (0.05)	147.3 (9.6)	14.94 (1.18)	21.4 (0.85)	35.0 (8.5)
	Wilted	35.44	34.75 (0.9)	268.7 (16.5)	3.70 (0.12)	22.8 (0)	14.0 (2.8)
Panicum maximum	Direct Cut	22.58	21.15 (0.04)	165.9 (11.2)	4.93 (1.21)	17.6 (0.21)	13.0 (2.8)
	Wilted	57.34	60.21 (1.3)	339.4 (9.0)	0.38 (0.42)	18.1 (0.07)	9.5 (3.5)
Statistical significance							
T-test of wilted vs direct cut.							
Pennisetum purpureum				***<0.001***	0.085	0.250	***0.025***
Brachiaria mutica				***<0.001***	***0.002***	0.129	***0.047***
Panicum maximum				***<0.001***	***<0.001***	0.079	***0.021***

DM: Dry matter, SD: Standard deviation

T-test was used to compare wilted and direct cut means. ***Italic*** p-values were significantly different at $p<0.05$

Chemical composition determined on a dry matter basis.

Table 3. 3 Effects of wilting on the fermentative characteristics and pH of silage.

		Chemical Composition (%DM)						
Plant Material	Treatment	Initial DM %	TA (SD)	LA (SD)	AA (SD)	BA (SD)	PA (SD)	pH (SD)
Pennisetum purpureum	Direct Cut	15.64	4.52 (0.30)	4.01 (0.20)	0.53 (0.09)	nd	nd	4.45 (0.1)
	Wilted	28.69	5.76 (0.21)	5.41 (0.37)	0.22 (0.06)	0.05 (0.07)	0.08 (0.03)	5.65 (0.1)
Brachiaria mutica	Direct Cut	18.15	5.89 (1.05)	2.81 (2.05)	3.08 (1.00)	nd	nd	5.3 (0.6)
	Wilted	35.44	1.04 (0.13)	0.25 (0.21)	0.41(0.04)	0.29 (0.02)	0.12 (0.03)	6.65 (0.1)
Panicum maximum	Direct Cut	22.58	5.77 (0.54)	4.96 (0.30)	0.72 (0.21)	nd	0.07 (0.01)	4.60 (0)
	Wilted	57.34	0.16 (0.06)	0.035 (0.02)	0.12 (0.04)	nd	0.01 (0.01)	6.50 (0)
Statistical significance								
T-test of wilted vs direct cut.			----------p-values----------					
Pennisetum purpureum			***0.025***	***0.033***	***0.036***	0.250	0.077	***0.002***
Brachiaria mutica			***0.036***	0.164	0.082	***0.017***	0.052	0.089
Panicum maximum			***0.021***	***0.014***	0.070	N/A	***0.025***	***0.009***

DM: Dry matter, TA: Total acid, LA: Lactic acid, AA: Acetic acid, BA: Butyric acid including iso-butyric acid, PA: Propionic acid, nd: Not detected, SD: Standard deviation, N/A: Not Applicable

T-test was used to compare wilted and direct cut means. ***Italic*** p-values were significantly different at $p<0.05$

Chemical composition determined on a dry matter basis.

DM Content and DM Loss

The DM content and silage density were increased by wilting in all grass species (Table 3.2). Although initial DM contents were similar for all grasses, DM content after 24h wilting greatly differed among grasses. The DM content of *P. purpureum* increased from 15.64 to 28.69% DM, *B. mutica* from 18.15 to 35.44% DM and *P. maximum* from 22.58 to 57.34% DM with wilting. The silage densities of both wilted and direct cut silage were in the range routinely reported in the literature (150 to 300 kg m^{-3}) (Buxton et al., 2003).

Although DM loss measurements are not used in the criterion of determining silage quality, they are an important measurement of the efficiency of the ensiling process. Loss of DM was significantly lower for wilted grasses in both *B. mutica* and *P. maximum*. The DM loss decreased from 14.94% to 3.70% in *B. mutica* and from 4.93% to 0.38% in *P. maximum*. Wilting *P. purpureum* decreased DM loss from 12.81% to 2.47% although this decrease was not statistically significant because of high variability in the DM loss measurements of direct cut.

There are two main sources of DM loss, effluent and fermentation (Buxton et al., 2003). Effluent losses arise when ensiling crops that are too wet and can be almost completely prevented by wilting the crop. Fermentation losses usually occur when a substrate is converted to CO_2 (g) by microorganisms and then lost from the system. High losses of DM with fermentation happen when microorganisms other than lactic acid bacteria are the dominant population in the silage.

Both *P. purpureum* and *B. mutica* had high DM loss (>12% of DM) when ensiled with direct cut grass. Effluent seepage from the silos was visible and not surprising as both grasses were quite wet (<20% DM) (Kung and Shaver, 2001). After wilting, however, seepage was not visible and DM loss was reduced. Direct cut *P. maximum*, however, had a higher DM before ensiling (22.58% DM) and did not exhibit visual signs of seepage and had a measured DM loss after ensiling of only 4.93% of DM. Miller and Clifton (1965) who ensiled herbage at different DM contents, reduced DM loss of silage from 9.5% to 1.5% of DM by wilting the herbage from 15% DM to 30% DM. They attributed the reduced DM loss to reducing the effluent loss by ensiling drier herbage. The results of Experiment 1 agree with their results and show that effluent loss due to excessively wet herbage was the main factor responsible for DM loss and can be prevented by wilting the grass prior to ensiling.

Silage pH

The mechanism for preservation of forage as silage is to create anaerobic conditions in order to reduce the pH (McDonald, 1981), therefore, silage pH is a main indicator of success of the ensiling process. Kung and Shaver (2001) reported a lower pH of silages occurred when ensiling wilted grass rather than using direct cut grass. In Experiment 1, wilting of the grass prior to ensiling was, however, an ineffective treatment to lower the pH of the tropical grasses (Table 3.3). In all grasses wilting of the herbage prior to ensiling resulted in a higher pH of the silage. Wilting of the grass prior to ensiling significantly increased *P. purpureum* silage pH from 4.45 to 5.56, significantly

increased *P. maximum* silage pH from 4.6 to 6.5 and increased *B. mutica* silage pH from 5.3 to 6.65, although not statistically significant. No silage of any treatment in Experiment 1 had a pH of <4.2, meaning that the preservation of the grass materials through ensiling was poor. High pH silages are highly susceptible to mold and DM loss (Buxton et al., 2003).

Successful silage requires suitable bacteria, moisture and carbohydrates (McDonald, 1981). The high pH of all silages in Experiment 1 was a result of a deficiency of one or more of these requirements for ensiling. Lactic acid bacteria (LAB) are the primary bacteria in silage that cause a pH drop. If these LABs are not present, lactic acid will not be produced and pH will not decrease. In Experiment 1, lactic acid content was measured in substantial concentrations in direct cut silages indicating that LAB were present in significant populations. After wilting, lactic acid production varied between the grasses. For *P. purpureum* lactic acid production increased while for both *B. mutica* and *P. maximum* wilting decreased lactic acid production. Results of *P. purpureum* show that wilting did not hinder LAB populations, while, for *B. mutica* and *P. maximum* wilting seems to be hindering LAB population to flourish. The likely difference is that wilting produced much higher DM content in the latter two grasses, leading to decreased microbial activity.

The moisture of the herbage to be ensiled can greatly influence the resulting silage. McDonald (1981) reported that unless high moisture plant material also had high sugar content and a low buffering capacity, it would be difficult to ensile because high

moisture content promotes clostridial fermentation, dilutes plant sugar concentration and slows the decline in silage pH. In contrast, wilted excessively (>45% DM) decreased fermentation in tropical grass silage (Catchpoole and Henzell, 1971). In Experiment 1, our results tended to agree with the latter report as all wilted grasses ensiled to a higher pH than direct cut. Both *B. mutica* and *P. maximum,* which after wilting had DM > 35%, and did not experience a pH drop after 30d of ensiling.

Water soluble carbohydrates (WSC) are the substrate used by LAB to produce lactic acid. If the carbohydrate content is insufficient, lactic acid production will be inadequate to lower silage pH to <4.2. Because tropical grasses usually contain low amounts of carbohydrates (Sarwatt et al., 1992) and are susceptible to large losses of sugar under high ambient temperatures due to respiration and aerobic decomposition during wilting (Wilson and Webster, 1980), wilting may also reduce WSC content. Results in Experiment 1 show that *P. purpureum*, which had the highest measured WSC content, produced silage of the lowest pH in both direct cut and wilted silage. *B. mutica* and *P. maximum* with lower carbohydrate content had higher silage pH.

Fermentation Products

The fermentation products measured was lactic acid, acetic acid, butyric acid and propionic acid. The amount of these acids in the silage can tell much about the ensiling process. For example, lactic acid is a stronger acid than the other acids produced in ensiling, and therefore is usually responsible for the majority of the drop in pH (Kung and Shaver, 2001). Silages that are dominant in lactic acid will usually be well preserved

(McDonald, 1981). High amounts of butyric acid (>0.5% of DM) indicates a silage has undergone clostridial fermentation and will likely be low in nutritive value and may detrimentally reduce animal performance (Kung and Shaver, 2001).

In Experiment 1, we hypothesized that wilting would positively affect the fermentation products because wilting may change the distribution of bacteria on the herbage. It has been shown that wilting hindered clostridia populations without affecting the LAB populations on herbage (Buxton et al., 2003), therefore producing lactic acid dominant silage. The effect of wilting the grass prior to ensiling on fermentation products differed among grasses, likely because DM content of wilted grasses greatly differed. Total acid production was significantly higher for wilted *P. purpureum* silage compared to direct cut silage (Table 3.3). Wilting increased total acid production from 4.52% to 5.76% of DM, while, wilting increased DM content to 28.69% the lowest of all the grasses. Total acid production was, however, significantly lower for wilted *B. mutica* and *P. maximum* silage compared to direct cut silage. Wilting the grass decreased total acid production from 5.89% to 1.04% DM and 5.77% to 0.16% DM for *B. mutica* and *P. maximum*, respectively and wilting increased DM content to 35.44% and 57.34% which is considerably higher than *P. purpureum*. Results show that the effect of wilting on total acid production is dependent on the extent of wilting, and if too much, will significantly decrease fermentation.

Lactic acid production was significantly higher for wilted *P. purpureum* silage compared to direct cut silage. Wilting the grass increased lactic acid production from

4.01% to 5.41% DM. All *P. purpureum* silage would be classified as lactic acid dominant type as over 88% of the acids produced were lactic. Lactic acid production was, however, significantly lower for wilted *P. maximum* silage compared to direct cut silage. Wilting decreased lactic acid production from 4.96% to 0.035% DM. Wilting *B. mutica* decreased lactic acid production from 2.81% to 0.25% DM although it was not statistically significant. Results of lactic acid production are similar to total acid production. Acid production in the silo was severely limited if wilting was excessive as it was for both *B. mutica* and *P. maximum*. In Experiment 1, there was a -0.70 correlation coefficient between DM% and lactic acid production implying wilting maybe hindering fermentation, especially when DM >35%. Previous studies have shown decreased fermentation of wilted tropical herbage but not to this extent (Catchpoole and Henzell, 1971; van Niekerk et al., 2007). Silage with lactic acid concentrations <1.0% of DM will be inadequately preserved and then be susceptible to harmful bacterial contamination (Kung and Shaver, 2001).

A high concentration of butyric acid in silage (>0.50% of DM) is an indicator of a clostridial fermentation, which is one of the poorest fermentations (Kung and Shaver, 2001). Butyric acid was detected in wilted *B. mutica* and *P. purpureum* silage in low amounts (<0.30% of DM) but not detected in other silages. Only *B. mutica* had significant differences of butyric acid between wilted (0.29% of DM) and direct cut (not detected). Butyric acid concentration measured in Experiment 1 should not negatively affect preservation or stability.

Large amounts of acetic acid (>4% of DM) may depress animal DM intake (Kung and Shaver, 2001). Acetic acid content of silage was less when the grasses were wilted, although only *P. purpureum* silage was significantly different between direct cut and wilted grass. All silage acetic acid concentrations were <4% of DM.

Crude Protein and the Amount of N as Ammonia

Crude protein is a measure of the total nitrogen in the silage. Crude protein content did not differ between wilted and direct cut silage of any grass. The amount of N that is ammonia (Amm-N%) is an indicator of protein breakdown in the silo (Kung and Shaver, 2001). Although in the silage, acidic condition would create ammonium not ammonia, because of the method of measurement it is reported as ammonia. The amount of N that is ammonia was significantly reduced by wilting in all grasses (Table 3.2). Wilting *P. purpureum* reduced the Amm-N % of silage from 17.5 to 12% of the total N. Wilting *B. mutica* reduced the Amm-N % of silage from 35 to 14% of total the N. Wilting *P. maximum* reduced the Amm-N % of silage from 13 to 9.5% of total the N. Results in Experiment 1 agree with those of Kung and Shaver (2001), who reported that wet silages (<30% DM) may have higher levels of Amm-N% than silages with higher DM%.

Experiment 2

Water Soluble Carbohydrates

Molasses was added to the grasses at the rate of 0, 40, and 100 L molasses tonne^{-1} fresh matter (FM) in order to determine if the water soluble carbohydrate (WSC) levels present in the grasses were sufficient for successful ensiling. Deficient WSC levels will limit the ability of lactic acid bacteria (LAB) to preserve the feed. Figure 3.3 shows the measured WSC content of the grasses ensiled at the three molasses rates.

Forage Analysis

In general forage quality was similar to that of Experiment 1 (Table 3.1), however, crude protein was slightly lower, ranging from 12.2 to 19.6% DM. Total WSC was slightly lower, ranging from 6.5 to 9.1% DM. Forages were analyzed immediately after harvesting for both *P. purpureum* and *B. mutica,* while for *P. maximum* forage analysis was done immediately after harvesting and after 24h of wilting. Some differences were measured between direct cut and wilted *P. maximum*. Most noticeable was the increase in WSC from 7.4 to 8.9% DM by wilting the grass.

Silage Density and DM loss

Silage density increased significantly with increasing molasses addition for direct cut *P. purpureum*, *B. mutica* and *P. maximum* (Table 3.3). A significant linear response to molasses addition was found in direct cut *P. purpureum* and *P. maximum* silage, and a significant negative quadratic response was found in direct cut *P. maximum* and *B.*

mutica silage. Silage densities were in the range routinely reported in the literature (Buxton et al., 2003).

Dry matter loss was generally less than in Experiment 1 as fermentation was more robust than in Experiment 1 (indicated by acid production), resulting in decreased DM losses from fermentation. There were no significant differences in DM loss at all molasses rates in *P. purpureum* silage. In all treatments *P. purpureum* silage had relatively low DM loss (<6% of DM) because of strong fermentation. In Experiment 1, direct cut *P. purpureum* silage showed considerable seepage and high DM loss. In Experiment 2, direct cut *P. purpureum* contained 19.84% DM as compared to 15.64% DM in Experiment 1 and the DM losses and seepage was considerably less. The effect of DM content on DM loss is consistent with Experiment 1. Results show a large decrease in DM loss when DM is increased from approximately 15 to 20% DM. In Experiment 2, seepage was only visually noticed at the highest molasses rate.

Dry matter loss significantly decreased with the addition of molasses in both direct cut *B. mutica* and *P. maximum* silage. The addition of molasses decreased the average DM loss of wilted *B. mutica* from 14.42% to 3.61%, although this was not statistically significant. Such an extreme reduction in DM loss of *B. mutica* with the addition of molasses can be explained by the significant silage pH and fermentation product changes. Without molasses *B. mutica* fermentation was acetic acid dominant and CO_2(g) was produced, but when molasses was added *B. mutica* silage pH decreased

and fermentation was almost completely lactic acid dominant resulting in little loss to CO_2 (g) (Table 3.3, 3.4 and 3.5).

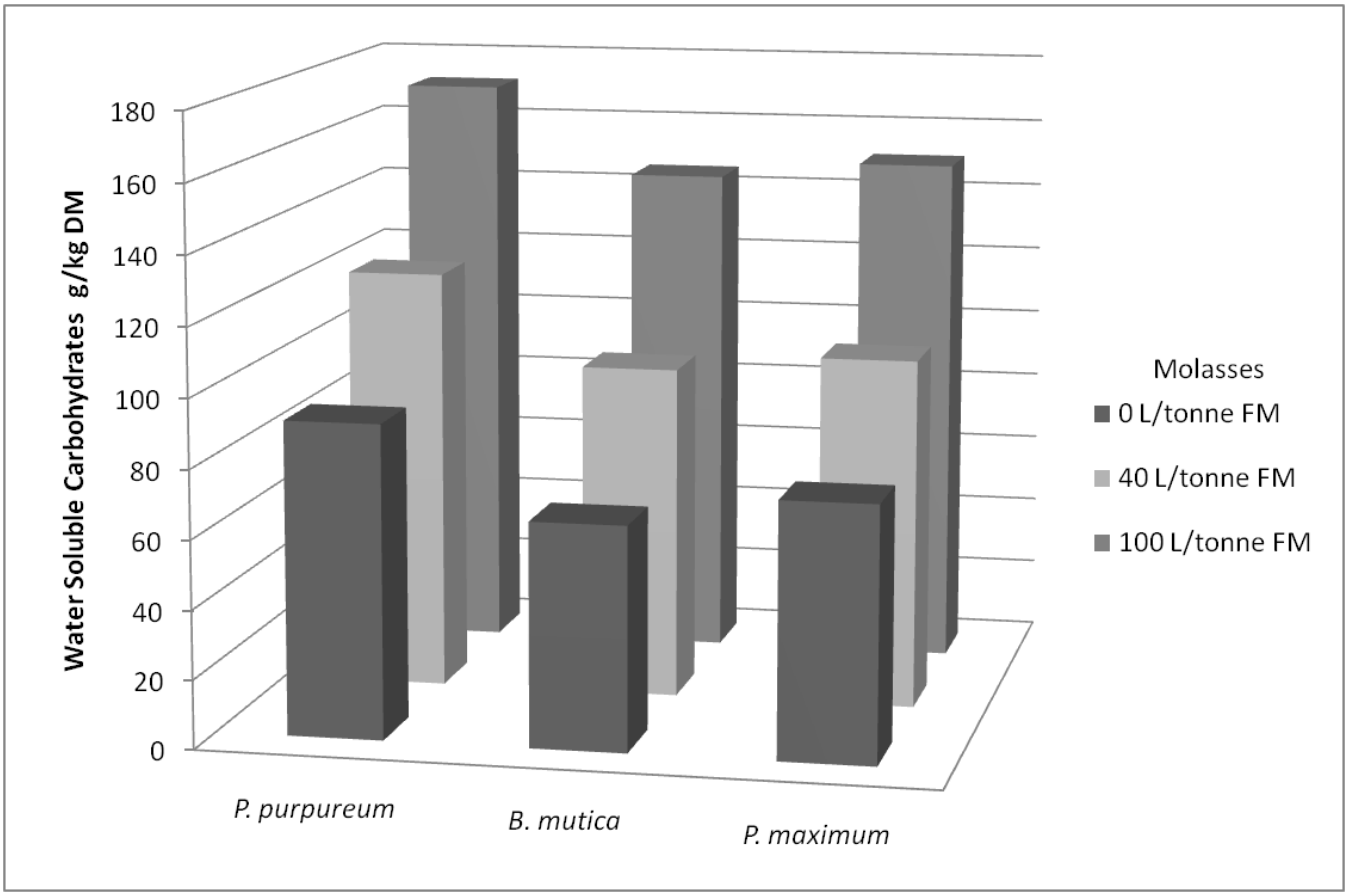


Figure 3. 3 Water soluble carbohydrates (WSC) levels of herbage at the three molasses rates ensiled in experiment 2.

Table 3. 4 Effects of wilting and molasses on silage density and dry matter loss of silages.

Plant Material	Treatment: Moisture	Molasses l ton^{-1} FM	Initial DM%	Final DM% (SD)	Silage Density kg m^{-3} (SD)	DM % Loss (SD)
Pennisetum purpureum	Direct Cut	0	19.84	19.59 (0.20)	149.60 (6.8)	3.44 (1.40)
		40	19.84	20.61 (0.44)	155.61 (5.9)	3.27 (2.91)
		100	19.84	24.22 (0.81)	168.91 (5.3)	4.34 (4.80)
	Wilted	0	28.17	26.59 (0.84)	218.52 (8.9)	5.58 (3.08)
		40	28.17	30.60 (1.25)	220.86 (6.6)	3.34 (1.33)
		100	28.17	30.86 (0.45)	228.67 (7.4)	5.07 (3.34)
Brachiaria mutica	Direct Cut	0	17.39	14.45 (0.65)	135.47 (6.2)	17.28 (1.32)
		40	17.39	16.51 (0.40)	142.93 (4.0)	3.36 (2.03)
		100	17.39	16.66 (0.52)	140.49 (3.2)	5.02 (2.47)
	Wilted	0	26.21	22.29 (0.53)	211.07 (8.2)	14.42 (2.04)
		40	26.21	25.05 (0.67)	207.88 (10.5)	3.61 (2.68)
		100	26.21	25.36 (1.72)	216.89 (11.9)	3.85 (5.45)
Panicum maximum	Direct Cut	0	26.31	26.50 (0.85)	182.95 (1.6)	9.15 (1.50)
		40	26.31	26.50 (0.14)	184.72 (1.4)	3.75 (0.64)
		100	26.31	30.02 (1.34)	197.32 (0.5)	1.55 (0.78)
	Wilted	0	40.15	33.50 (0.57)	239.29 (5.5)	4.25 (0.35)
		40	40.15	37.40 (1.41)	238.93 (1.0)	4.15 (0.50)
		100	40.15	38.10 (1.91)	238.92 (7.2)	3.05 (0.07)

Significance of molasses and test for linear or quadratic response

			p-values	
Pennisetum purpureum	Direct Cut	Molasses Level	***0.007***	***0.912***
		Linear	***0.002***	***0.751***
		Quadratic	0.690	0.802
	Wilted	Molasses Level	0.301	***0.602***
		Linear	0.147	***0.826***
		Quadratic	0.640	***0.344***
Brachiaria mutica	Direct Cut	Molasses Level	***0.027***	***<0.001***
		Linear	0.053	***<0.001***
		Quadratic	***0.035***	***0.002***
	Wilted	Molasses Level	0.255	***0.112***
		Linear	0.162	***0.090***
		Quadratic	0.374	***0.145***
Panicum maximum	Direct Cut	Molasses Level	***0.003***	***0.011***
		Linear	***0.002***	***0.005***
		Quadratic	***0.017***	0.172
	Wilted	Molasses Level	0.208	0.073
		Linear	0.144	***0.043***
		Quadratic	0.285	0.201

DM: Dry Matter, SD: Standard Deviation

Contrasts were used in Anova to test if molasses addition produced either linear or quadratic response. ***Italic*** p-values are significant at p<0.05

Table 3. 5 Effects of wilting and molasses on pH, crude protein and ammonia concentration of silages

Plant Material	Treatment: Moisture	Molasses l ton^{-1} FM	pH (SD)	CP (SD)	Amm-N % of Total N (SD)
Pennisetum purpureum	Direct Cut	0	4.2 (0)	17.0 (0.42)	11.5 (2.1)
		40	3.95 (0.07)	15.1 (1.27)	10.0 (4.2)
		100	3.8 (0)	15.2 (0.14)	9.5 (2.1)
	Wilted	0	5.1 (0)	16.3 (0.92)	11.5 (3.5)
		40	4.9 (0)	15.6 (0.28)	15.5 (4.9)
		100	4.25 (0.07)	15.5 (1.34)	10.5 (2.1)
Brachiaria mutica	Direct Cut	0	5.45 (0.21)	20.6 (0.35)	33.0 (7.1)
		40	3.6 (0)	17.9 (0.28)	7.0 (0)
		100	3.7 (0)	16.5 (0.28)	6.0 (2.8)
	Wilted	0	5.35 (0.21)	18.0 (0.21)	27.5 (6.4)
		40	3.85 (0.07)	17.5 (0.28)	6.0 (1.4)
		100	3.8 (0)	16.5 (0.21)	5.5 (0.7)
Panicum maximum	Direct Cut	0	5.6 (0.14)	11.7 (0.42)	9.0 (1.4)
		40	4.9 (0)	11.1 (0.42)	10.0 (5.7)
		100	4.6 (0.14)	10.1 (0.07)	5.5 (2.1)
	Wilted	0	6.2 (0)	12.4 (0.92)	13.0 (0)
		40	6.15 (0.07)	11.9 (0.07)	11.0 (1.4)
		100	5.75 (0.35)	11.4 (1.13)	5.5 (2.1)
Statistical significance of molasses and test for linear or quadratic response					
			----------p-values----------		
Pennisetum purpureum	Direct Cut	Molasses Level	***0.005***	0.152	0.799
		Linear	***0.002***	0.104	0.553
		Quadratic	0.252	0.235	0.860
	Wilted	Molasses Level	***0.005***	0.644	0.461
		Linear	***<0.002***	0.415	0.805
		Quadratic	***0.008***	0.741	0.268
Brachiaria mutica	Direct Cut	Molasses Level	***0.001***	***0.002***	***0.014***
		Linear	***0.046***	***0.009***	***0.009***
		Quadratic	***0.003***	0.086	***0.046***
	Wilted	Molasses Level	***0.002***	***0.018***	***0.016***
		Linear	***0.001***	***0.008***	***0.010***
		Quadratic	***0.007***	0.404	***0.049***
Panicum maximum	Direct Cut	Molasses Level	***0.007***	***0.046***	0.503
		Linear	***0.003***	***0.021***	0.400
		Quadratic	0.139	0.603	0.441
	Wilted	Molasses Level	0.205	0.534	***0.030***
		Linear	0.119	0.301	***0.015***
		Quadratic	0.403	0.975	0.263

DM: Dry Matter, SD: Standard Deviation, CP: Crude protein % of DM, Amm-N% of total N: Percentage of total nitrogen as ammonia

Table 3. 6 Effects of wilting and molasses on the fermentative characteristics of silage.

	Treatment		Chemical Composition %DM				
Plant Material	Moisture	Molasses L ton^{-1} FM	TA (SD)	LA (SD)	AA (SD)	BA (SD)	PA (SD)
Pennisetum purpureum	Direct Cut	0	6.78 (0.17)	6.17 (0.24)	0.58 (0.03)	nd	0.10 (0)
		40	7.21 (0.81)	6.53 (0.16)	0.68 (0.96)	nd	nd
		100	9.59 (2.05)	8.64 (0.89)	0.87 (1.15)	nd	0.05 (0.06)
	Wilted	0	3.19 (0.09)	2.77 (0.16)	0.38 (0.09)	nd	0.02 (0.03)
		40	3.75 (0.17)	3.30 (0.18)	0.46 (0)	nd	nd
		100	5.98 (0.02)	5.74 (0.27)	0.24 (0.24)	nd	0.05 (0)
Brachiaria mutica	Direct Cut	0	9.47 (0.12)	1.70 (0.37)	7.67 (0.49)	nd	0.03 (0.04)
		40	15.10 (0.30)	14.21 (0.14)	0.89 (0.16)	nd	nd
		100	11.59 (0.39)	11.17 (0.87)	0.38 (0.53)	nd	0.08 (0.01)
	Wilted	0	6.30 (0.09)	1.92 (0.31)	4.37 (0.24)	nd	0.02 (0.03)
		40	10.69 (2.00)	10.23 (1.44)	0.46 (0.55)	nd	nd
		100	11.02 (0)	10.59 (0.54)	0.39 (0.54)	nd	nd
Panicum maximum	Direct Cut	0	2.59 (0.22)	2.04 (0.11)	0.39 (0.01)	0.07 (0.09)	0.09 (0)
		40	6.38 (2.77)	5.96 (2.71)	0.30 (0.06)	nd	0.12 (0.01)
		100	7.26 (3.50)	6.95 (3.52)	0.28 (0.06)	nd	0.03 (0.04)
	Wilted	0	1.48 (0.28)	1.22 (0.24)	0.26 (0.02)	nd	nd
		40	1.91 (0.53)	1.63 (0.45)	0.28 (0.04)	nd	nd
		100	2.89 (2.36)	2.65 (2.63)	0.22 (0.04)	nd	0.03 (0.04)
Statistical significance of molasses and test for linear or quadratic response							
			----------p-values----------				
Pennisetum purpureum	Direct Cut	Molasses Level	0.205	***0.036***	0.943	N/A	0.121
		Linear	0.115	***0.020***	0.756	N/A	0.168
		Quadratic	0.442	0.158	0.953	N/A	0.092
	Wilted	Molasses Level	***<0.001***	***0.002***	0.430	N/A	0.465
		Linear	***<0.001***	***<0.001***	0.401	N/A	0.305
		Quadratic	***0.004***	***0.013***	0.335	N/A	0.535
Brachiaria mutica	Direct Cut	Molasses Level	***<0.001***	***<0.001***	***<0.001***	N/A	0.174
		Linear	***0.006***	***<0.001***	***<0.001***	N/A	0.270
		Quadratic	**<0.001**	***<0.001***	***0.003***	N/A	0.116
	Wilted	Molasses Level	***0.045***	***0.004***	***0.006***	N/A	0.118
		Linear	***0.026***	***0.002***	***0.003***	N/A	0.164
		Quadratic	0.136	***0.014***	***0.018***	N/A	0.089
Panicum maximum	Direct Cut	Molasses Level	0.303	0.274	0.196	0.465	0.083
		Linear	0.170	0.151	0.104	0.308	0.103
		Quadratic	0.563	0.556	0.506	0.530	0.075
	Wilted	Molasses Level	0.634	0.623	0.301	N/A	0.465
		Linear	0.388	0.381	0.326	N/A	0.308
		Quadratic	0.837	0.819	0.225	N/A	0.503

DM: Dry Matter, SD: Standard Deviation, TA: Total acid, LA: Lactic acid, AA: Acetic acid, BA: Butyric acid including iso-butyric acid, PA: Propionic acid, ND: Not setected, N/A: Not applicable for statistical analysis

Contrasts were used in Anova to test if molasses addition produced either linear or quadratic response. ***Italic*** p-values are significant at p<0.05

Silage pH

The pH of the resulting silage significantly decreased with the addition of molasses in all grasses and treatments except wilted *P. maximum* (Table 3.4). Direct cut silage made from all grasses showed a significant linear response while wilted *P. purpureum* and *B. mutica* silage showed both a significant linear and positive quadratic response to molasses. Average pH of all silage at the 0 L molasses tonne^{-1} FM was 5.3 which was reduced to 4.5 at the 40 L molasses tonne^{-1} FM rate and again reduced to 4.3 at the 100 L molasses tonne^{-1} FM rate.

The silage pH of *P. purpureum* and *B. mutica* after molasses additions was <4.2, indicating that these silages will be highly stable because adequate acidity was produced which would prevent clostridia and other spoilage microorganisms from growing. The change in pH of *P. purpureum* and *B. mutica* silage was significantly positively quadratic. This implies that if molasses rates continued to increase, final silage pH may rise.

The lower pH of direct cut silage contrasts with Kung and Shaver (2001), who reported a higher pH for silage from direct cut herbage than from wilted herbage. In the present experiment when wilting the herbage exceeds 35% DM, fermentation was greatly restricted, resulting in unstable silage. It seems that the effectiveness of wilting depends on many factors among which are the inherent variations between species in terms of wilting rate, WSC and DM content.

Fermentation products

Fermentation products are the acids produced during ensiling. With the addition of molasses total acid content of silage was significantly increased in wilted *P. purpureum* silage and in both direct cut and wilted *B. mutica* silage (Table 3.5). Wilted *P. purpureum* showed both significant linear and quadratic response to molasses additions, while direct cut and wilted *B. mutica* showed a significant linear increase. No *P. maximum* silage, however, showed a significant effect on total acid production from the addition of molasses.

Lactic acid content of silage was significantly increased in all *P. purpureum* and *B. mutica* silage with the addition of molasses. Lactic acid content of wilted *P. purpureum* and both direct cut and wilted *B. mutica* showed significant negative quadratic responses indicating that high levels of molasses may hinder fermentation. *P. maximum* silage, however, did not show a significant effect on lactic acid production from the addition of molasses. For direct cut *P. maximum,* however, there was an increase in lactic acid production with molasses although high variability resulted in no significance. For wilted *P. maximum* a high DM content of 40% likely hindered fermentation. Our results, with the exception of *P. maximum*, agree with previous findings that reported increased lactic acid and decreased acetic acid production in tropical grasses with the addition of molasses (Yunus et al., 2001).

Acetic acid production was low and did not significantly change with molasses additions in *P. purpureum* and *P. maximum* silages*. B. mutica* silage, however, contained

high amounts of acetic acid (7.67 % DM for direct cut and 4.37 % DM for wilted silage) at the 0 L molasses tonne^{-1} FM rate but exhibited significant decreases as the molasses rate increased (Table 3.5). These changes in acetic acid indicate a major change in the ensiling system after the addition of molasses. In *B. mutica* at the 0 L molasses tonne^{-1} FM rate, the fermentation would be classified as acetic acid dominant for both direct cut and wilted; but at both the 40 and 100 L molasses tonne^{-1} FM rate the silage is clearly lactic acid dominant (Figure 3.4). Acetic acid dominant silages produce low quality silage, as acetic acid buffers against a decline in silage pH below 4.8 (Bates et al., 1989). This agrees with results in Experiment 2 as *B. mutica* silage pH was above 5.3 at the 0 L molasses tonne^{-1} FM rate. *B. mutica* silage showed significant linear decreases to the acetic acid concentration with increasing molasses additions.

A high concentration of butyric acid (>0.50% of DM) is an indicator of a clostridial fermentation which is one of the least desirable fermentations (Kung and Shaver, 2001). Butyric acid was detected in very small concentration (0.07% of DM) in direct cut *P. maximum* at the 0 L molasses tonne^{-1} FM rate. Butyric acid was not detected in the silage of any other grasses and treatments. Propionic acid was measured in some samples in low quantities (<0.075 % DM). Propionic and butyric acid contents measured in Experiment 2 were low and should not affect animal performance.

Crude Protein and the Amount of N as Ammonia

Average crude protein decreased with increasing molasses application in all grasses, however, only *B. mutica* and direct cut *P. maximum* silage showed a significant

decrease with increasing molasses additions. The amount of N that is ammonia (Amm-N%) is an indicator of protein breakdown in the silo, which is caused by a slow drop in pH or clostridial fermentation (Kung and Shaver, 2001). The amm-N% in *P. purpureum* silage did not significantly differ with different rates of molasses additions. The amm-N% for both direct cut and wilted *B. mutica* silage was high at the 0 L molasses tonne^{-1} FM rate (33% and 27.5%) and was significantly decreased to 6% and 5% of total N with molasses additions. Acetic acid ensiling such as that of *B. mutica* at the 0 L molasses tonne^{-1} FM rate have higher rates of deamination of amino acids, which results in higher ammonia levels (McDonald, 1981) than lactic acid dominant silage. The amm-N% showed both a linear and quadratic response with increasing molasses addition in *B. mutica* silage. For wilted *P. maximum* amm-N% also significantly decreased, indicating a more highly desirable ensiling process.

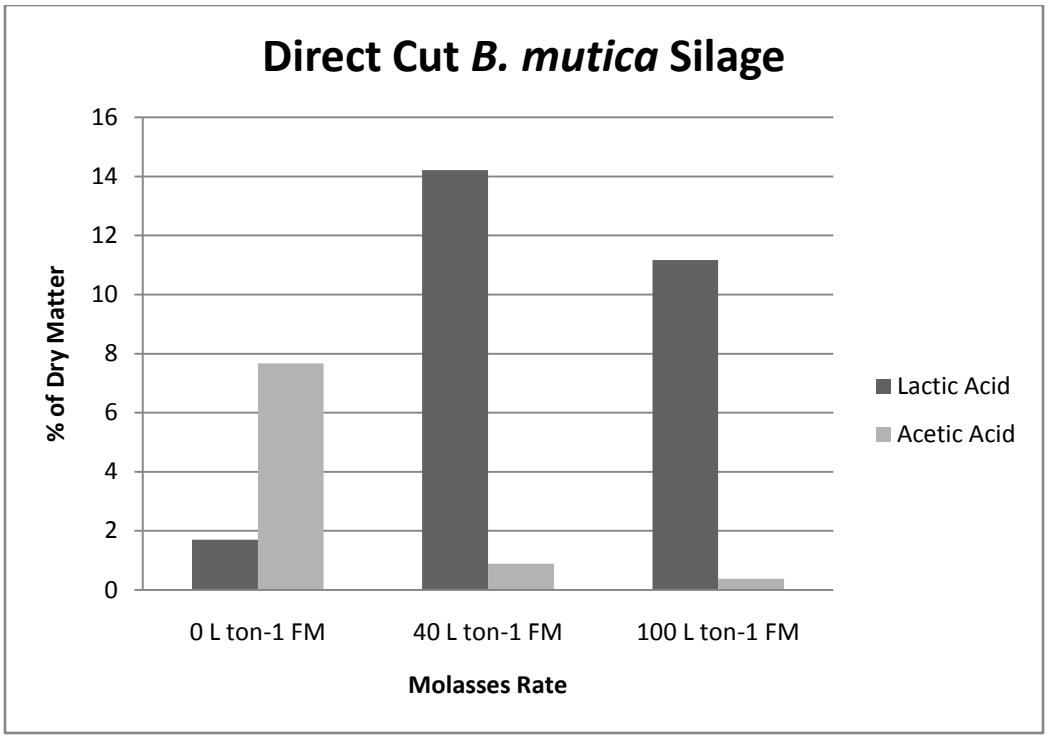
Although CP of *B. mutica* was significantly reduced with increasing molasses addition, the significantly reduced Amm-N% actually corresponded to a net gain of animal available protein. For example, assuming that crude protein consists of true protein and ammonia, *B. mutica* direct cut silage at the 0 L molasses tonne^{-1} FM rate had on average 20.65% CP in DM but 33% was ammonia resulting in only 13.8% true protein in DM. *B. mutica* direct cut silage at the 40 L molasses tonne^{-1} FM rate had total CP of 17.9% of DM, which was significantly lower, but the Amm-N % was significantly lower at 7% of total N resulting in 16.6% of true protein in DM, approximately a 20% increase in true protein.

Additional Discussion of Molasses

In addition to the large influence of molasses on the fermentation and thus, the ensiling process, molasses supplementation to silage increases silage intake (Bolsen et al., 1996). Molasses additions can, therefore, enhances animal performance in ruminants (Bertilsson and Burstedt, 1983). Lattemae (1997) reported increases in ruminant digestion at the highest molasses rate (100 L molasses tonne^{-1} FM), likely resulting in increased intake. Further, milk yield tended to increase with increasing level of molasses without affecting fat and protein concentrations. Improved aerobic stability of silage can also be expected when the pH is lowered quickly (McDonald, 1981). This will occur when the ensiled material contains high levels of WSC and LAB flourish. According to Aguilera et al. (1997), in molasses treated silage the utilization of NDF and lignocellulose complex by fermenting microorganisms is expected to be low due to the presence of high levels of WSC from molasses. More nutrients may, therefore, be retained in the silages treated with molasses than those without molasses addition.

Statistical analysis showed that increasing molasses additions often showed a quadratic effect. For example, pH, lactic acid and total acid production all showed quadratic effects in wilted *P. purpureum* and direct cut *B. mutica* indicating reduced fermentation at the highest molasses rate. Dry matter loss of direct cut *B. mutica* silage also showed a negative quadratic effect indicating increased amount of DM loss at the highest molasses rate likely due to increased seepage of effluent. Molasses at the two higher rates both favored fermentation although at the highest rate, fermentation was

often slightly restricted. In all cases silage at the 100 L molasses tonne^{-1} FM rate was still of higher quality than at the 0 L molasses tonne^{-1} FM rate. Although rates in excess of 100 L molasses tonne^{-1} FM were not tested, if the negative quadratic trend continued molasses may begin to hinder fermentation and silage quality. Lattemae (1997) reported similar results of a negative quadratic effect under the same rates of molasses and attributed this to osmosis and buffering properties of molasses.



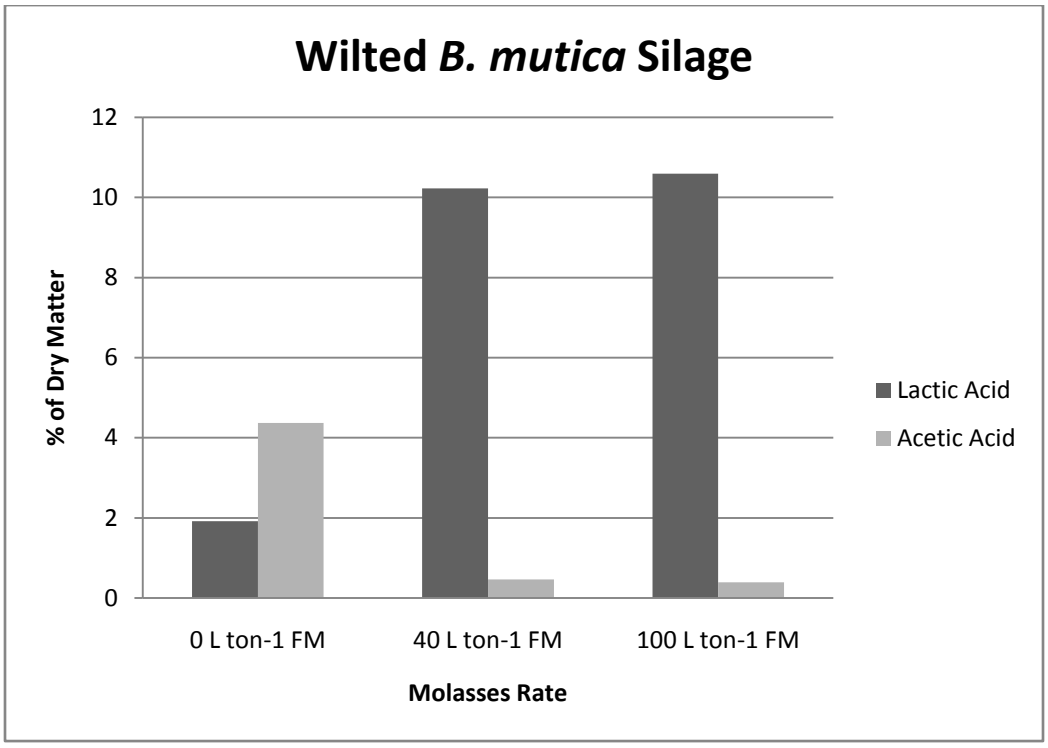


Figure 3. 4 Changes in fermentative characteristics of B. mutica silage with different rates of molasses.

Conclusions

In a series of two experiments, silage made from direct cut and wilted *Pennisetum purpureum* (Schumach.), *Brachiaria mutica* (Forssk.) and *Panicum maximum* (Jacq.) with three rates of molasses was analyzed to determine fermentative characteristics. Results showed that if wilting increased dry matter to >35%, fermentation was greatly reduced. Although wilting of the grass hindered fermentation in Experiment 1, it offered benefits of increased silage density, lower DM loss and lower ammonia content.

Molasses was shown to benefit the ensiling process by reducing pH and increasing fermentation. Of the three grasses only *P. purpureum* contained sufficient WSC for fermentation, although inconsistent and only when ensiled directly. When WSC was increased by adding molasses, fermentation intensity of *B. mutica* increased and a major shift in the type of fermentation took place. For example, *B. mutica* was an acetic acid dominant fermentation without molasses, but when molasses was added to the plant material, the fermentation became overwhelmingly lactic acid dominant. With the addition of molasses in Experiment 2, wilted silage, except that of *P. maximum* was of low pH and lactic acid dominant while maintaining the advantages of wilting. *P. maximum* was excessively wilted (>35% DM), which resulted in weak fermentation.

In Experiment 2, increasing molasses additions often resulted in a quadratic effect on silage quality. The 40 L molasses tonne^{-1} FM rate produced satisfactory results in accordance with the standards referenced. Although similar results were also found

at the 100 L molasses tonne^{-1} FM rate, little to no additional benefits do not justify the extra costs of this extra addition. Rates above 100 L tonne^{-1} FM may begin to hinder fermentation.

Although butyric acid was generally not detected, it should be noted that this was a controlled experiment on a very small scale. High amounts of butyric acid would indicate that the silage has undergone clostridial fermentation, which results in low nutritive value (Kung and Shaver 2001). When animal manure and/or soil are in contact with plant material, the number of clostridial spores usually increase (Buxton et al., 2003). During mowing and harvesting, soil or manure may come in contact with the plant material especially following a rain event. The best way to ensure a clostridial free fermentation is through a strong and fast lactic acid fermentation, which is possible with the addition of a WSC source such as molasses.

Chapter 4. The Buffering Capacity of the Three Tropical Grasses and the Effect of Wilting

Introduction

The buffering capacity of herbage, i.e., its ability to resist pH change is an important factor in ensiling. Herbages of high buffering capacity will require more acid to achieve anaerobic stability, assuming that fermentable substrate is not limiting. Roughly 80 to 90% of the buffering of herbages can be attributed to the anionic fraction of herbage (organic acid salts, orthophosphates, sulfates, nitrates, and chlorides) (McDonald, 1981), while plant proteins account for the remaining 10 to 20%.

The buffering action of organic acids and their salts are strongest over the pH range 6.0 to 4.0 which, unfortunately, is the usual pH range of ensiled herbage (Woolford, 1984). This, in combination with organic acid concentrations of approximately 2 to 8% of DM result in organic acids being the main source of buffering action in herbage. Grasses, which have less organic acids than legumes (2 to 6% of DM vs. 6 to 8% of DM) have lower buffering capacities (Woolford, 1984) and are thought to have higher ensilability than legumes.

Buffering capacity is experimentally defined as the amount of lactic acid needed to lower the pH of a herbage to 4.0. This value is used because silage preserved at this level is regarded as satisfactory (Catchpoole and Henzell, 1971). Wilted herbage should have a lower buffering capacity than direct cut material because during wilting, organic

acids will be respired to CO_2 and H_20. The objective of this research reported in this chapter was to:

1. Determine the buffering capacity of direct cut and wilted *Pennisetum purpureum* (Schumach.), *Brachiaria mutica* (Forssk.) and *Panicum maximum* (Jacq.)

Materials and Methods

Buffer Capacity

Buffering capacity measurements were performed as described in McDonald and Henderson (1962). 25.0g of fresh matter (FM) direct cut and 20.0g of FM wilted *P. purpureum*, *B. mutica* and *P. maximum* were macerated in a blender for 3 minutes with 250 ml deionized water and the mixtures were transferred to 600 ml glass beakers. Initial pH of the mixtures was measured with a glass electrode pH meter and then the mixtures were titrated with 0.1 *M* lactic acid ($C_3H_6O_3$) while being continually mixed until pH 4.0. All titrations were done in triplicate. For a blank, 250 ml of deionized water was titrated with 0.1 *M* lactic acid, and the amount of lactic acid was subtracted from the herbage titration results.

A subsample of all direct cut and wilted herbage was taken to determine the dry matter (DM) content according to the procedure described in Chapter 3. The DM content was used to calculate the DM amount of herbage used in the titration. The buffering capacity was then defined as the amount of 0.1 *M* lactic acid needed to lower the pH of 1.0 g of DM herbage to pH 4.0.

Statistical Analysis

The data was analyzed statistically using a t-test of the means of the volume of 0.1 M lactic acid needed to lower the pH of 1 g of DM direct cut and wilted herbage with the null-hypothesis that the means were equal. The null hypothesis was rejected if p-values were ≤0.05.

Results and Discussion

In all grasses, initial pH of wilted grasses was higher than that of direct cut (Table 4.1). The higher pH may be the result of more dry matter (DM) in the wilted grass mixture, which contained more proteins and amino acids that could decompose to $NH_3$and raise the pH (Woolford, 1984).

The buffering capacity of wilted and direct cut *P. purpureum* and *P. maximum,* however, did not statistically differ (Table 4.1). Buffering capacity of wilted *B. mutica* was significantly lower (39.3 mg $C_3H_6O_3$ g^{-1} DM) than that of direct cut (47.1 mg $C_3H_6O_3$ g^{-1} DM). Wilted herbage is expected to have less buffering capacity than direct cut because wilting stimulates respiration of organic acids that contribute to buffering (Woolford, 1984).

The buffering capacities of the three tropical grasses, both direct cut and wilted ranged from 38.1 to 56.3 mg of $C_3H_6O_3$ g^{-1} DM (Table 4.1). McDonald and Henderson (1962) quantified the buffering capacity of six temperate grasses and five legumes and reported that the grasses ranged from 23 to 42 mg of $C_3H_6O_3$ g^{-1} DM, while the legumes

ranged from 38 to 66 mg of $C_3H_6O_3$ g^{-1} DM. The three tropical grasses therefore tended to have higher buffering capacity than temperate grasses and were similar to the range of legumes. Legumes are known to be difficult to ensile because of their high buffering capacity from higher concentrations of organic acids and proteins (Lattemae, 1997).

Table 4. 1. The buffering capacity of the three tropical grasses either wilted or direct cut.

Grass Material	Treatment	Initial pH (SD)	Buffering capacity (SD)
Pennisetum purpureum	Direct cut	5.28 (0.10)	38.1 (7.4)
	Wilted	5.79 (0.02)	44.6 (4.7)
Brachiaria mutica	Direct cut	6.03 (0.03)	47.1 (3.2)
	Wilted	6.23 (0.02)	39.3 (1.4)
Panicum maximum	Direct cut	6.06 (0.04)	49.0 (7.4)
	Wilted	6.24 (0.05)	53.6 (2.9)
Statistical significance			
T-Test for differences between direct cut and wilted.			
Pennisetum purpureum			0.121
Brachiaria mutica			***0.015***
Panicum maximum			0.262

Dm: dry matter, SD: standard deviation, Buffering capacity:mg of 0.1 *M* $C_3H_6O_3$ needed to lower 1g DM to pH 4.0.

T-test to test the hypothesis that wilted and direct cut means are equal. **Italic** p-values significantly different at $p<0.05$

Conclusions

Of the three tropical grasses, only the buffering capacity of *B. mutica* was significantly reduced by wilting. All three grasses had high buffering capacities compared to temperate grass. If tropical grasses have fewer carbohydrates (Yunus et al., 2001) and

higher buffering capacity, ensiling of tropical grasses could be difficult without increasing the carbohydrate content.

Chapter 5. A Closer Look at Water Soluble Carbohydrates

Introduction

Water soluble carbohydrates (WSC) are the substrate used by the lactic acid bacteria to produce lactic acid which in turn, preserves the herbage through a drop in pH. In Chapter 3, we showed that a lack of WSC resulted in low-quality silage; and when carbohydrates were increased, fermentation quality significantly increased. Because carbohydrates are central to silage fermentation and tropical grasses are known to be low in carbohydrates (Sarwatt et al., 1992), it may be beneficial to silage producers to closely examine the variability of the carbohydrates and the relationship with forage quality. In this chapter the objective was to:

1. Quantify the water soluble carbohydrate content of the same grass (*Panicum maximum* (Jacq.)) at different locations and to observe if there were any correlation between carbohydrates and forage quality measurements.

Materials and Methods

Materials

Five samples of *Panicum maximum* were taken in the late afternoon at 5 locations at Island Dairy in O'okala, Island of Hawaii, HI in August 2008. At each location the grass was described by color, height and morphological characteristics (i.e. leafy or stemmy) and elevation was estimated using a digital elevation map. Samples were taken

by hand with a sickle and were immediately put on ice until they could be frozen (approximately 4h).

WSC Analysis

The following day they were weighed and then dried at 70°C in a forced draft oven, DM was determined as in Chapter 3. Samples were then sent to Dairy One (DHI Forage Testing Lab, Ithaca, NY) for forage quality analysis as described in Chapter 3. A correlation analysis was performed to determine if WSC were correlated with other forage quality measurements.

Results and Discussion

Table 5.1 shows the grass species, sample description and the results from the forage analysis which included: WSC, crude protein (CP), net energy of lactation (NEL), acid detergent fiber (ADF) and neutral detergent fiber (NDF) and the correlation coefficients of the above measurements with WSC.

Crude Protein

The correlation coefficient between crude protein and WSC was low at 0.30. The low correlation was likely due to the high CP but low WSC of *B. mutica.* One sample of *B. mutica* had the highest CP although the measured WSC was among the lowest. Although the correlation was low, it was positive indicating that CP increased with higher WSC.

ADF and NDF

The correlation coefficient of both ADF and NDF to WSC was negative and both were nearly the same at -0.59 and -0.58. The negative correlation although moderately low shows that as NDF or ADF increases as WSC decreases.

NEL

The correlation coefficient of NEL with WSC was moderately 0.60. The positive correlation implies that NEL increases with higher amounts of WSC.

Conclusions

The correlation between WSC and the other measurements of forage quality was low. There was no definite indicator of WSC from another measurement. The general results do show that increased carbohydrates are correlated with increased forage quality. For example, there was a positive correlation between both CP and NEL with WSC while a negative correlation between ADF and NDF to WSC was observed. Forage of high quality will have high CP and NEL, and low ADF and NDF (Bogdan, 1977), which should then result in higher carbohydrate content.

The Island Dairy samples were taken in the late afternoon which is when the WSC content of the plant is at its highest (Woolford, 1984). Although WSC were likely at their highest, overall WSC content of the Island Dairy *P. maximum* were low from an ensiling standpoint. The WSC ranged from 4.2 to 7.2% of DM, which was in the same range as the *P. maximum* harvested in Manoa and ensiled in Chapter 3. This amount of

WSC in the plant material at Island Dairy is likely not enough substrate to ensure optimal fermentation. A molasses addition (or another sugar source) may be needed for optimum fermentation intensity and to ensure preserved and stable silage of herbage from this location at O'okala, Hawaii.

Table 5. 1. Forage analysis including water soluble carbohydrates of selected forage grasses.

Grass	Description	Approx. Elevation	WSC	CP	ADF	NDF	NEL
		m	----------% of DM----------				mcal kg^{-1}
P. maximum	Medium to dark green, 0.5 to 1.0 m, stemmy	427	4.2	10.0	41.4	69.7	0.99
P. maximum	Dark green, 0.5 to 1.0 m, leafy	412	4.7	16.4	35.5	64.2	1.17
P. maximum	Dark green, 0.5 to 0.8 m, leafy	226	7.2	16.5	35.2	60.7	1.19
P. maximum	Green to slightly yellow, 0.5 to 1.0 m, slightly stemmy	236	5.8	8.3	40.6	67.5	1.03
P. maximum	Light to medium green, 0.75 to 1.25 m, slightly stemmy	183	5.9	12.7	38.4	66.0	1.12
P. purpureum	Medium to dark green, 0.5 to 1.0 m, leafy	44	9.2	18.0	32.6	60.2	1.16
P. Purpureum	Medium to dark green, 0.5 to 1.0 m, leafy	44	9.1	16.2	34.7	59.8	1.30
B. mutica	Green to slightly yellow, 0.5 m, slightly stemmy	44	5.7	22.2	34.4	59.8	1.18
B. mutica	Green to slightly yellow, 0.5 m, slightly stemmy	44	6.5	19.6	33.3	62.8	1.23
P. maximum	Green to slightly yellow, 1.0 m, stemmy	44	7.0	15.5	34.0	64.1	1.21
P. maximum	Green to slightly yellow, 1.0 m, stemmy	44	7.4	12.8	37.7	68.4	1.10
		Correlation Coefficient		0.30	-0.59	-0.58	0.59

DM: Dry Matter, WSC: Water soluble carbohydrates, CP: Crude protein, ADF: acid detergent fiber, NDF: Nuetral detergent fiber, NEL: Net energy for lactation Correlation coefficients is of WSC and the constituent above the coefficent

Chapter 6. General Summary, Conclusions and Recommendations

General Summary

Costs of operating a livestock operation in Hawai'i continually rise, as a result of increasing energy and importation costs. Dairies must become more feed sustainable in order to survive. The utilization of local grass-based forages is becoming a viable alternative. When growing forages on a commercial scale, harvested forage is often in surplus and must be conserved. The main goal of conservation is to preserve high nutritive forage with minimal losses and a good hygienic quality. This study assessed the ensilability of three tropical grass, *Pennisetum purpureum* (Schumach.), *Brachiaria mutica* (Forssk.) and *Panicum maximum* (Jacq.) which have been selected as high potential for use as dairy forage in Hawaii.

In a series of experiments the effects of wilting and applying molasses to the herbage prior to ensiling were observed on fermentative characteristics. The results showed that wilting the grass significantly reduced dry matter (DM) loss and the ammonia content in all silages, however, wilting significantly raised the silage pH of *B. mutica* from 5.3 to 6.65 and *P. maximum* from 4.6 to 6.5. However, for *P. purpureum,* wilting, increased total acid production although the pH remained higher (5.65) than direct cut (4.45). The difference between the results of wilting on the different grasses was from the extent of drying during wilting, both *B. mutica* and *P. maximum* where

wilted to >35% DM which resulted in decreased fermentation and acid production. When molasses was added, pH decreased and lactic acid production increased in both direct cut and wilted grass. Although when wilting increased DM to >35%, fermentation was greatly reduced even with molasses.

The buffering capacity dictates how much acid is needed to lower the pH to an acceptable level. Results showed that the three grasses had high buffering capacities when compared to temperate grasses. Wilting the herbage is expected to decrease buffering capacity, however, only *B. mutica* showed a significant decrease. High buffering capacity of grasses will decrease ensilability especially when water soluble carbohydrates are low.

Water soluble carbohydrates are the substrate that is converted to lactic acid during ensiling. Herbages of low carbohydrate contents will be difficult to preserve. Results in this thesis show that tropical grasses often contain low amounts of carbohydrates which result in poorly ensiled herbage.

Conclusions

In general, ensiling tropical grasses is hindered by their high buffering capacity and low carbohydrate content. When a carbohydrate source was added to the herbage prior to ensiling, fermentation intensity was dramatically increased and the silage pH was reduced to <4.2, the level that defines stable silage. Wilting decreased DM loss and

the amount of ammonia but when wilting increased DM to >35%, fermentation was greatly reduced.

Recommendations

P. purpureum

Of all the grasses *P. purpureum* had the highest concentration of water soluble carbohydrates (WSC) which, in turn produced the most lactic acid during ensiling. *P. purpureum* also had the lowest DM content which led to high losses of DM from seepage of effluent. It would be recommended to lightly wilt *P. purpureum* herbage to between 20 to 25% DM to reduce effluent, chop the herbage to <1cm to allow for good compaction and to add molasses at no higher than the 40L tonne^{-1} fresh *P. purpureum* rate to ensure ample WSC for fermentation. Results from this recommendation should ensure a stable silage with pH between 3.8 to 4.0.

B. mutica

Results of Experiment 2 show that *B. mutica* benefitted greatly from a WSC addition. At the 0 molasses rate, *B. mutica* silage was high in pH and dominated by an acetic acid fermentation which resulted in high amount of DM loss from fermentation. But once molasses was added, fermentation was nearly all lactic acid and pH of silage was <4.2. It is recommended that *B. mutica* herbage should be chopped to <1cm and amended with molasses at the rate of 40L molasses tonne-1 fresh *B. mutica*. Wilting is not recommended unless DM content is <20% as dry matter losses of silage were from

fermentation not effluent. If DM is <20%, lightly wilting to 20 to 25% DM is recommended.

P. maximum

Of all the grasses P. maximum had the lowest ensilability, as silage pH was continually >4.2. In Experiment 2, lactic acid production was too low for preservation, even though the WSC content was sufficient to overcome the buffering capacity. It is recommended that a lactic acid bacteria (LAB) inoculant be added to determine if LAB were the limiting factor in ensiling. From the research reported here, P. maximum will not ensile satisfactory and silage be avoided as a potential preservation method.

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