THE INFLUENCE OF GROWTH STAGE ON THE NUTRITIONAL VALUE OF *PANICUM MAXIMUM* (CV. *GATTON*) AND *DIGITARIA ERIANTHA* SPP. *ERIANTHA* SILAGE FOR SHEEP

by

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LIST OF ABBREVIATIONS

DM	-	dry matter
DMI	-	dry matter intake
DOMI	-	digestible organic matter intake
MFN	-	metabolic faecal nitrogen
N	-	nitrogen
NAN	-	non-ammonia-nitrogen
NDF	-	neutral detergent fibre
NDF-N	-	nitrogen bound to neutral detergent fibre
NH_3-N	-	ammonia-nitrogen
NPN	-	non protein nitrogen
OM	-	organic matter
OMADR	-	organic matter apparently digested in the rumen
OMI	-	organic matter intake
TD	-	true digestion
TN	-	total nitrogen
VFA	-	volatile fatty acids
WSC	-	water soluble carbohydrates

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DECLARATION

I, Federico Martino Bechaz, declare that this thesis for the degree M.Sc (Agric) (Animal Science) at the University of Pretoria, has not been submitted by me for a degree at any other University.

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ABSTRACT

The influence of growth stage on the nutritional value of *Panicum maximum* (cv. *Gatton*) and *Digitaria eriantha* spp. *eriantha* silage for sheep.

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The objective of this study was the evaluation of grass silage made from *P. maximum* cv. *Gatton* (*P. maximum*) and *Digitaria eriantha* spp. *eriantha* (Smutsfinger) grass. The study was conducted in two phases. In phase one the influence of sugar (molasses) addition, wilting, growth stage and plant species, on the quality of the silages obtained, were investigated. Three growth stages were incorporated, an early (physiologically young), medium (boot) and late (full bloom) stage. Four different treatments were applied during ensiling, namely direct cut, direct cut + sugar, wilted and wilted + sugar.

The second phase comprised of a partial digestibility study to evaluate the differences between plant species and growth stage, within a specific treatment. The silages used were made from the medium and late growth stages, where prior wilting and sugar addition occurred.

The addition of sugar during ensiling, even with prior wilting, had a positive effect on silage quality, leading to a lower pH, ammonia-nitrogen concentration and a higher lactic acid concentration in the silage. These effects were less pronounced with the plant material ensiled at a late growth stage. These silages tended to undergo secondary fermentation between day 21 and day 120, when sugar was added.

When comparing the three growth stages, within the direct cut + sugar and wilted + sugar treatments, the following was observed. The silages made from early and medium growth stages tended to be of better quality compared to the silages made from the late growth stage. In most of the comparisons the silages made from the medium growth stage were of a better quality than that of the silages made from the younger plant material.

In the comparison between *P. maximum* and *D. eriantha* silages, the silages made from *P. maximum* grass tended to be of a better quality than that made from *D. eriantha* grass. The difference between the two species was smaller than the differences observed between growth stages within a specie.

In the partial digestibility study, there was a difference in OMI, with sheep receiving the late growth stage silages having higher intakes. When expressed as DOMI / $W^{0.75}$ no significant differences were observed between the four diets. There were no significant differences in the total digestibility of OM across the four diets. There were differences in the rumen ammonia and VFA's concentrations across the diets. There was a significant difference in the intake of nitrogen across the four diets, resulting in differences in the disappearance of nitrogen, ammonia and non-ammonia-nitrogen across the four diets. There was no difference in the total true nitrogen digestibility across the four diets.

UITTREKSEL

Die invloed van groeistadium op die voedingswaarde van *Panicum maximum* (cv. *Gatton*) en *Digitaria eriantha* spp. *eriantha* kuilvoer vir skape.

deur

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Die doel van die studie was om kuilvoer, gemaak van *P. maximum* cv. *Gatton* (*P. maximum*) and *Digitaria eriantha* spp. *eriantha* (Smutsvinger) gras, se voedingswaarde vir skape te bepaal. Die studie is in twee fases uitgevoer. In die eerste fase is die invloed van suiker (melasse) byvoeging, verwelking, groeistadium en plantspesie op kuilvoer kwalitiet bepaal. Drie groeistadiums, 'n vroeë (fisiologies jong), medium (pyp) en laat (volblom) stadium is ondersoek. Vier verskillende behandelings is toegepas tydens inkuiling, naamlik direk, direk + suiker, verwelk en verwelk + suiker.

Tydens die tweede fase is 'n parsiële veteringstudie uitgevoer ten einde die invloed van groeistadium en plant spesie op die vertering van kuilvoer te bepaal. Die kuilvoer is gemaak van die medium en laat groeistadia en nadat dit verwelk is asook, suiker byvoeging, is dit aan skape gevoer.

Die byvoeging van suiker, selfs met verwelking, het 'n voordelige effek op die kuilvoer kwaliteit gehad. Dit het gelei tot 'n verlaging in die pH en ammoniak-N inhoud en 'n verhoging in die melksuur inhoud van die kuilvoer. Die kuilvoer gemaak van die laat groeistadium grasse, was van 'n swakker kwaliteit as dié van die ander groeistadia. Die laat groeistadia kuilvoere het geneig na 'n toestand van sekondere fermentasie tussen dag 21 en dag 120.

Met die evaluasie van die drie groeistadiums, binne die direk + suiker en verwelk + suiker behadelings, is dit gevind dat die vroeë en medium groeistadiums 'n beter kwaliteit kuilvoer as die laat groeistadiums gelewer het. In meeste van die vergelykings was die kwaliteit van die medium groeistadium kuilvoer beter as die van die vroeë groeistadiums.

Die kwaliteit van die *P. maximum* kuilvoer het geneig om beter te wees as die van die *D. eriantha* kuilvoer. Die verskille in kwaliteit tussen die *P. maximum* en *D. eriantha* was kleiner as die verskille binne 'n spesie wanneer die drie groeistadiums vergelyk word.

Daar was verskille in OMI tydens die parsiëlevertering studie, met 'n hoër inname by die kuilvoer gemaak van die gras ingekuil tydens die laat groeistadium. As die inname uitgedruk word as VOMI / $W^{0.75}$, is daar geen betekenisvolle verskille tussen die diëte gevind nie. Daar was ook geen betekenisvolle verskille in die totale vertering van OM en stikstof nie. Daar was wel betekenisvolle verskille in die inname van stikstof tussen die diëte, en dit het gelei tot verskille in stikstof, ammoniak en ware proteïen verdwyning in die spysverteringsstelsel. Daar was geen betekenisvolle verskille in die totale ware proteïen vertering tussen die diëte nie, maar wel betekenisvolle verskille in die rumen ammoniak- en vlugtige vetsuurinhoud tussen die verskillende diëte.

CHAPTER 1

Introduction and liturgy review

1.1 Introduction

The goals of the National Grazing Strategy and the Land Conversion Scheme have emphasised the need for information concerning animal production systems. Information pertaining to all suitable pasture species and cultivars needs to be collected.

In their evaluation of the use and potential of agricultural land in the Highveld region, Ludick and Wooding (1991) concluded that approximately 819 680 ha which is currently being used for crop production, would be more suited to cultivated pastures. This area comprises approximately 7.1% of the total surface area of the Highveld region, and it is recommended that this area be withdrawn for use as cultivated pastures. The requirements for more information concerning animal production systems and cultivated pastures will increase when these marginal maize areas are converted to cultivated pastures.

The winter climate in South Africa forces farmers to make full use of winter pastures and stored feeds. Winter pastures can often not be cultivated due to water shortages and low temperatures. On the other hand, the storage of grass in the form of hay or foggage has certain limitations. Varying climatic conditions in the summer months cause hay of varying nutritional value to be produced. Foggage must be utilised in the early and middle winter. Foggage can not be left till late winter or early spring, since the animals then select the new regrowth of the plants (Rethman, 1983). The result is a great need for quality roughage during the late winter and early spring period. Silage production using excess summer pastures can greatly increase the success of bridging this late winter/early spring period and in some cases grass silage is indispensable. Silage production in South Africa is largely limited to maize silage, but considering the economic climate of the last few years, grass silage has become more popular.

Recent South African research on the ensiling of tropical grasses highlighted the potential of tropical grass silage. This also contributed to the renewed interest in the use of grass silage

for animal production. The need for more research, however, was emphasised (Meeske, Basson, & Cruywagen, 1999; Meeske, 2000).

In an intensive ruminant production system, feed costs make up the largest percentage of "fluctuating" costs. The economic value of silage in a production system is dependent on the level of animal production that it supports. The level of animal production is in turn dependent on the nutritional value and fermentation characteristics of the silage. The type and level/amount of fermentation has a great influence on both the preservation process and voluntary feed intake (Wilkins, Hutchinson, Wilson & Harris, 1971). Rumen fermentation patterns for silage-based diets may differ substantially from non-silage diets as result of the fermentation end-products already present in the silage when consumed (Chamberlain, Martin & Robertson, 1989; Martin, Chamberlain, Robertson & Hirst 1994). The great variability in the composition of farm silages is likely to cause a wide range of rumen fermentation patterns especially for diets containing high proportions of silage. Thus, variation in rumen fermentation pattern, may be the prime cause for the variation in productivity often observed in animals given silage-based diets (Offer & Percival, 1998). The question is to what degree the physiological maturity of the grass plant is influencing the fermentation process and thus the quality of the silage.

Silage is classified according to moisture level:

- High-moisture silage, direct cut, 70 percent moisture
- Wilted silage, 60-70 percent moisture
- Low-moisture silage (haylage), 40-60 percent moisture (Smith, Cruywagen & Maree, 1993).

According to Tainton (1988), silage has the advantage over hay of generally being less wasteful of plant nutrients and of requiring less exact climatic conditions during curing, although it is true that even silage can best be made during dry weather when the material can be wilted to a degree before it is ensiled. Silage should be made from good-quality young pasturage if it is to provide forage of good quality.

Nelson & Moser (1994) stated that in a global sense, forage quality is a result of species present, amount of forage available, and composition and texture of each species. The species present depend on their adaptation, thus, potential productivity, composition, and texture of the forage are limited by decisions made within the constraints of adapted species. Understanding the biological potential of a species at a given site will help to evaluate its limitations in production, quality, and persistence, and give valuable insight into breeding and management strategies necessary to improve its performance and quality. The biological potential of adapted species depends on the climate.

1.2 Principles of ensilage

The first essential objective in preserving crops by natural fermentation is the achievement of anaerobic conditions. This can be obtained by various methods of which the most efficient way is to store the material in a hermetically sealed container, and under these conditions the oxygen trapped in the herbage is rapidly removed by respiratory enzymes in the plant. In the open-type silo, the efficiency with which anaerobiosis can be obtained depends upon the degree of consolidation and the effectiveness of the final sealing. The main aim of sealing is to prevent re-entry and circulation of air during storage. Where oxygen is in contact with herbage for any period of time, aerobic microbial activity occurs and the material decays to a useless, inedible and frequently toxic product (McDonald, Edwards & Greenhalgh, 1990; Church, 1991; McDonald, Henderson & Heron, 1991). Under ideal crop and storage conditions this phase will last only a few hours. With improper management this phase may last for several weeks (McDonald *et al.*, 1991).

McDonald *et al.* (1991), stated that the second main objective is to discourage the activities of undesirable micro-organisms such as clostridia and enterobacteria. Clostridia are usually present on the harvested forage in the form of spores, but start to multiply as soon as conditions in the silo become anaerobic. The growth of these organisms is undesirable, as they produce butyric acid and degrade amino acids to a variety of products, which are of poor nutritional value. The enterobacteria are non-spore forming, facultative anaerobes (able to grow in both the presence and absence of oxygen), which ferment sugars to acetic acid and other products and also have the ability to degrade amino acids. The commonest way of

inhibiting the growth of these undesirable micro-organisms is to promote lactic acid fermentation.

The lactic acid bacteria are also normally present on harvested crops and like the enterobacteria, are facultative anaerobes. These organisms ferment the naturally occurring sugars (mainly glucose and fructose) in the crop to a mixture of acids, but predominantly lactic acid. The lactic acid produced, increases the hydrogen ion concentration to a level at which the undesirable bacteria are inhibited. This inhibition is caused not only by the hydrogen ion concentration but also by the undissociated acids themselves. It is difficult to state an exact pH value of the silage at which this inhibiting effect occurs, as the inhibition depends not only on pH but also on the moisture concentration and the temperature. The wetter the material, the lower the critical pH value will be. With unwilted grass crops of DM concentration of about 200 g/kg, it is normally accepted that the achievement of a pH value of about 4.0 will preserve the crop satisfactorily, provided the silo remains airtight and is free from penetration by rain (Church, 1991; McDonald *et al.*, 1991).

According to Woolford (1984), an alternative method of inhibiting the growth of undesirable bacteria is to reduce the moisture concentration of the crop by wilting prior to ensiling. Lactic acid bacteria have a relatively high tolerance to low moisture conditions and are able to dominate the fermentation in high DM crops. Clostridia are known to be particularly sensitive to water availability and they require very wet conditions for active development. With very wet crops, i.e. those with a DM concentration of about 150 g/kg, even the achievement of a pH value as low as 4.0 may not inhibit clostridial growth (McDonald *et al.*, 1991).

The rate of lactic acid production is an important factor inhibiting the growth of undesirable bacteria and in reducing fermentation losses, and this depends upon the initial lactic acid bacterial population on the ensiled crop and upon the substrate availability. This in turn is influenced by the degree of physical damage (bruising, laceration, chopping and mincing). Modern precision-chop forage harvesters are capable of chopping herbage into particle lengths <25mm. With such material, plant sap is rapidly liberated and the lactic acid bacterial growth stimulated. Finely chopped silage of this type is more readily consumed by ruminant animals than long or coarsely chopped material (McDonald *et al.*, 1991).

1.3 Plant attributes and nutritional value

1.3.1 Carbohydrate components

Plant carbohydrates are usually divided into non-structural and structural types. The non-structural compounds are the readily available carbohydrates - sugars, starches and fructosans. In herbage, glucose and fructose are the principal free single sugars, being found in a 1:1 ratio and 1 to 3 percent of dry matter (**DM**). Sucrose is the only other sugar found in appreciable amount; it may often be present at about 4 to 5 percent. Other sugars are only found in trace amounts (Church, 1991). These carbohydrates are all soluble in cold water and are collectively known as the 'water soluble carbohydrates' (**WSC**) (McDonald *et al.*, 1991). Starches and fructosans are the most common polysaccharides in this class. For the plant these compounds are a form of stored energy. The starch concentration of temperate grasses is usually within the range of 1 to 6 percent (Church, 1991). Grasses of tropical and subtropical origin accumulate starches in their vegetative tissues, but grasses of temperate origin tend to accumulate greater amounts of fructosans (Church, 1991; McDonald *et al.*, 1991). Whereas in temperate grasses fructans are stored mainly in the stems, in grasses of tropical origin starches are stored primarily in the leaves. In contrast to fructans, starches are only sparingly soluble in cold water (McDonald *et al.*, 1991).

The WSC can be fermented rapidly by lactic acid bacteria provided they are readily available. It has been suggested that rates of diffusion of soluble carbohydrates from the intact and ruptured cells into the aquas phase, may be more important than absolute amounts in the crop. It is generally considered that most naturally occurring lactic acid bacteria do not have the ability to ferment starch directly (Woolford, 1984).

According to Church (1991), plant structural carbohydrates, which are all polysaccharides, range from homogenous to highly varied molecules, which may be linear or highly branched and form amorphous to crystalline structures. Structural carbohydrates are grouped into three major groups, the pectic substances, which are believed to function as intercellular cement, the non-cellulytic polymers (hemicelluloses), which are composed primarily of 5-carbon and 6-carbon sugars, and cellulose, a linear polymer composed of simple sugar units. The latter two are relatively insoluble and are resistant to digestion because the sugar units are

chemically linked in a different manner (β 1-4 linkage) which are more available carbohydrates such as the starches.

Church (1991), stated that variations in the amount of these different fractions are observed within the plant cells, between plant parts (celluloses are especially higher in stems), between different species and in type of climate adaptation. With grasses, there is generally a rise in cellulose with a lesser increase in hemicellulose in the stems. Less marked changes are seen in tropical grasses, but the cellulose level is usually considerably higher than in temperate species.

1.3.2 Nitrogenous components

About 75 to 90 percent of the total nitrogen (**TN**) in fresh grass is present as true protein. As in all other living cells, this protein is made up of some 20 amino acids (**AA**) linked through peptide bonds, the *a*-amino group of one acid and the carboxyl group of the next. The proteins present in forage, as distinct from those of seeds, are almost all enzymatic by nature and are thus concerned with the growth and biochemical function of the cells. The AA concentration of herbage protein does not vary greatly with plant species. The main factor influencing the protein concentration of herbage is stage of growth, although the application of nitrogenous fertilisers can also have a marked effect in increasing the protein concentration. In general, the protein concentrations of tropical grasses tend to be lower than those of temperate species (McDonald *et al.*, 1991).

It was reported by McDonald *et al.* (1991), that some 10 to 25 percent of the TN in herbage is made up of non-protein nitrogenous (**NPN**) components. These include free amino acids and the amides glutamine and asparagine, peptides of varying chain length, amines, ureides, ucleotides, chlorophyll and nitrates. In forage crops the free amino acid composition is very variable and bears little relationship to the amino acid composition of the protein fraction, which is relatively stable. The free amino acids are influenced by many factors, such as species, stage of growth and environmental conditions. In addition to the amino acids normally found as components of proteins, a number of non-protein amino acids also occur free in grasses and other forage crops. The amide and nitrate concentration of grasses is influenced by nitrogenous fertilisation.

1.3.3 Factors influencing the nutritional value of the grass plant

1.3.3.1 Species and Climate

In temperate areas, having a reasonably uniform distribution of rainfall, grasses grow and mature relatively slowly and can thus be utilised at an early stage of growth when their nutritive value is high. In warmer climates, however, grasses mature more rapidly, their protein and phosphorous concentrations falling to very low levels, and their fibre concentration rising. In the wet tropics the herbage available is commonly fibrous but lush (i.e. high in water concentration). In drier areas the mature herbage becomes desiccated and is grazed as 'standing hay'. In both cases digestibility is low, typical values for tropical herbage being 0.1 to 0.15 units lower than for temperate herbage (McDonald *et. al.*, 1990).

According to McDonald *et al.* (1990), the differences in composition between temperate and tropical grasses are not only a result of climate. Temperate species of grasses belong to the C_3 category of plants in which the three-carbon compound, phosphoglycerate is an important intermediate in the photosynthetic fixation of carbon dioxide. Most tropical grasses have a C_4 pathway of photosynthesis in which carbon dioxide is first fixed in a reaction involving the four carbon compound oxalacetate. The low protein concentration often found in tropical grasses is an inherent characteristic of C_4 plant metabolism, which is associated with survival under conditions of low soil fertility.

Another factor of nutritional importance is that the mesophyll cells in the leaves of tropical grasses and intercellular spaces represent only 3 to 12 percent of leaf volume compared with 10 to 35 percent in temperate species. This may partly explain why tropical grasses have a higher tensile strength than temperate ones, a feature which results in both a slower mechanical and slower microbial degradation in the rumen. A consequence of this is relatively low voluntary dry matter intakes (**DMI**) by ruminants consuming these plants (McDonald *et al.*, 1990).

The WSC concentrations of tropical species of grasses are generally considered to be lower than those of temperate species. This difference may, in part, be due to a lack of fructans in the former (McDonald *et al.*, 1991). In temperate grasses fructans are the main storage carbohydrates while in tropical species these are replaced by starch. Further differences in

WSC concentration among cultivars within a species are known to exist. A reduction in light intensity reduces the WSC concentration in grasses (McDonald *et al.*, 1990). It has also been found by Wilkinson (1984), that sunny weather leads to higher sugar levels in the forage, resulting from photosynthesis, and it also means less water is present at the time of cutting.

1.3.3.2 Growth stage

Stage of growth is the most important factor influencing the composition and nutritive value of the grass plant. As plants grow there is a greater need for structural tissues, and therefore the structural carbohydrates (cellulose and hemicellulose) and lignin increase. This is reflected in the neutral detergent fibre (**NDF**) concentration, which increases as the plant matures. As the plant ages the concentration of protein decreases. There is therefore a reciprocal relationship between crude protein and crude fibre concentrations in a given species, although this relationship can be upset by the application of nitrogenous fertilisers. In addition to the changes in the crude protein and carbohydrate contents, changes also occur in the mineral or ash constituents. The total ash concentration decreases as the plant matures. (Thomas & Thomas, 1985; McDonald *et al.*, 1990; Church, 1991).

It was stated by McDonald *et al.* (1990), that the digestibility of the organic matter (**OM**) is one of the main factors determining the nutritive value of forage, and this may be as high as 0.85 in young spring pasture grass and as low as 0.50 in winter forage. Although it decreases as the plant matures, it is further influenced by the leaf:stem ratio.

The concentration of WSC in grasses of temperate origin is influenced greatly by the leaf:stem ratio. Concentration appears to increase with advancing maturity as the proportion of stem tissue increases and is also affected by light intensity and temperature (McDonald *et al.*, 1991; Donald, Fenlon & Seddon, 1995). According to McDonald *et al.* (1991), this increase in WSC is due primarily to an increase in fructan concentration.

1.3.3.3 Leaf:stem ratio

Differences in digestibility of grasses are influenced by leaf:stem ratios. In very young grass the stem is more digestible than the leaf, but whereas with advancing maturity the digestibility of the leaf fraction decreases very slowly, that of the stem fraction falls rapidly.

As plants mature, the stem comprises an increasing proportion of the total herbage and hence has a much greater influence on the digestibility of the whole plant than the leaf (McDonald *et al.*, 1990).

1.3.3.4 Fertiliser application levels

The application of nitrogenous fertilisers can increase the crude protein of pasture herbage and influence the amide and nitrate concentration (McDonald *et al.*, 1990). The WSC concentration of grass is reduced by the application of nitrogenous fertilisers (Thomas & Thomas, 1985; McDonald *et al.*, 1991). McDonald *et al.* (1991), considered the decrease to be caused by the acceleration of herbage growth rate promoted by the N fertilisation and appears to be the result of a decrease in fructan concentration rather than that of total sugars.

1.3.3.5 Diurnal variations

Concentrations of total sugars and WSC in temperate grasses appear to increase during the morning hours until sometime in the afternoon, and then to decrease until daylight the next day. Most of the diurnal variation appears to be due to changes in sucrose concentration (McDonald *et al.*, 1991)

1.4 The initial aerobic phase

Immediately after cutting and during the early stages of ensiling changes occur, resulting from the continuing metabolism of plant cells and from the activity of the enzymes of dead tissue. The processes of respiration and proteolysis are of particular importance.

1.4.1 Influence of plant enzymes on the carbohydrate component

Respiration was defined by McDonald *et al.* (1991), as the oxidative degradation of organic compounds to yield usable energy. In higher plants oxygen is the terminal electron acceptor. Although plants can and do respire proteins and lipids, carbohydrates are the major respiratory source. The substrate for oxidation is usually a hexose sugar, which undergoes glycolysis before entering the aerobic phase.

Plant respiratory enzyme activity will continue in the ensiled herbage as long as conditions are aerobic and the pH is not drastically altered. The WSC in the crop will be oxidised to

carbon dioxide and water, with the production of heat sufficient to cause a considerable rise in temperature of the mass (McDonald *et al.*, 1990; Rotz & Muck, 1994). The changes in WSC concentration may be relatively small since the oxidised soluble sugars may be compensated for by sugars released from the hydrolysis of polysaccharides (Thomas & Thomas, 1985). Under more normal conditions, when anaerobiosis is rapidly achieved, the lactic acid bacteria ferment the WSC, available after hydrolysis mainly as glucose and fructose, to lactic acid and other products. Some hydrolysis of hemicellulose also occurs, liberating pentoses, which may be fermented to lactic acid (McDonald *et al.*, 1990). As sugars are the main substrates for respiration, changes to the WSC components of herbage are likely to occur during the aerobic phase of ensiling. Immediately after harvesting both sucrose and fructans are rapidly hydrolysed to glucose and fructose (McDonald *et. al.*, 1991).

1.4.1.1 Factors affecting the rate of respiration

1.4.1.1.1 Temperature

McDonald *et al.* (1991), concluded that the rate of respiration is controlled mainly by temperature and thus the two are interdependent. Two different effects of temperature occur. Firstly, the initial velocity of the reaction increases and, secondly, the destruction of the enzyme, usually by denaturation at the higher temperatures, produces a continuous drop in active enzyme concentration. In the early stages of ensilage, during the aerobic phase, temperature can have an important influence on the rate of respiration since much of the heat generated in the mass is retained, resulting in a progressive temperature increase with time. Temperatures of 40°C or more are not uncommon in farm silos. The final temperature is dependent on the quantity of air present, the insulating properties of the silo and the DM concentration of the crop, since the specific heat capacity of the plant material increases with increasing moisture concentration. The effect of ambient temperature is far less than that of plant respiration in controlling herbage temperature. A rise in temperature in the silo also accelerates the activities of other enzymes such as proteases, leading to further nutrient losses and a reduction in the digestibility of the silage. In some instances up to 50 percent of plant protein may be broken down during this process.

1.4.1.1.2 Oxygen and carbon dioxide concentrations

According to McDonald *et al.* (1991), the effect of oxygen concentration on the intensity of respiration appears to vary in different tissues. Increased concentration of carbon dioxide in the atmosphere brings about a marked depression in respiration. During the initial period of ensiling, oxygen is perhaps more important with regard to its effect on the extent, rather than the rate, of respiration. The former is controlled mainly by the sugar and oxygen supplies, but in practise sugars are usually abundant and the limiting factor is the oxygen supply. In a well consolidated, sealed silo it is estimated that the trapped oxygen is of little significance and will lead to a temperature rise of only 3 to 4°C, with a loss of only 10 g/kg hexose. It was also noted that oxygen was more rapidly utilised in silos filled with direct-cut rather than pre-wilted herbage, which presumably reflected a reduction in the respiration rate of the wilted material owing to the increased DM concentration.

1.4.1.1.3 Hydrogen ion concentration

In general, enzymes are only active over a limited range of pH and, in most cases, for each enzyme there is a pH value at which the rate of enzymatic activity is optimal. The effect of pH on enzymes, like all pH effects, is a result of changes in the state of ionisation of the components of the system as the pH alters. The free enzyme, the enzyme-substrate complex or the substrate may undergo such changes. In silage studies, pH determinations are usually carried out on whole plant macerates, and with most fresh plant materials, pH values usually lie within the range 5.5 to 6.5. Such determinations, however, ignore the pH variations which normally occur within the living plant. The aerobic phase of ensiling consists of the period in the field during wilting and the initial period in the silo, which may last only a few hours. During field wilting, overall pH values of the crop do not appear to be affected to any extent. In the silo, lactic acid bacteria grow rapidly on the released plant juices and even in the aerobic phase produce lactic and acetic acids, which result in a rapid fall in pH. There is evidence that the respiration rate declines as the pH falls (McDonald *et. al.*, 1991).

1.4.1.1.4 Dry matter concentration

On the whole, the general opinion seems to be that respiration decreases with increasing DM (McDonald *et al.*, 1991). Mitchell and Shepperson (1955) as cited by McDonald *et al.* (1991), considered that respiration losses were directly proportional to the initial moisture

concentration and inversely proportional to the rate of drying. Thus, under favourable wilting conditions, when the DM increase is rapid, respiration losses would be expected to be low. Under moist wilting conditions, however, losses may be significant. This was confirmed by Honig (1980) as cited by McDonald *et al.* (1991), who found that respiration intensity (respiration loss per hour) of wilting grass increased with decreasing DM concentration and increasing temperature according to quadratic functions. This author observed that it was especially high at low DM concentrations directly after cutting, and can thus be decreased most effectively by speeding up the drying process during the early stages of wilting.

McDonald *et al.* (1991), concluded that the temperature during wilting affects the losses incurred, as the respiration intensity of most plant materials increases rapidly with rising temperature, especially at low DM levels. The stage of maturity of the harvested plant is also important as younger plants have a higher rate of respiration. Since immature plants also have a lower concentration of WSC, the respiration losses are relatively more important. When ensiling wilted herbage, respiration losses can be reduced by chopping the material finely, as this allows it to compact more easily and hence reduces the amount of air trapped. Despite some unavoidable DM losses, wilting under good conditions is generally beneficial to the fermentation quality of the silage and usually results in a reduction in overall DM losses.

1.4.2 Influence of plant enzymes on the nitrogen component

In fresh herbage, 75 to 90 percent of the total N is present as protein, the rest being mainly in peptides, free amino acids, amides, ureides, nucleotides, chlorophyll and nitrates. After harvesting, rapid proteolysis (hydrolysis of peptide bonds) takes place (McDonald *et. al.*, 1990; McDonald *et. al.*, 1991). The extent of true protein degradation varies with plant species, rate and extent of pH changes, DM concentration and temperature, but it may reduce the protein concentration by 50 to 60 percent, even in a well-preserved silage. Further breakdown of amino acids may also occur. Such changes in the nitrogenous components adversely affect the subsequent utilisation of the nitrogen (N) by ruminants as well as inhibiting the acidification process of fermentation. It has been considered that rapid, extensive proteolysis was only halted by attainment of a high DM concentration or a low pH, as the material was dried or ensiled respectively (McDonald *et. al.*, 1991).

1.4.2.1 Factors affecting proteolysis

1.4.2.1.1 Dry matter

It was reported by McDonald *et al.* (1991), that the extent of proteolysis during ensiling is influenced by several factors, including DM concentration, pH and temperature. During wilting, since there is little change in pH, any reduction of proteolysis depends on reaching a high enough DM. It has been shown that lightly wilted material may show increased levels of proteolysis due to the inhibition of acidification.

1.4.2.1.2 Temperature

Since plant proteases have high temperature optima, a rise in temperature in the silo will tend to increase their activity. The degree of heating is normally controlled by respiration, therefore it is important that the herbage should be well compacted and the silo filled rapidly and well-sealed to prevent entry of air (McDonald *et. al.*, 1991).

1.4.2.1.3 pH

According to McDonald *et al.* (1991), it is well-known that the rate of fall of pH is important in determining the extent of proteolysis. If it is slow to fall, then more protein will be broken down. It has been stated that once a pH of 4.3 has been reached, further proteolysis would be negligible, but several workers have shown that even direct acidification to a pH below 4 will not prevent proteolysis, although it will reduce it. It has been suggested that the extent of proteolysis during ensiling depends mainly on the 'proteolytic potential' and the rate of fall of pH. The former is a measure of the total protease activity, the substrate availability and the substrate affinity. It varies between species and will probably also be affected by crop management and environmental conditions.

1.5 The subsequent anaerobic phase

The major objective in silage fermentation is to achieve a stable pH at which biological activity virtually ceases. In this way, preservation is obtained while minimising nutrient losses and avoiding adverse changes in the chemical composition of the material. This is achieved by discouraging the activities of undesirable micro-organisms and encouraging the development of bacteria which produce lactic acid (Gordon, 1989).

According to Gordon (1989), the undesirable micro-organisms are mainly clostridia, coliforms and yeasts and if these proliferate, they will compete with lactic acid bacteria for sugars. In addition they will metabolise lactic acid to produce end-products such as acetate, butyrate, propionate, ethanol and butanol. Clostridia and coliforms will also metabolise amino acids to produce a variety of products including higher volatile fatty acids (**VFA**), amines and ammonia. Since butyric acid is a weaker acid than lactic acid, and many of the products of amino acid breakdown are bases, clostridial activity slows, or reverses, the normal reduction in pH, which occurs in the silo.

1.5.1 Factors influencing the growth and activity of micro-organisms in the silage

Donald *et al.* (1995) stated that it is the relationship between grass quality, as expressed by grass maturity and DM, and the degree of aerobiosis/anaerobiosis which influences the efficiency of fermentation. It is these factors together which determine the relative populations of the epiphytic micro flora on the ensiled grass. Silage is a non-homologous substrate and natural variations in microbial concentration between samples occur.

1.5.1.1 Particle size

Substrate availability is influenced by the degree of physical damage (bruising, laceration, chopping and mincing). Modern precision-chop forage harvesters are capable of chopping herbage into particle lengths <25 mm. With such material, plant sap is rapidly liberated and lactic acid bacterial growth is stimulated. When ensiling wilted herbage, respiration losses can be reduced by chopping material finely, as this allows it to compact more easily and hence reduces the amount of air trapped (McDonald *et al.*, 1991).

1.5.1.2 Plant species

It was concluded by McDonald *et al.* (1991), that a grass species with a high WSC concentration and a low protein concentration, is more advantageous for the production of a good quality silage, as low WSC concentration mainly leads to the production of acetic acid silage. As tropical grass species tend to have lower levels of WSC, they usually produce acetic acid silage, while grasses of temperate origin produce lactic acid silage.

1.5.1.3 Growth stage

Stage of growth is the most important factor influencing the composition and nutritive value of the pasture herbage. As the plant matures, the stem comprises an increasing proportion of the total herbage and hence has a much greater influence on the digestibility of the whole plant than the leaf. The digestibility of grasses are thus influenced by leaf:stem ratios (McDonald *et al.*, 1990). According to McDonald *et al.* (1991), there is a positive relationship between stage of maturity and the WSC concentration of the plant. As the plant matures, the WSC concentration increases. Thus the stage of maturity of the plant and the leaf:stem ratio play an important role in determining the fermentation process resulting in the silage, due to the availability of substrates for fermentation. This has a direct influence on the quality of the silage produced.

1.5.1.4 Original micro-organism population

Bacteria are present on the aerial parts of plants. The great majority of these bacteria are strict aerobes which contribute little or nothing to silage preservation and, since anaerobiosis is achieved rapidly after the silo is sealed, their growth is soon inhibited. Lactic acid bacteria, which are the most important species during ensiling, are usually present in much lower numbers than other micro-organisms (McDonald *et al.*, 1991).

1.5.1.5 Dry matter concentration, pH, water soluble carbohydrates, temperature and buffering capacity

Prewilted grass samples show high levels of enterobacteria which increase during the first few days of ensilage. In well-fermented grasses with a low pH, enterobacteria die out quickly. It has also been observed that with delayed fermentation (due to poor quality grass or oxygen availability) that the enterobacteria persisted (Donald *et al.*, 1995).

Micro-organisms vary in their optima ranges of pH, temperature and moisture concentration, where they grow optimally. An initial low DM concentration (high water concentration), high temperature, high buffering capacity and a low WSC concentration, all favour the growth of clostridia bacteria. This leads to a silage with a high butyric acid concentration and a low nutritive value (McDonald *et al.*, 1991).

The buffering capacity of plants, or their ability to resist pH change, is an important factor in ensilage. Buffering capacity is expressed as milliequivalents (meq) of alkali required to change the pH of 1 kg DM from 4 to 6. Most of the herbage buffering properties can be attributed to anions present (organic acid salts, orthophosphates, sulphates, nitrates and chlorides), with only about 10 to 20 percent resulting from the action of plant proteins. Quantitatively, the most important acids occurring in grasses are malic, citric and quinic acids. Oxalic acid may also be present, but in temperate grasses the level is usually regarded as being low. However, in some tropical species, levels as high as 70 g/kg have been reported (McDonald *et al.*, 1991).

1.5.2 Lactic acid silage

Lactic acid bacteria, which are the most important species during ensiling, are usually present on grass in numbers 1000 times lower than their main competitors, fungi and enterobacteria. After ensiling, the micro-organisms capable of anaerobic growth (lactic acid bacteria, enterobacteria, clostridia, some *Bacillus spp.*, yeasts) begin to grow and compete for available nutrients. The changes in the first few days are critical to the success or failure of the subsequent fermentation. If conditions are suitable, the lactic acid bacteria will quickly acidify the environment to such an extent that the competing organisms will not be able to survive and the end result will be a stable, low pH silage. If the pH is not lowered quickly enough, the undesirable micro-organisms (mainly enterobacteria, clostridia and yeasts) will be able to compete for nutrients and, in doing so, reduce the chances even further of obtaining a stable silage, since many of their products do not aid preservation. They are also capable of producing end-products which may reduce the nutritive value of the silage (McDonald *et al.*, 1991).

Under anaerobic conditions the lactic acid bacteria can ferment a wide range of substrates using a variety of pathways. The pathway followed for fermentation of hexose sugars is used as a basis for their identification. The lactic acid bacteria can be divided into three physiologically distinct groups:

A) Obligate homofermenters ferment hexoses almost exclusively to lactic acid but pentoses and gluconate are not fermented. They contain fructose biphosphate (FBP) aldolase but not phosphoketolase.

B) Facultative heterofermenters possess FBP aldolase and also ferment hexoses almost exclusively to lactic acid, but they are in addition able to ferment pentoses to lactic and acetic acids using an inducible phosphoketolase.

C) Obligate heterofermenters ferment hexoses to lactic acid, acetic acid/ethanol and carbon dioxide, and possess phosphoketolase but not FBP aldolase.

Thus the presence of aldolase helps to differentiate the bacteria, as does the stereo-isomer of lactic acid produced during fermentation. Unlike higher animals and plants which produce exclusively the L(+) isomer, species of lactic acid bacteria produce either D(-), L(+) or a mixture of both (DL) (McDonald *et al.*, 1991).

In general, Schaadt & Johnson (1968), as quoted by McDonald *et al.* (1991), stated that most of the lactic acid produced during ensilage is in the D(-) form, and there is concern that this isomer is nutritionally inferior to the L(+) form because it is metabolised more slowly by the host animal for gluconeogenesis. Unlike the L-isomer which is oxidised in the cytosol, the D-isomer must first cross the mitochondrial membrane to be oxidised within the mitochondria. However, even on silage diets the lactate concentration in the rumen is low since it is rapidly metabolised by the rumen micro-organisms. Both isomers are metabolised at equal rates in the rumen. It has been found, that only 10 percent of the rumen lactic acid are absorbed from the rumen, the rest being metabolised mainly to acetate as well as propionate and butyrate. These VFA's are then absorbed from the rumen and used for production (Newbold, Chamberlain & Williams, 1984).

There is considerable variation in the actual species dominating in particular silages and both numbers and species present are bound to be affected by the many variables associated with ensiling. There is evidence to show that members of most of the genera of lactic acid bacteria are represented at some stage during ensiling, with *Lactobacillus plantarum* being the most frequently found species. Of all lactic acid bacteria, *L. plantarum* can most successfully colonise freshly ensiled forage, as this species can ferment a wide variety of substrates, is highly competitive and produces large amounts of acid quickly. Other dominant species are *L. Brevis* and the *Pediococcus spp.* (McDonald *et al.*, 1991).

McDonald *et al.* (1991), concluded from the literature, that a distinctive feature of the lactic acid bacteria is their high acid tolerance. The pH range for growth is about 4.0 to 6.8, although some species will grow at pH 3.5. Most of the lactic acid bacteria normally maintain a pH differential (higher inside) across the cytoplasmic membrane, although the internal pH decreases along with the external pH. The pH gradient is probably maintained using an H^+ATPase but eventually the accumulation of acidic end-products of fermentation lowers the internal pH below the threshold level, resulting in cessation of growth and metabolism. The level of this threshold is characteristic for each organism. The temperature range is very variable, growth occurring within the range 5 to 50 °C, but the optimum for most strains is about 30°C. Most species of lactobacilli grow at 15°C but not at 45°C.

Lactic acid bacteria are virtually non-proteolytic and, as they have limited powers of amino acid synthesis, an external supply of amino acids is necessary for their growth. Their ability to ferment amino acids also appears to be restricted and it is thought that only two, serine and arginine, are extensively attacked by some, but not all, of these organisms. There is some evidence to indicate that certain lactic acid bacteria can decarboxylate amino acids to form amines. In well-preserved silages a small amount of nitrate is reduced to nitrite and most of this is lost from the silo as nitrogen oxide gases, where as in badly-preserved silages, a larger amount of nitrate is reduced to nitrite which is then further reduced to ammonia (McDonald *et al.*, 1991).

1.5.3 Acetic acid silage

Enterobacteria represent a minor part of the micro flora on grass but they are usually present in significantly higher numbers than the lactic acid bacteria, and their numbers increase substantially during the first days of ensiling. The development of lactic acid bacteria and the subsequent fall in pH normally leads to a rapid decrease in their numbers (Heron, Wilkinson & Duffus, 1993). According to McDonald *et al.* (1990), under certain ill-defined conditions, acetic acid producing bacteria may dominate the fermentation. Acetate silages contain high levels of acetic acid and relatively low levels of lactic acid. Deamination of amino acids is usually extensive, and consequently ammonia levels in these silages are higher than those found in lactate silages. Because of the negative correlation of acetic acid concentration and

DM intake, it is reasonable to assume that the latter will be low in animals given these silages *ad libitum*.

1.5.4 Butyric acid silage

McDonald *et al.* (1991), stated that the clostridia bacteria can be divided into two major physiological groups based on their substrates. Saccharolytic clostridia, for example *Clostridium butyricum*, ferment mainly sugars and organic acids and possess little activity against proteins or amino acids. Proteolytic clostridia, such as *Clostridium sporogenes*, ferment mainly amino acids. Some clostridia, for example *Clostridium perfringes*, have both types of activity. Apart from their presence in silage, clostridia also occur in faeces and in soil. The presence of clostridia in silage is probably the result of soil contamination, since clostridial numbers on green plant material are generally quite low.

Silages with high concentrations of butyric acid have undergone a clostridial fermentation. Clostridial growth is stimulated by high storage temperatures, low DM concentration (< 30%), low WSC concentration, high buffering capacity of the crop and delayed sealing (Thomas & Thomas, 1985; Jonsson, 1991). It is generally assumed that clostridia multiply either during the initial phase of silage fermentation or at a later stage in secondary-fermented silages (Jonsson, 1991). They usually have pH values within the range of 5 to 6, and contain low concentrations of lactic acid and WSC. Butyric acid is usually the dominant fermentation product, although acetic acid concentrations are also frequently high (McDonald *et al.*, 1990). As butyric acid is a much weaker acid than lactic acid and as only one mole of butyrate is produced from two moles of lactate, it follows that the pH will increase. This makes conditions more suitable for the growth of proteolytic clostridia, which are inhibited at a higher pH than saccharolytic clostridia (McDonald *et al.*, 1991). Because of the extensive breakdown of amino acids caused by clostridia, McDonald *et al.* (1990), concluded that silages of this type will contain high concentrations of ammonia-N, usually in excess of 200 g/kg total N. Decarboxylation of amino acids to amines also occurs. As a result of these changes, the subsequent utilisation by ruminants of the nitrogenous compounds in butyrate silages is likely to be low. The DM intake of ruminants given these silages is low, but whereas there is a close negative correlation between DM intake and the concentration of silage ammonia-N, the exact cause of these reduced intakes by animals is unknown.

1.6 Losses during ensilage

The efficiency of any conservation system must take into account not only the nutritional value of the product but also the losses which occur between the harvesting and feeding stages.

1.6.1 Field losses

According to McDonald *et al.* (1990), crops cut and ensiled the same day, nutrient losses are negligible and even over a 24 hour wilting period, losses of not more than 1 or 2 percent DM can be expected. Over periods of wilting longer than 48 hours, considerable losses of nutrients can occur depending upon weather conditions. DM losses as high as 6 percent after 5 days and 10 percent after 8 days of wilting in the field have been reported. The main nutrients affected are the WSC and proteins which are hydrolysed to amino acids.

According to McDonald *et al.* (1991), three main causes of field losses have been identified, ie mechanical, biochemical and leaching. In crops that are cut and directly harvested, biochemical and leaching losses can be expected to be minimal. It has been found that mechanical losses do not account entirely for field losses in forage cut directly with a flail harvester nor with pre-mown material picked up with a precision-chop harvester. It has been suggested that losses during loading and transportation probably account for the difference between the mechanical and field losses. The situation is entirely different with forage that is wilted in the field. Mechanical losses reflect the number of times the herbage is turned or tedded. Biochemical losses result mainly from respiration and other enzymatic processes occurring in the plant after harvesting. The effect of rain on losses during wilting shows that increasing amounts of rain resulted in increasing DM losses. Further it was shown that there was an interaction between the effect of mechanical treatment and rainfall losses. Bruising of the grass prior to wilting increased the ill effects of the rain (McDonald *et al.*, 1991).

1.6.2 Fermentation losses

The losses arising from fermentation depend upon the nutrients fermented and the organisms responsible. Although considerable biochemical changes occur during fermentation, especially to the WSC and proteins, overall DM and energy losses arising from the activities of lactic acid bacteria are low. DM losses can be expected to be less than 5 percent and gross

energy losses, because of the formation of high energy compounds such as ethanol, are even less (McDonald *et al.*, 1990; McDonald *et al.*, 1991).

If clostridia or enterobacteria dominate the fermentation, losses of DM and energy will be much greater than if lactic acid bacteria had been dominant. Such high losses arise from the extensive production of carbon dioxide and hydrogen, from the fermentation of lactate or hexoses, and from the deamination and decarboxylation of amino acids (McDonald *et al.*, 1990; McDonald *et al.*, 1991). McDonald *et al.* (1991), concluded that if yeasts are very active, as they may be in carbohydrate-rich crops such as maize, ethanol production can result in very high DM losses. However, the production of ethanol by these organisms, results in the loss of very little energy.

1.6.3 Effluent losses

The factors influencing the production of silage effluent include DM concentration of the ensiled crop, type of silo, degree of consolidation and pre-treatment of the crop. Of these, DM concentration is the most important factor. With very wet crops effluent DM losses can exceed 10 percent, whereas with crops ensiled with DM concentrations of 250 to 350 g/kg and higher very little effluent will be produced, except in tall tower silos where pressures can be very high (McDonald *et al.*, 1990; McDonald *et al.*, 1991).

Since effluent contains highly digestible components such as WSC, organic acids, minerals and soluble nitrogenous compounds, it follows that production of large volumes of effluent will tend to increase the concentration of the nutritionally less desirable cell wall components in the silage (McDonald *et al.*, 1990; McDonald *et al.*, 1991). The clear advantage in wilting wet crops prior to ensiling as a means of reducing effluent production is obvious, but unless it is carried out quickly in good weather conditions, it can result in increasing nutrient losses instead of reducing them (McDonald *et al.*, 1991).

1.6.4 Oxidation losses

Oxidation losses result from the action of plant and microbial enzymes on substrates such as sugars in the presence of oxygen, leading to the formation of carbon dioxide and water. In a silo which has been rapidly filled and sealed, the oxygen trapped within the plant tissues is of

little significance causing a DM loss of about 1 percent only. The continuous exposure of herbage to oxygen, as sometimes occurs on the sides and upper surfaces of ensiled herbage, leads to the formation of inedible composted material (McDonald *et al.*, 1990; McDonald *et al.*, 1991).

When a silage clamp is opened, the exposed face comes into contact with oxygen from the atmosphere and aerobic deterioration begins. Aerobic and facultative organisms, such as yeasts, oxidise substrates, starting with simple substrates such as lactic and acetic acids (Woolford, 1990; Ruxton & Gibson, 1994). In extreme cases, structural carbohydrates may ultimately be degraded (Williams, Lowe & Reeds, 1994). Donald *et al.* (1995), concluded that big bale silages are particularly susceptible to aerobic spoilage due to their large surface area to volume ratio compared to clamp silages. This makes them vulnerable to air infiltration.

1.7 Nutritional value of grass silage

According to Wilkins (1981), the fermentation quality is responsible for much of the variation in the intake of silages currently produced on farms. The pH value can be measured easily, but the relationship between pH and fermentation quality is complicated by the high pH of both badly-preserved silages which have undergone a clostridial fermentation and well-preserved silages in which little fermentation has taken place. The concentrations of either ammonia or of total VFA's are much more satisfactory indices of fermentation quality and both are related to intake.

1.7.1 Well-preserved unwilted silage

These silages are characterised by having low pH values, usually between pH 3.7 and 4.2, and containing high concentrations of lactic acid. In grass silages the lactic acid concentration normally lies in the range 80 to 120 g/kg DM, although higher amounts may be present if silages are made from wet crops rich in WSC. Although lactic acid is the main fermentation acid present in these silages, appreciable amounts of acetate also occur. Small amounts of propionic and butyric acids are frequently found in well-preserved silages, the concentration of butyrate depending very much on the rate at which lactate is produced. As a result of the formation of fermentation acids, the buffering capacity of the ensiled crop may increase as

much as three- or four-fold during the period of ensilage. The nitrogenous components of well-preserved silages are mainly in a non-protein soluble form in contrast to those present in fresh forage crops, where some 70 to 90 percent of the TN is present as protein (McDonald *et al.*, 1990; McDonald *et al.*, 1991).

The stage of growth of the crop at the time of harvesting is the main factor influencing the digestibility of forages, although other factors, such as particle size and level of feeding, play a lesser but important role. The decline in digestibility with increasing maturity of forages, is mainly a result of the increasing concentration of structural carbohydrates, which are less digestible than the soluble components of plants. The effects of silage fermentation on digestibility are generally considered to be small. With sheep, fine chopping resulted in a decreased digestibility, especially of the crude fibre and crude protein, presumably as a result of a higher rate of passage through the digestive tract (McDonald *et al.*, 1991). By decreasing particle length, DM intake is increased (Teller, Vanbelle, Kamatali, Collignon, Page & Matatu, 1990; Van Vuuren, Huntanen & Dulphy, 1995). In cattle, particle length plays a lesser role than in sheep (Van Vuuren *et al.*, 1995).

The voluntary DM intake of forages is a major factor influencing their value for animal production. Intake is influenced by the characteristics of the animal and also of the forage. Although ruminants possess, in common with other animals, chemostatic mechanisms for regulating the intake of concentrate foods, the intake of forages is limited by the rate of removal of forage particles from the reticulo-rumen. This rate of removal is related to the chemical composition of the forage, to its particle size, to the digestion rate of its digestible constituents and to the reduction rate of its indigestible components. The intake of silage DM by ruminants is thought to be controlled by the same basic mechanisms regulating fresh forage intake. It has been noted that intakes of silage DM are generally lower than those of fresh or dried forage made of similar material (Church, 1991; McDonald *et al.*, 1991; Thiago, Gill & Dhanoa, 1992; Van Os, Dulphy & Baumont, 1995). On average this reduction is 30 percent (McDonald *et al.*, 1991). Ensiling appears to have a much greater depressing effect on intake by sheep than by cattle and the extent of reduction is much greater with grass than with legume or maize silages (McDonald *et al.*, 1990; McDonald *et al.*, 1991).

According to McDonald *et al.* (1991), several attempts have been made to correlate various chemical parameters of silage DM intake. Water *per se* seems to have very little influence. It has been found that crude fibre is negatively correlated with DM intake. A positive correlation has been obtained between the residual WSC concentration of silages and DM intake in lambs. The correlation was improved by including the protein-N concentration of the silages and the WSC concentration of the grasses, which both had positive effects on intake, and the ethanol concentration of the silages, which had a negative effect. There is evidence to indicate that low intakes of silages are associated with high organic acid concentration. It seems further that osmolality may also be an important factor regulating DM intake. Lactic acid *per se* does not appear to influence the voluntary intake of silage DM, although when expressed as a concentration of the total organic acids, the concentration can give a relative indication of silage intake. However, receptors in the sheep's duodenum are particularly sensitive to the duodenal concentration of lactic acid. Thus, silages that tend to be high in lactic acid (immature) would be expected to depress intake more than those that are low in lactic acid (more mature) (NRC, 1987). In silage diets containing high levels of crude protein (>210 g/kg DM) and a high proportion of NPN, excessive absorption of ammonia from the rumen can result in a significant reduction in DM intake (McDonald *et al.*, 1991).

Several workers have shown conclusively that the intake of silage DM by ruminants can be increased by reducing the chop length of the silage (McDonald *et al.*, 1991; De Boever, De Smet, De Brabander & Boucque, 1993). Chopping increases silage intake in two ways: firstly, through improving the fermentation quality and, secondly, through increasing the rate of passage of food through the rumen (McDonald *et al.*, 1991). It may be that the sheep's reticulum posesses stretch receptors that are sensitive to distention of the gut after a meal. This factor, when coupled with the longer lag time before distention begins, slower rate of digestion and longer retention time of long vs chopped forages may explain the unique effect of length of forage on intake in sheep (NRC, 1987). Compared with wilted silages, direct-cut silages had higher eating and total chewing indexes because of lower palatability and higher water concentration, but the ruminating index was only slightly affected by the preservation method (De Boever *et al.*, 1993).

According to McDonald *et al.* (1991), it has been found that well-preserved grass silages have higher gross energy (GE) values compared with fresh herbage. Consideration of the metabolic pathways of fermentation suggests that the increases in GE during ensiling result from losses of DM without concomitant losses of energy. There is also evidence to indicate that in wet crops DM losses in the effluent are comparatively greater than losses of GE. Of the deductions (faecal, urinary and methane) made from GE in the calculation of ME, faecal energy is the greatest. Losses of energy in the urine are usually higher on silage diets than on fresh herbage diets. This can be attributed mainly to the increased N excretion arising from inefficient utilisation of silage-N in the rumen. There is no evidence to indicate that methane energy losses in ruminants are increased as a result of ensiling. Because the magnitude of the increase in GE during fermentation is normally greater than the increased urinary energy loss, well-preserved silages can be expected to have higher ME values than the original parent materials.

Following ingestion of food by the ruminant, nitrogenous compounds undergo hydrolysis and deamination in the rumen under the influence of microbial enzymes to yield ammonia-N, which itself is one of the major nitrogenous substrates for the growth of rumen micro-organisms. The efficiency with which food-N is converted into microbial protein depends upon the relative rates of ammonia release and ammonia assimilation. If the former is faster than the latter, then the excess ammonia will be absorbed into the blood, carried to the liver and converted to urea. Although some of this urea is recycled to the rumen, generally most of it is excreted in the urine. The two main factors in this process are the rumen degradability of the dietary-N compounds and the level of energy available for microbial growth. Because a high proportion of the nitrogenous components of naturally fermented silages are in a non-protein, soluble form, they are extensively and rapidly degraded in the rumen, resulting in extreme ammonia patterns, manifested by higher peak values immediately after feeding and lower minimum values before feeding than those found in sheep given grass diets (McDonald *et al.*, 1991). Ammonia has been shown to be a protein fermentation product negatively correlated with intake (Van Os *et al.*, 1995).

McDonald *et al.* (1991), stated that the optimal utilisation of ammonia by rumen micro-organisms depends upon the synchronisation of both nitrogen and a suitable energy source.

The energy sources available in silage are not ideal, as most of the soluble carbohydrates present in the original forage have already been fermented to reduced products, which themselves may be end-products of rumen fermentation. An exception is lactate which, in the L(+) isomeric form, appears to be rapidly fermented in the rumen. Unfortunately, in most silages the D(-) isomer predominates and this form of lactic acid is metabolised by the animal more slowly than the L(+) form, which may lead to acidosis. However, there is evidence that the lactate concentration in the rumen is maintained at a low level due to its rapid metabolism by the rumen micro-organisms, and therefore little lactate is absorbed by the animal. Protozoa play a central role in ruminal lactate metabolism; the major end-product of this fermentation appears to be acetate.

As a consequence of the rapid production of ammonia in the rumen, combined with the deficiency of a suitable energy source, ruminal microbial protein synthesis in animals given silage diets, is lower than that in animals given fresh forage or hay diets (McDonald *et al.*, 1991; Van Vuuren *et al.*, 1995). The ARC (1984) accepts an average efficiency of microbial protein synthesis of 32g N/kg organic matter apparently digested in the rumen (**OMADR**) compared to an average of 23 g N/kg OMADR for grass silage based diets. Lower yields for silage based diets might be partly explained on the basis that readily fermentable OM has already been fermented to lactic acid and VFA's. These acids can account for up to 0.17 of DM and do not provide much energy for microbial growth.

Thus, because of a deficiency of suitable energy sources and the highly degradable nature of the nitrogenous compounds in naturally-fermented silages, it is reasonable to assume that the addition of carbohydrate-rich or protein-rich foods to silage diets may improve the overall utilisation of nitrogen. Supplementation of grass silage with barley has, however, proved largely ineffective in stimulating the efficiency of microbial synthesis, whereas consistent responses have been obtained by supplementing silage given to cattle with protein concentrates such as soya bean meal. Although starch, in the form of barley, appears to be an unsuitable energy source for stimulation of microbial protein synthesis in ruminants on silage diets, several workers have shown that a more readily fermentable carbohydrate source, such as sucrose, can be beneficial (Chamberlain *et al.*, 1989; McDonald *et al.*, 1991; Chamberlain, Robertson & Choung, 1993).

The beneficial effects of adding a protein supplement to silage diets on nitrogen utilisation and animal performance can be explained in a number of ways. Firstly, it has been suggested that the efficiency of microbial protein synthesis is considerably increased, or indeed optimised, when the rumen micro-organisms have access to both amino acid-N (as protein) and ammonia-N (Chamberlain, *et al.*, 1989; McDonald *et al.*, 1991; Robinson & McQueen, 1993). In models such as the Cornell Net Carbohydrate and Protein System (**CNCPS**) bacteria are categorised into those that ferment structural carbohydrates and those that ferment non-structural carbohydrates. Structural carbohydrate bacteria ferment cellulose and hemicellulose and use ammonia as their primary nitrogenous nutrient. Non-structural carbohydrate bacteria ferment sugars, starch and soluble fiber and use ammonia, AA's and peptides as nitrogenous nutrients for optimal microbial synthesis of protein (Russell, O'Connor, Fox, Van Soest & Sniffen, 1992). Since the proportion of nitrogen present as protein in silage diets is normally low, it is possible that absorption of intact amino acids by rumen micro-organisms is a limiting factor in their growth (Chamberlain, *et al.*, 1989; McDonald *et al.*, 1991).

Secondly, for efficient utilisation of acetate and butyrate, which are produced in the rumen and which may also be present in silage, the body tissues need an adequate supply of gluconeogenic substrates to supply the necessary amounts of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and glycerol for fat synthesis. Gluconeogenic amino acids, surplus to requirements for tissue protein synthesis, may play an important role in this respect in sparing glucose. Finally, that part of the dietary protein which is undegraded in the rumen may provide the animal with essential amino acids necessary for tissue synthesis. Amino acids which appear to be of particular importance are methionine, threonine and lysine (McDonald *et al.*, 1991).

1.7.2 Well-preserved wilted silage

McDonald *et al.* (1991), describes the term 'wilted silage' as silage made from herbage usually cut with a mower and left for varying periods of time in the field prior to lifting and ensiling, regardless of weather conditions. Under poor weather conditions, DM concentrations may increase very little, if at all (Haigh, 1988), and if the wilting period is

extended over several days, soluble carbohydrates and protein-N concentrations may be reduced and deamination of amino acids may increase (Carpintero, Henderson & McDonald, 1979; Steen, 1984; Gordon, 1986). The main effects of wilting under good weather conditions on the crop and on the composition of the silage in general, is that fermentation is restricted as DM concentration increases, and this is reflected in higher pH and soluble carbohydrate values, and lower levels of fermentation acids. The latter leading to a lower buffering capacity (McDonald *et al.*, 1990; McDonald *et al.*, 1991). Wilting by increasing the DM concentration reduces fermentation. Clostridia are particularly restricted in dry conditions with the net result that the WSC concentration required to produce a stable silage, which does not undergo clostridial fermentation, is reduced with an increase in DM concentration (Wilkins, 1981).

McDonald *et al.* (1991), concluded that the results of animal trials suggest that the digestibility is not influenced by wilting to any marked extent, although some workers have noted reductions in digestibility as a result of wilting, while others have obtained increases. The effect of wilting on digestibility is likely to be influenced very much by weather conditions. If wilting is prolonged and carried out under poor weather conditions, losses of highly digestible nutrients through oxidation and leaching can be relatively high and this could have an effect in reducing DM digestibility.

Several workers have reported increases in voluntary DM intake with increasing DM concentration, although in some studies increases in DM intake as a result of wilting have not always been obtained. It seems likely that the effect of wilting on DM intake is less with short-cut silage than with long material. The response in intake of silage DM concentration is likely to be small when low DM silages are well-preserved (McDonald *et al.*, 1991).

Because wilting a crop prior to ensiling restricts fermentation, both GE and ME values of wilted silages are likely to be less than those of silages made from unwilted material. It might be reasonable to assume that silages made from high DM herbages, and containing relatively high protein-N and WSC levels, would provide a better substrate for microbial protein synthesis. However, there is little evidence to indicate that the nitrogenous components in

wilted silages are utilised any more efficiently by ruminants than those in silages made from direct-cut material (McDonald *et al.*, 1991).

1.7.3 Badly-preserved silages

The term badly-preserved silages refers to silages in which either enterobacteria or clostridia or both have usually dominated the fermentation. It does not include those silages which have deteriorated as a result of oxidation. Badly-preserved silages are produced from crops which are either ensiled too wet or which contain low levels of fermentable carbohydrates. They may also be produced if the ensiled forage is deficient in lactic acid bacteria. In general, badly-preserved silages are characterised by having high pH values, usually within the range 5.0 to 7.0. The main fermentation acid present is either acetate or butyrate. Lactate and WSC are present in relatively low concentrations or frequently absent. The ammonia-N levels are usually high, often above 200 g/kg TN. This ammonia, which is derived from the catabolism of amino acids, is accompanied by other degradation products such as amines and various keto- and fatty-acids (McDonald *et al.*, 1991). Amines, have also been suggested as being responsible for the reduction in intake of poor-quality silages (Van Os *et al.*, 1995).

McDonald *et al.* (1991), stated that it is conceivable that when losses from the silo are high, as they usually are in the production of badly-fermented silages, concentrations of crude fibre, including lignin, can increase and this may result in a significant reduction in digestibility in silages made from relatively mature herbage. In young herbages the fibre is often as highly digestible as the other nutrients. It is generally accepted that intakes of DM by ruminants fed badly-preserved silages, are less than those by animals fed well-preserved silages.

From a consideration of the fermentation pathways, DM losses during the fermentation of sugars and lactic acid by enterobacteria and clostridia are very high. These losses are only partially balanced by energy losses and, as a consequence, the GE values of badly-preserved silages are likely to be slightly higher than those of the original herbage. When feeding the badly-preserved silages, the faecal energy losses are unlikely to be markedly different from those obtained when feeding the grass material, unless the grass material is very mature. On the other hand urine energy losses can be expected to be high from badly-preserved silage

because of the poor utilisation of silage nitrogen. Acetate is known to be used less efficiently as an energy source for tissue growth than propionate or butyrate, especially if there is a deficiency of glucose or glucose precursors. Both acetate and butyrate are themselves end-products of rumen fermentation and consequently are unlikely to feature as suitable energy sources for rumen micro-organisms. A further consequence of feeding silages low in gluconeogenic substances is the possibility of increasing the risk of hyperketonaemia (McDonald *et al.*, 1991).

According to McDonald *et al.* (1991), a large part of the nitrogenous components of badly-preserved silages is in a highly degradable form and this, coupled with a deficiency of suitable energy substrates, is likely to result in poor utilisation of silage nitrogen by the rumen micro-organisms. Inefficient utilisation of ammonia-N in the rumen may lead to increases in blood urea levels. It has been suggested that this may be associated with poor conception rates and reduced reproductive efficiency (Bruckental, Drori, Kaim, Lehrer & Folman, 1989; McCormick, French, Brown, Cuomo, Chapa, Fernandez, Beatty & Blouin, 1999). The potential toxic nature of the amines in badly-preserved silages, together with the possible contamination of milk and cheese with clostridial spores, make it imperative that the production of badly-fermented silage is avoided at all costs (McDonald *et al.*, 1991).

1.7.4 Aerobically deteriorated silages

Many silages when exposed to air immediately start to deteriorate. If the period of aerobic exposure is long, extensive changes in the composition of the silages can occur and this is likely to influence adversely their nutritional value. The changes are brought about firstly, by bacteria and yeasts and subsequently by moulds. Initially the soluble components of silages, WSC, organic acids and soluble nitrogenous compounds, act as substrates for the development of these micro-organisms. The losses of these nutrients result in corresponding increases in crude fibre and ash concentrations. Increases in pH and ammonia-N also frequently occur, although changes in the latter are inconsistent because of variable losses through volatilisation. In the terminal stages of deterioration, structural carbohydrates can also be decomposed (Woolford, 1990; McDonald *et al.*, 1991).

In a study with grass silages, Futjita, Matsuoka, Takahashi, Fukazawa & Takase (1980), as cited by McDonald, *et al.* (1991), compared the nutritional values of silages aerobically deteriorated for three to seven days with those of undeteriorated silages. Significant decreases in the digestibilities of organic constituents were observed in the deteriorated silages. Cows fed the latter had greater nitrogen losses in their faeces and urine, and significantly lower nitrogen retention values. Oxygen consumption, carbon dioxide production and heat production per unit metabolic weight were increased in animals fed the deteriorated silages, suggesting that there was a decrease in the availability of net energy (NE). High losses of ß-carotene can occur from silages exposed to air (McDonald *et al.*, 1991). In practice, once aerobic deterioration has begun, there is nothing that can be done to stop it (Woolford, 1990).

In addition to a reduction in nutritional value, aerobic deterioration of silages can result in serious health risks through the development of mycotoxin-producing moulds, such as *Aspergillus spp.* and *Fusarium spp.*, and undesirable pathogens such as *Listeria monocytogenes* (Woolford, 1990; McDonald *et al.*, 1991; Van Vuuren *et al.*, 1995).

CHAPTER 2

Materials and Methods

2.1 Introduction

The study was conducted at the Potchefstroom Agricultural College during the period December 1990 through to December 1993. The area has an exclusively summer rainfall, dry autumn and winter.

In this investigation attention was paid to:

1. The influence of physiological maturity on the process of fermentation.
2. Silage quality as measured by means of a partial digestibility trial.

2.2 Establishment of pastures

2.2.1 Soil sampling

Top soil samples (0-200 mm) and lower soil samples (200-400 mm) were taken on 14 December 1990 and analysed . See Table 2.1 and Table 2.2 for details of soil samples.

Table 2.1 pH and mineral analysis of topsoil samples

Topsoil (0-200 mm)					
Sample	1	2	3	4	5
pH H_2O	6.6	7.1	6.8	6.4	6.8
pH KCl	5.6	6.0	5.8	5.5	5.8
P mg/kg	67	49	104	32	48
K mg/kg	53	48	69	36	57
Ca mg/kg	467	495	549	464	496
Mg mg/kg	293	375	344	365	416
Na mg/kg	11	12	15	13	18

Table 2.2 pH and mineral analysis of lower soil samples

Lower soil (200-400 mm)					
Sample	1	2	3	4	5
pH H_2O	6.4	6.8	6.6	6.2	6.5
pH KCl	5.4	5.7	5.7	5.2	5.5
P mg/kg	14	9	7	8	4
K mg/kg	65	31	83	27	54
Ca mg/kg	448	393	600	620	388
Mg mg/kg	264	291	223	364	291
Na mg/kg	14	18	12	21	17

No fertiliser was applied before the pastures were planted.

2.2.2 Establishment of *Digitaria eriantha* and *Panicum maximum*

On 4 February 1991, 0.8 ha *Digitaria eriantha* spp. *eriantha* (*D. eriantha*) and 0.8 ha *Panicum maximum* cv. Gatton (*P. maximum*) were planted under irrigation. The pastures were allowed to reach maturity and on 26 August 1991 all the material was cut and removed.

2.2.3 Fertilisation

On 1 October 1991, 350 kg LAN/ha (100 kg N/ha) was applied to both pastures, after which 25mm irrigation was applied. A further 50 kg N/ha was applied on 9 January 1992.

2.2.4 Irrigation

To ensure a fair amount of DM-production on these small areas, at least 25mm of rainfall a week was simulated by means of irrigation.

2.3 Ensiling of pastures in laboratory silos

2.3.1 Hypothesis

It is expected that the fermentation process will differ between the young physiological stage (early, treatment A), the boot stage (medium, treatment B) and the full bloom stage (late, treatment C), with respect to the end products of fermentation.

Thus the three separate physiological stages were simulated to determine the effect of physiological maturity on the fermentation process.

2.3.2 Procedure

Treatment A, B and C were randomly distributed over the trial plot (area). For each pasture, treatment A was allocated $10m^2$, treatment B 0.5 ha and treatment C 0.3 ha.

For ensiling in fruit jars (1l capacity) and plastic bags (2.1 x 2.75m), each treatment of each pasture was cut at a height of 10cm above ground level. The pasture was chopped (10 to 15mm) by a maize silage harvester before it was ensiled in either fruit jars or plastic bags.

The first fertilisation of 100 kg N/ha took place on 1 October 1991 and the second of 50 kg N/ha on 9 January 1992. The pastures were cut on the days as follows:

1 To simulate the young physiological stage (A) (21 days regrowth) all pastures were harvested in the $10m^2$ plots on 9 January 1992. *D. eriantha* was ensiled on 30 January 1992 and *P. maximum* on 6 February 1992. This treatment was only ensiled in fruit jars and was not used in the digestibility trial.

2 The boot stage (B) of 0.5 ha for each pasture was harvested on 30 December 1991. *P. maximum* (treatment B) was ensiled on 5 February 1992 (30 days active regrowth). *P. maximum* only showed signs of active regrowth a week after it had been harvested, thus causing it to be ensiled a week after the *D. eriantha*. Treatment B of *D. eriantha* was ensiled on 30 January 1992 (31 days active regrowth). This treatment was ensiled in fruit jars as well as in plastic bags.

3 To simulate the full bloom stage (C) of each 0.3 ha pasture, they were cut on 13 December 1991. Treatment C of *P. maximum* was ensiled on 4 February 1992 (45 days active regrowth). Treatment C of *D. eriantha* was ensiled on 28 January 1992 (46 days active regrowth). This treatment was ensiled using fruit jars and plastic bags.

2.3.3 Determination of dry matter concentration

Before a certain stage was harvested for ensiling, ten tufts were clipped off at a height of 10cm above soil level. The material obtained from these tufts, were pooled and a sample was analysed for DM concentration. The DM concentration determination was essential to be able to estimate the amount of sugar that had to be added to each treatment of pasture during ensiling.

Since tropical pastures have a lower sugar concentration than temperate pastures, they tend towards an acetate fermentation (McDonald, *et al.*, 1991), thus an addition of 12 kg sugar per ton of grass is recommended if the DM concentration of the pasture is between 20 to 25%. If the DM concentration is higher, perhaps between 30 to 40%, it is recommended that 8 kg sugar per ton grass be added to ensure sufficient fermentation to produce a lactate type of silage (De Figueiredo, 1987).

2.3.4 Monitoring of the fermentation process

The following applies to treatment B and C:

1. As a control, 640 g of the harvested, chopped, material of each treatment of each pasture was ensiled in 1 l fruit jars without wilting or any sugar addition.
2. Secondly, 640 g unwilted, chopped material of each treatment was ensiled in 1l fruit jars but with a sugar addition of 7.6 g (11.8 kg/ton).
3. Thirdly, the chopped, harvested material of each treatment was left to wilt to a DM concentration of $\pm$30% after which 640 g was ensiled in 1 l fruit jars with no additions.
4. Lastly, the material was allowed to wilt as above, and 640 g chopped material was ensiled in 1 l fruit jars with an addition of 5.2 g sugar (8 kg/ton).

Due to the lack of material obtained from treatment A, 640g of this material was ensiled directly with 7.6 g sugar, and a further 640g of material was ensiled after it had been wilted (±30% DM) with 5.2 g sugar. Twelve samples per treatment (A, B & C) per pasture (*D. eriantha* or *P. maximum*) were taken. Three samples were immediately oven-dried, and analysed in the same way as the ensiled samples. These samples represent zero-fermentation. The other nine ensiled samples were analysed on day 7, day 21 and day 120 after ensiling. Three samples were allocated to each of these periods. After 7 days each sample representing each physiological stage (A, B & C) of each pasture (*D. eriantha* or *P. maximum*) was treated as follows. One fraction of each of the three samples was oven-dried at 60°C and analysed in duplicate for DM concentration, OM concentration and N concentration. The second fraction of each of the three samples was stored in a freezer at -13°C until it was analysed for buffering capacity, lactate concentration, acetate concentration, ammonia-nitrogen (NH_3-N) concentration, pH and total VFA's. The same procedures were followed after 21 and 120 days.

2.3.5 Analytical methods

The samples, which were oven-dried at 60°C, were used to determine DM concentration, ash concentration, OM concentration and N concentration. The samples which were frozen, were used to obtain a liquid supernatant by means of an extraction process. This extract was then used to determine pH value, buffering capacity, ammonia-N, VFA's and lactic acid concentration of the silage.

2.3.5.1 Dry matter concentration

This sample was oven-dried in porcelain crucibles at 100°C for 24 h, cooled in the moisture-free air of a dessicator containing silica gel and weighed. DM concentration was calculated as follows (AOAC, 1990):

$$\%DM = (\text{Dry mass g})/(\text{Sample mass g}) \times 100$$

2.3.5.2 Ash concentration

After weighing, the sample used for determining DM concentration, were incinerated in a muffle furnace at 600°C for 4h, cooled in a dessicator for one hour and weighed. The ash concentration was expressed as follows (AOAC, 1990):

%Ash = (Ash mass g)/(Sample mass g) x 100

Correction for DM was made and reported as:

%Ash (DM) = (%Ash x 100 / %DM)

2.3.5.3 Organic matter concentration

Utilising the DM and ash concentration of the samples the OM concentration was calculated as follows:

%OM (DM) = 100% - %Ash (DM)

2.3.5.4 Nitrogen concentration

The N concentration of the silage samples was determined by the macro-Kjedahl method (AOAC, 1990). Percentage N was calculated as follows:

% N = ((T - Bl) x F / sample mass) x 100

where
- T = titration figure
- Bl = blank value (0.01)
- F = factor

Percentage crude protein (CP) was calculated as follows:

% CP = % N x 6.25

Corrections were made for the DM concentration of the samples.

2.3.5.5 Extraction process

Extracts were made of all the silage samples for the determination of fermentation characteristics. Exactly 40 g of wet silage was weighed off from each jar and placed in 1000 ml containers to which 160 ml distilled water and 4 ml saturated mercuric chloride were

added. The latter served as a preservative. The containers were put in cold storage (7°C) overnight. The following morning they were shaken for 6 hours (180 rpm) using a horizontal shaker. The extracted silage was then filtered through four layers of cheesecloth to remove the plant matter. The supernatant was then transferred to 250 ml plastic bottles and stored in a freezer (-15°C) for later analysis. This extract was further divided into three sub-samples for analysis:

1. For the analysis of pH, WSC and nitrates. The extract was not treated further.
2. For the analysis of ammonia-N. The extract was treated as follows, 30 ml extract + 5 ml HCL (0.5M).
3. For the analysis of VFA's and lactic acid concentrations. The extract was treated as follows, 10 ml extract + 1 ml NaOH (10%).

These extracts were all frozen until time of analysis.

2.3.5.6 Silage pH

The above mentioned extracts were removed from the freezer and allowed to stand overnight to reach room temperature. The pH was measured by means of a pH-meter using a wet glass electrode after the meter had been calibrated by means of pH 4 and pH 7 buffer solutions. Readings were noted to the nearest 0.01 pH unit.

2.3.5.7 Buffering capacity

Buffering capacity is expressed as the number of milli-equivalents alkali required to change the pH of silage (100g DM) from 4 to 6 (Payne & McDonald, 1966). Five gram of the silage sample was placed in a plastic honey jar, 100 ml distilled water added and the sample macerated in a blender for 2 min, switching on and off every 20 sec. The next step was to filter through a Whatman No.1 filter paper and use an aliquot of the filtrate (50 ml) to determine the pH. The filtrate was then titrated to pH 3 with 0.1 M hydrochloric acid in order to release bicarbonate as carbon dioxide. The same filtrate was then titrated to pH 4 with 0.2 M sodium hydroxide and the burette reading (R1) recorded. The titration with 0.1 M sodium hydroxide to pH 6 was continued and the burette reading (R2) recorded.

The buffering capacity is calculated as follows:

Buffering capacity (meq / 100 g DM) = (390 / (R2 - R1)ml) x DM% sample

A correction must be made for the titration value of a 50 ml water blank.

2.3.5.8 Ammonia Nitrogen concentration

The clear supernatant of the silage extract was diluted when necessary and the NH_3-N concentrations determined using a Technicon Autoanalyser. The procedure described by Davie (1989) and Technicon Auto Analyser II Industrial Method No. 334-74W/B, Jan. 1976/revised March 1977 was used. The NH_3-N concentrations were reported as g/kg DM.

2.3.5.9 Volatile fatty acids concentration

The frozen extracts were removed from the freezer and allowed to reach room temperature. Of the extract, 2 ml was placed in a centrifuge tube. To this 0.2 ml ortho-phosphoric acid (50%) was added and mixed. These treated samples were centrifuged in a cooled chamber (less than 10° C) for 20 min at 4500 rpm. The clear supernatant was transferred to plastic containers and frozen until they were analysed.

The plastic containers were allowed to reach room temperature, before 1 µl of the supernatant was injected into a capillary column of a gaschromatograph. The specifications of the capillary column are as follows:

Chromopack, Wcot Fused Silica; 25m x 0.53 mm ID;
Coating CP-Wax 58 (FFAP) - CBDF=2.0
Cat. No. 7654.
The carrier gas was nitrogen at 15 ml/min.

The temperature programme used is as follows:

Programme 1. Starting temperature 100°C
Final temperature 160°C
Temperature increase 6°C / min

Programme 2. Final temperature 210°C
Temperature increase 12°C / min
Hold for 2 min

The standard used to calibrate the instrument contained the following:

Acetic acid	1.210 mmol /100 ml
Propionic acid	0.127 mmol /100 ml
n-Butyric acid	0.101 mmol /100 ml

iso-Valeric acid	0.036 mmol /100 ml
n-Valeric acid	0.039 mmol /100 ml

2.3.5.10 Lactic acid concentration

The method is based on the conversion of lactic acid to acetaldehyde by heating with sulphuric acid. The acetaldehyde is reacted with p-hydroybiphenyl to form a coloured complex which is read spectrophotometrically. By dissolving the p-hydroxybiphenyl in dimethylformamide instead of an alkali, a reagent is obtained which is indefinitely stable (Pryce, 1969).

The procedure followed is described below.

Pipette 7.9 ml of the precipitating reagent into a centrifuge tube and then add 0.10 ml of sample fluid. Mix and centrifuge for 5 min at 2000 rpm. Transfer 1.0 ml of this supernatant to a test tube. Rapidly add 6 ml sulphuric acid (AR grade). Allow the tube to stand for about 2 min and mix. Place the tube in tap water to cool for 2 to 3 min (below 20°C). Add 0.1 ml of p-hydroxydiphenyl solution to the tube, mix well and allow to stand for 10 min. Place the tube in a boiling water bath for 90 seconds. Cool again in tap water and read the absorbance values at 564 nm. The colour is stable for at least 1 hour after development.

2.3.6 Statistical analysis

The GLM (General Linear Models) procedure of SAS (1989), was used to test for statistical differences between treatments (D, D+S, W and W+S) and between sampling periods (Day 0, Day 7, Day 21 and Day 120). The three bottles per sampling period were used as replicates for statistical analysis. Significance of difference between least squares means was determined by Bonferroni's test (Van Ark, 1981).

2.4 Partial digestibility study

2.4.1 Hypothesis

It is expected that the differences that may occur in the fermentation process of the boot stage (B) and full bloom stage (C) may have an effect on nutrient supply to the animal.

2.4.2 Procedure

Only treatment B (± 30 days regrowth) and C (± 54 days regrowth) which were ensiled at a DM concentration of ±30% with an addition of sugar (8 kg/ton), were used for the partial digestibility study. Every half hour the DM concentration of the material was determined, using a microwave oven (Narasimhalu, Kunelius & Winter, 1982) until it had reached the required DM concentration. Then it was ensiled in air tight plastic bags (2.1m x 2.75m) from which the air was removed with a vacuum pump.

The partial digestibility study was done on an *ad libitum* intake basis. Four multi-fistulated sheep per treatment (ruminal, abomasal and ileal cannulas) were fitted with faecal collection bags and randomly allocated to treatments B and C. The animals were housed in individual metabolism crates and were fed three times daily, in the morning, afternoon and evening.

A constant infusion of markers with time interval sampling was used. The double marker technique of Faichney (1980), where the particulate and liquid fractions are marked simultaneously but separately, was used. An adaptation period of 14 days on the respective silage was allowed before sampling began. After ten days a prime dose of the marker was infused and thereafter a normal dose. Marker infusion then continued for 4 days to obtain a steady state condition before sampling for a period of 4 days began (Faichney, 1980). Marker infusion proceeded according to the procedure recommended by Siddons, Paradine, Beever & Cornell (1985), where ± 100mg Yb/animal/day and ± 240mg Cr/animal/day are infused. A peristaltic pump was used to infuse the markers and it was calibrated to infuse one litre of marker solution or 240 mg Cr and 100 mg Yb per day. The actual amounts of Chromium and Ytterbium in the prepared solutions were measured by atomic absorption spectrophotometry, and this was used to calculate the actual amounts of each marker infused daily.

Abomasal vs duodonal cannulas:

The abomasum can serve as a collecting pool with differential passage of digesta phases – abomasal contents may not be representative of those passing from the rumen and could yield biased data. Duodenal cannulation preferred – more representative of the material leaving the rumen (Titgemeyer, 1997)

2.4.3 Sampling methods

To simulate the effect of continuous flow through the digestive tract, sampling needs to be done at approximately three hour intervals to obtain representative samples of the digesta. Unfortunately such frequent sampling tends to interfere with the feeding behaviour of the animals, as well as the accuracy of flow determinations and is often not practical. To overcome this problem, sampling was conducted over a four day period. Sampling commenced on the fifth day after the onset of infusion and continued until day eight.

Digesta samples were taken from the rumen, abomasum and ileum. The samples were taken and pooled over four days in the following way:

First day	6 am and 6 pm
Second day	9 am and 9 pm
Third day	12 am and 12 pm
Fourth day	3 am and 3 pm

Faecal samples were collected twice daily, in the morning and afternoon. Provided steady state conditions have been achieved, it can be assumed that the marker is distributed evenly through the entire digestive tract.

Samples were preserved in the following way:

Rumen sample: Approximately 60ml rumen fluid was withdrawn and filtered through a double layer of cheese cloth. At this stage the pH value of the rumen fluid was measured. After this two samples, 30 ml for ammonia and 10 ml for VFA, were preserved by the addition of 5 ml of 0.5M HCL and 0.6 ml NaOH (10%) respectively. These were subsequently frozen.

Abomasal sample: This sample is naturally acid (pH $\pm$2) and does not need preservation. Thus it was only stored in a freezer.

Ileal sample: By freezing the sample directly after sampling.

Faecal sample: Faecal bags were emptied twice daily, the faeces were weighed and a grab sample (10% of total mass) was taken and added to the previous days frozen sample.

2.4.4 Analytical methods

Flow estimates of digesta and components thereof was determined according to a practical guide compiled by Smuts (unpublished) based on the dual-phase marker system technique described by Faichney (1975; 1980).

Abomasum and ileal samples were thawed and mixed. From this a homogenous sample was taken for the determination of DM concentration in the wet digesta. A further sample was taken and centrifuged, the supernatant was decanted and cool stored for the analysis of Cr and Yb concentrations (see section 2.4.4.4). The rest of the sample was dried in an oven at 60°C until dry. The dried sample was milled by a laboratory mill to pass through a 1mm sieve. The dried sample was further used for analysis. A second DM analysis was conducted on the dried sample, and this value was also used when reporting values.

2.4.4.1 Ammonia-nitrogen concentration

Ammonia-N concentration of the silage was determined according to the method described in section 2.3.5.8.

Preserved rumen fluid, abomasal and ileal samples were thawed and subsamples centrifuged at 4500 rpm for 20 min in a cooled chamber (10°C). The clear supernatant was diluted when necessary and the NH_3-N concentrations determined by a Technicon Autoanalyser. The procedure described by Davie (1989) and Technicon Auto Analyser II Industrial Method No. 334-74W/B, Jan. 1976/revised March 1977, was used. The NH_3-N concentrations were reported as mg /100ml:

$$[NH_3\text{-}N]\ (mg/100\ ml) = (Reading \times Dilution\ factor \times 1.2159) / 10$$

2.4.4.2 Volatile fatty acids concentration

The VFA's of the silage was determined according to the method described in section 2.3.5.9.

The VFA's of the rumen samples were determined on a packed column and not on a capillary column. The specifications of the packed column are as follows:

Column material - Deact glass; length 2m; OD 5 mm; ID 3 mm;

Support - Chromosorp WHP - 5P, Mesh range 80/100;

Liquid phase CW20M + H_3PO_4; Temperature range 60 - 225 °C.

The carrier gas was nitrogen at 35 ml/min.

The temperature programme used for the analysis was as follows:

Programme 1. Starting temperature 80°C
Hold for 2 min
Final temperature 130°C
Temperature increase 5°C / min
Hold for 2 min

Programme 2. Final temperature 150°C
Temperature increase 10°C / min
Hold for 1 min

The same standard as described in section 2.3.5.9. was used to calibrate the instrument, utilising the capillary column.

To 10.0 ml of the rumen sample/ NaOH-mixture, 1.0 ml 50% v/v ortho-phosphoric acid was added and mixed. These samples were then centrifuged in a cooled chamber (less than 10°C) at 4500 rpm for 20 min. Exactly 2 ml of the clear supernatant was pipetted into a clean bottle and to this exactly 1 ml internal standard solution (pivalic acid) and 5 ml distilled water was added. These mixtures were stored in a refrigerator until analysed. If the samples were to be held for longer than 48 hrs they were stored in the freezing compartment.

2.4.4.3 Lactic acid concentration

The method described in section 2.3.5.10 was used to determine the lactic acid concentration of the silage fed to the sheep during the experimental period.

2.4.4.4 Determination of Cr and Yb concentrations

Since the markers, Cr and Yb, were not expected to behave ideally, the concentrations of both were measured in the fluid and the solid phase. Samples of the wet abomasal and ileal digesta were centrifuged for 20 min at 4500 rpm in a cooled chamber (10°C). The supernatant was decanted and the Cr and Yb concentrations determined promptly by atomic absorption spectrophotometry. A Perkin-Elmer 2380 atomic absorption spectrophotometer was used.
The remainder of the abomasal samples were dried at 60°C and the ileal samples freeze-dried. Cr and Yb measurements were made on the solid phase after a wet digestion procedure. Approximately 1.5 g of sample was weighed, exactly 25 ml of nitric acid (HNO_3) added and digested at 230°C in a fume cabinet. After cooling, a further 10 ml of concentrated perchloric acid ($HClO_2$) was added and digested until the solution changed colour from clear through green to yellow/red. This indicates that the Cr has been oxidised to dichromate. This solution was diluted to 250 ml with potassium chloride solution (KCl). The potassium acts as an ionization suppressant to ensure the alkaline conditions necessary for Yb measurements. About 20 ml were decanted into clean glass bottles for later analysis.

The Cr was determined with a hollow cathode lamp at a wavelength of 357.9 nm. The slit setting was 0.7 nm and an air-acetylene flame was used. Yb was determined with a hollow cathode lamp at a wavelength of 398.8 nm. The slit setting was 0.2 nm and a nitrous oxide-acetylene flame was used. Where appropriate, the readings were multiplied by the dilution factor. Concentrations were reported in mg/l.

2.4.4.5 Feed intake of the animals used in the partial digestibility study

The feed intake of each sheep was determined by weighing the fresh feed offered and weighing the orts every morning before feeding. The difference yielded the intake of each individual animal.

2.4.4.6 True digesta flow in the digestive tract

The flow of digesta (referred to here as "True digesta" or "**TD**") through the digestive tract was calculated by reconstitution from the concentrations of the particulate and fluid markers (Ytterbium and Chromium-EDTA respectively) in the dried samples and supernatants (Smuts, unpublished; based on Faichney, 1975). The markers do not associate exclusively

with their respective media (Faichney, 1975), and since both the supernatants and dried samples contained Cr and Yb, corrections had to be made in calculating the particulate matter flow and the fluid flow rates.

By multiplying the TD value with the concentration of each nutrient (nitrogen) in the small and large intestine, the flow of that particular nutrient could be calculated for each of the two sections of the digestive tract. Simple subtraction yielded the disappearance of the nutrient in the particular part of the digestive tract, hence the term "partial digestibility" study. The disappearance of a nutrient in the rumen was calculated from the difference in intake of the nutrient and the flow of the nutrient in the abomasum. Similarly, the disappearance in the large intestine was calculated from the difference in flow of the nutrient in the ileum and the amount voided in the faeces.

As an example, the disappearance of N in the small intestine was calculated as follows:

N disappearance (g/d) = N flow at abomasum (g/d) - N flow at ileum (g/d)

2.4.4.7 Non ammonia nitrogen flow at the abomasum and ileum

NAN was calculated as:

NAN = Total N flow - NH_3-N flow

NAN gives an indication of the true protein in the small intestine and consists of protein or peptides from both microbial and dietary origin.

2.4.4.8 Nutrient disappearance as a proportion of nutrient intake

This was calculated as follows using OM as an example:

OM disappearance (proportion of intake) = (OM disappearance (g/d) / OM intake (g/d)) x 100

2.4.5 Statistical analysis

Tukey's studentized range test for factorial designs was used to test for differences between the two silages, separately for each harvesting stage (medium and late). Statistical significance was accepted at $p \leq 0.05$.

CHAPTER 3

Results and Discussion

Treatment differences within a specific growth stage and a specific grass

With the ensiling of the *D. eriantha* and *P. maximum* grass at an early growth stage, only two treatments were applied. Both these treatments included the addition of sugar (molasses), one without prior wilting (D+S) and the other with prior wilting (W+S). At the ensiling of the *D. eriantha* and *P. maximum* grass, (medium and late growth stages), four treatments were applied. These treatments were as follows:

1 Grass ensiled directly with no sugar addition (D)

2 Grass ensiled directly with sugar addition (D+S)

3 Grass ensiled with prior wilting and no sugar addition (W)

4 Grass ensiled with prior wilting and sugar addition (W+S)

In this chapter, the results obtained between treatments, within a specific growth stage and specific grass species, will be discussed.

3.1 *D. eriantha* grass silage ensiled at an early growth stage

The average DM concentration of the unwilted plant material was 23%, whilst that of the wilted material had a higher DM concentration of 31%.

3.1.1 Silage pH and buffering capacity

There was a significant decrease in the pH value of the D+S silage between day 0, and day 7, from 4.98 to 4.11 (Table 3.1). The pH value decreased further non-significantly until day 21 and increased significantly until day 120. The value on day 120 was significantly higher than the values on day 7 and day 21, but was significantly lower than the value on day 0. The pH value of the W+S silage decreased significantly between day 0 and day 7, with a further significant decrease between day 7 and day 21. Between day 21 and day 120 the pH value did not differ.

Table 3.1 The pH and buffering capacity values of *D. eriantha* grass ensiled at an early growth stage

Parameter	Day	D+S		W+S	
pH	0	$4.98^{1}{}_{a}$	±0.01	$4.82^{1}{}_{b}$	±0.03
	7	$4.11^{2}{}_{b}$	±0.02	$4.70^{2}{}_{a}$	±0.02
	21	$4.08^{2}{}_{b}$	±0.02	$4.24^{3}{}_{a}$	±0.03
	120	$4.25^{3}{}_{a}$	±0.04	$4.32^{3}{}_{a}$	±0.03
Buffering capacity (meq/100 g DM)	0	49.42_{b}	±0.77	56.55_{a}	±0.49

The following information will apply to all the tables in Chapter 3.

1: Values with different superscripts (1,2,3,4) down a column differ significantly at $P \leq 0.05$.
2: Values with different subscripts (a,b,c,d) across a row differ significantly at $P \leq 0.05$.
3: Values after the ± sign are standard deviation values.
4: Three observations per treatment.

The pH value of the W+S silage was significantly lower than that of the D+S silage on day 0. On day 7 and 21 the pH values of the D+S silage was significantly lower than those of the W+S silage. The D+S silage had a more rapid drop in pH between day 0 and day 7 compared to the W+S silage. This indicates a better rate of fermentation.

There were no significant differences in the pH values of the D+S and W+S silages on day 120, with the W+S silage (4.32) having a numerically higher pH value than the D+S silage (4.25). These values are slightly higher than the recommended 4.20 for good quality unwilted silage (McDonald *et al.*, 1991). This could have had a negative effect on the silage quality. According to Woolford (1999), silage made from a green crop like grass, will generally stabilise at a pH of around 4.0 when the DM concentration at the outset is 200 g/kg. Thus the value for the D+S silage (day 120) is slightly above these recommendations.

The buffering capacity value of the D+S silage was significantly lower than that of the W+S silage. The higher the buffering capacity of the plant material being ensiled, the more lactic acid needs to be produced to reach the critical pH where clostridia growth will be inhibited (Carpintero, Holding and McDonald, 1969). However, the higher osmotic pressure associated in general with wilting can inhibit clostridial growth so that a stable wilted silage can be achieved at pH values of 5 and above (McDonald *et al.*, 1991). This does not, however, mean that pH values of wilted silages are always high (Marsh, 1979). The slightly higher pH value of the wilted silage compared to the direct cut silage does not imply that the wilted silage is of an inferior quality. The pH value for the W+S silage (day 120) would be acceptable.

3.1.2 Lactic acid and acetic acid

There was a significant increase in the lactic acid concentration of the D+S silage between day 0, day 7 and day 21 (Table 3.2). The value on day 120 was not significantly higher than the value on day 21. There was a significant increase in the lactic acid concentration of the W+S silage from day 0 to day 7, from day 7 to day 21 and from day 21 to day 120.

There was no significant difference in the lactic acid concentration of the D+S and W+S silages on day 0, 7 and 21 respectively. The W+S silage had a significantly higher lactic acid concentration on day 120 (32.14 g/kg DM) compared to the D+S silage (27.68 g/kg DM). In a study by Meeske *et al.* (1999), the lactic acid concentration of the *D. eriantha* silages varied from 10 g/kg DM (control) to 31 g/kg DM (bacterial inoculant - Sil-All®). The lactic acid concentrations, of the D+S and W+S silages are within the above range.

There was a numerical decrease in the acetic acid concentration of the D+S silage as the ensiled period progressed. The acetic acid concentration of the W+S silage decreased non-significantly between day 0 and day 7, where after it increased significantly until day 21. There was a non-significant increase between day 21 and 120.

Table 3.2 The lactic and acetic acid concentrations of *D. eriantha* grass ensiled at an early growth stage

Parameter	Day	D+S	W+S
Lactic acid (g/kg DM)	0	3.86^{3}_{a} ±0.71	2.95^{4}_{a} ±0.07
	7	15.39^{2}_{a} ±1.24	13.58^{3}_{a} ±1.33
	21	22.73^{1}_{a} ±1.63	21.52^{2}_{a} ±0.53
	120	27.68^{1}_{b} ±1.81	32.14^{1}_{a} ±1.30
Acetic acid (g/kg DM)	0	4.23^{1}_{a} ±0.71	4.09^{2}_{a} ±1.49
	7	4.12^{1}_{a} ±0.60	3.34^{2}_{a} ±0.65
	21	4.06^{1}_{b} ±0.46	8.53^{1}_{a} ±0.84
	120	3.95^{1}_{b} ±0.55	9.07^{1}_{a} ±1.89

There were no significant differences in the acetic acid concentration of the D+S and W+S silages on day 0 and day 7. On day 21 and day 120 the W+S silage had a significantly higher acetic acid concentration compared to the D+S silage. The acetic acid concentrations of the D+S and W+S silages are similar to those obtained by Meeske *et al.* (1999), 9.9 g/kg DM (control silage) and 6.4 (inoculated silage).

With the higher buffering capacity value of the W+S silage compared to the D+S silage, more acid molecules would have to be produced to decrease the pH of the W+S silage (Carpintero *et al.*, 1969). From the data obtained there was a higher production (day 120) of lactic and acetic acid in the W+S silage.

3.1.3 Nitrogen and ammonia-nitrogen

The N concentration of the D+S silage decreased as the ensiled period progressed, with a significant decrease occurring between day 7 and day 21 (Table 3.3). The N

concentration of the W+S silage decreased significantly between day 0 and day 7, with no other significant differences occurring between day 7 and day 120. There were no significant differences in the N concentrations of the D+S and W+S silages on day 0, day 7 and day 21. On day 120 the D+S silage (24.74 g/kg DM) had a significantly lower N concentration compared to the W+S silage (28.42g/kg DM).

Table 3.3 The N and NH_3-N concentrations of *D. eriantha* grass ensiled at an early growth stage

Parameter	Day	D+S	W+S
N (g/kg DM)	0	29.73^{1}_{a} ±0.13	31.81^{1}_{a} ±0.07
	7	29.47^{1}_{a} ±1.82	28.77^{2}_{a} ±2.11
	21	25.75^{2}_{a} ±1.28	27.61^{2}_{a} ±1.06
	120	24.74^{2}_{b} ±1.59	28.42^{2}_{a} ±1.25
NH_3-N (g/kg N)	0	$46.71^{1,2}_{a}$ ±4.91	25.67^{2}_{b} ±3.90
	7	35.42^{2}_{a} ±1.50	36.72^{2}_{a} ±5.41
	21	55.27^{1}_{a} ±0.82	36.26^{2}_{b} ±0.81
	120	56.52^{1}_{b} ±12.20	98.79^{1}_{a} ±5.93

The NH_3-N concentration of the D+S silage decreased non-significantly between day 0 and day 7, with a significant increase occurring between day 7 and day 21. There was a non-significant increase between day 21 and day 120. The NH_3-N value of the W+S silage increased non-significantly between day 0 and day 21, with a significant increase occurring between day 21 (36.26 g/kg N) and day 120 (98.79 g/kg N). This significant increase could be a result of the slower drop in the pH of the W+S silage between day 0 and 21, with more clostridia growth occurring, breaking down the protein fraction to NH_3-N, as found by Meeske (1998).

The NH_3-N concentration of the D+S silage was significantly higher than that of the W+S silage on day 0 and day 21, with no significant difference on day 7. While on day 120 the D+S silage had a significantly lower NH_3-N concentration compared to the W+S silage.

A good index of protein degradation during ensiling is the NH_3-N concentration of the silage and it should be less, for a good quality silage, than 11% or 110 g/kg N of the TN concentration (McDonald *et al.*, 1991). Wilkinson (1988) and Orr & Treacher (1990), classified silages either as 'well-fermented' (50 to 100 g/kg N or less) or 'satisfactorily fermented' (100 to 150 g/kg N), using NH_3-N as a proportion of total N as an indicator of fermentation quality. Using the above criteria, the D+S and W+S silages can be classified as well-fermented silages.

To summarise both the D+S and W+S silages can be classified as well-fermented silages, having low pH values, higher lactic acid concentrations compared to acetic acid and having NH_3-N values less than 110 g/kg N.

3.2 *D. eriantha* grass silage ensiled at a medium growth stage

The average DM concentration of the unwilted plant material was 19%, whilst that of the wilted material had a higher DM of 30%.

3.2.1 Silage pH and buffering capacity

The buffering capacity of the D and D+S silages was significantly higher than that of the W and W+S silages on day 0 (Table 3.4). The implication of this is that more acid molecules would be needed to decrease the pH of the D silages compared to the W silages (Carpintero *et al.*, 1969). This difference between the D and W silages is relatively small compared to differences, which occur between legumes and grasses (McDonald *et al.*, 1991).

Table 3.4 The pH and buffering capacity values of *D. eriantha* grass ensiled at a medium growth stage

Parameter	Day	D	D+S	W	W+S
pH	0	5.17^{1}_{a} ±0.21	5.17^{1}_{a} ±0.21	4.91^{1}_{b} ±0.04	4.91^{1}_{b} ±0.04
	7	4.93^{2}_{a} ±0.04	4.02^{2}_{d} ±0.02	4.74^{3}_{b} ±0.01	4.33^{2}_{c} ±0.02
	21	4.83^{2}_{a} ±0.04	$3.99^{2,3}_{c}$ ±0.01	$4.77^{2,3}_{a}$ ±0.02	4.31^{2}_{b} ±0.03
	120	4.70^{3}_{b} ±0.02	3.90^{3}_{d} ±0.02	$4.88^{1,2}_{a}$ ±0.02	4.33^{2}_{c} ±0.03
Buffering capacity (meq/100 g DM)	0	43.80_{a} ±0.02	43.80_{a} ±0.02	32.05_{b} ±0.12	32.05_{b} ±0.12

The pH of the *D. eriantha* medium growth stage ensiled directly (D), decreased significantly between day 0 and day 7, with a non-significant decrease until day 21, followed by a significant decrease until day 120 (Table 3.4). The final pH value of 4.70 (day 120), is higher than the recommended 4.20 for good quality unwilted silage (McDonald *et al.*, 1991) This could have a negative influence on the quality of the D silage.

There was a significant decrease in the pH of the *D. eriantha* medium silage, ensiled directly, with sugar addition (D+S), between days 0 and day 7, followed by a significant decrease until day 120. This improvement in the lower pH value of the D+S silage compared to the D silage could possibly be attributed to an increased amount of water-soluble carbohydrates due to the addition of sugar. This leads to more energy substrate being available for the micro-organisms, leading to a higher production of lactic and/or acetic acid molecules, resulting in a lower silage pH value (McDonald *et al.*, 1991; Meeske, 1998). The final pH value of the D+S silage (3.90) is lower than the recommended 4.20 for good quality unwilted silage (McDonald *et al.*, 1991), and this silage can be classified as good quality silage.

The pH decrease of the W silage resembled that of the D silage. However, the initial pH was significantly lower than that of the D silage. The final pH of the W silage (4.88) was significantly higher than that of the D silage (4.70), this was not the case between day 0, 7 and 21. The variation in the pH value of the D and W silages corresponds with data obtained by Fitzgerald (1996), who obtained a variation in pH value of between 0.15 and 0.40 units, with the unwilted material having a lower pH compared to the wilted material.

In effect wilting had no effect on the pH as the ensiling period progressed and hence silage quality in this respect was not improved. However, according to McDonald *et al.* (1991), a stable wilted silage can be obtained with a pH value higher than 5.0. This does not, however, mean that pH values of wilted silages are always high (Marsh, 1979).

The initial pH value of W+S silage was the same as the W silage, but significantly lower than the D and D+S silages. During wilting micro-organisms present on the plant material will start to ferment the WSC in the plant material under aerobic conditions and produce acetic acid. This would lead to the decrease in pH of the wilted material compared to the direct ensiled material (McDonald *et al.*, 1991).

The pH of the W+S silage decreased significantly between day 0 and day 7, whereafter it remained constant until day 120. The pH values for day 7, 21 and 120 of the W+S silage was significantly lower than the values for the D and W silages. Thus wilting counteracted in someway the improvement, which was obtained with sugar addition, as the D+S silage had significantly lower pH values compared to the W+S silage on days 7, 21 and 120.

According to McDonald *et.al.* (1991), a dramatic decrease in pH occurs within the first three to four days after ensiling, whereafter the pH should remain constant or decrease slightly in well preserved silages. The pH data for the D+S and W+S silages follows this trend.

3.2.2 Lactic acid and acetic acid

The lactic acid concentration of the D silage increased significantly from 1.41 g/kg DM to 24.47 g/kg DM between day 0 and day 7, whereafter it remained fairly constant (Table 3.5). There was a significant increase in the acetic acid concentration of the D silage between day 0, day 7 and day 21, whereafter it decreased significantly until day 120.

Table 3.5 The lactic and acetic acid concentrations of *D. eriantha* grass ensiled at a medium growth stage

Parameter	Day	D	D+S	W	W+S
Lactic acid (g/kg DM)	0	1.41^{2}_{a} ±0.05	1.41^{4}_{a} ±0.05	1.37^{2}_{a} ±0.05	1.37^{2}_{a} ±0.05
	7	24.47^{1}_{b} ±3.77	53.47^{3}_{a} ±5.66	17.67^{1}_{c} ±1.44	24.08^{1}_{b} ±7.78
	21	24.52^{1}_{b} ±3.18	64.24^{1}_{a} ±2.85	12.71^{1}_{c} ±0.32	22.86^{1}_{b} ±3.94
	120	25.22^{1}_{b} ±1.26	58.97^{2}_{a} ±1.52	12.83^{1}_{c} ±0.32	24.14^{1}_{b} ±0.62
Acetic acid (g/kg DM)	0	5.28^{3}_{a} ±1.01	5.28^{2}_{a} ±1.01	6.19^{3}_{a} ±1.33	6.19^{1}_{a} ±1.33
	7	17.72^{2}_{a} ±0.43	10.31^{1}_{b} ±1.72	$8.76^{2,3}_{bc}$ ±3.11	6.35^{1}_{c} ±1.37
	21	23.71^{1}_{a} ±2.24	11.89^{1}_{b} ±3.42	11.43^{2}_{b} ±4.29	5.08^{1}_{c} ±0.56
	120	19.42^{2}_{a} ±3.47	11.58^{1}_{b} ±1.52	16.80^{1}_{a} ±4.14	5.34^{1}_{c} ±2.44

The addition of sugar to the *D. eriantha* (D+S) at ensiling resulted in a significant increase in lactic acid concentration of the silage between day 0 (1.41 g/kg DM) and day 7 (53.47 g/kg DM), and a further significant increase until day 21. There after it decreased significantly until day 120, although it was still high in comparison to day 0. The lactic acid concentration of the D+S silage was significantly higher than that of the D silage for days 7, 21 and 120 (Table 3.5). Keady, Murphy & Harrington (1996), concluded that the addition of molasses (sugar) to plant material before ensiling resulted

in a decrease in the pH, increase in the lactic acid concentration and a lower NH_3-N concentration of the silage.

In contrast, the acetic acid concentration of the D+S silage was significantly lower than that of the D silage on day 7, day 21 and day 120. The acetic acid concentration of the D+S silage increased significantly between day 0 and day 7 whereafter it remained fairly constant. This difference in the fermentation pattern indicates that the addition of sugar, favoured lactic acid production and not acetic acid production (McDonald *et al.*, 1991). The higher initial pH of the D+S silage compared to the W and W+S silages could have favoured the growth of bacteria which produce lactic and acetic acid (McDonald *et al.*, 1991). This could explain the higher production of acetic acid between day 0 and day 7 compared to the W and W+S silages.

The W silage had a significant increase in lactic acid concentration between day 0 and day 7, with a non-significant decrease until its final value of 12.83 g/kg DM on day 120. The acetic acid concentration, of the W silage, increased significantly as the ensiled period progressed.

The W silage had significantly lower lactic acid concentrations when compared to D and D+S for days 7, 21 and 120. The acetic acid concentration of the W silage was significantly lower than that for the D silage on day 7 and day 21. However, in contrast, the acetic acid concentration of the W silage did not differ significantly from the D+S silage, except for the W silage, having a significantly higher value on day 120.

The data obtained, indicated that wilting of *D. eriantha*, before ensiling, resulted in a fermentative process favouring acetic acid production. This was reflected in the higher acetic acid (16.80 g/kg DM) concentration compared to the lactic acid (12.83 g/kg DM) concentration of the W silage on day 120.

The lactic acid concentration of the W+S silage increased significantly between day 0 and day 7 and then remained fairly constant until day 120. The acetic acid concentration

of the W+S silage did not change very much as the ensiled period progressed, as it fluctuated around an average of 5.74 g/kg DM.

The lactic acid concentration of the W+S silage, on day 7, 21 and 120, was significantly higher than that of the W silage, but did not differ significantly from that of the D silage, and was significantly lower than that of the D+S silage. The acetic acid concentration of the W+S was significantly lower than that of the D, D+S and W silages towards the end of the ensiling period (day 21 and 120).

In a major study conducted in Northern Ireland, where one hundred and thirty six grass silages were analysed, the lactic acid concentration varied between 0 and 144 g/kg DM, and the acetic acid concentration varied between 4 and 63 g/kg DM (Steen, Gordon, Mayne, Poots, Kilpatrick, Unsworth, Barnes, Porter and Pippard, 1995). The values in the current study were within the above mentioned minimum and maximum levels, but towards the lower end of these ranges, indicating that the production of lactic and acetic acid was on the low side, compared to some of the other values obtained by Steen *et al.* (1995). The pH values obtained by Steen *et al.* (1995), varied between 3.5 and 5.5, compared to those of the current study which varied from 3.90 to 4.88 on day 120.

The data sets presented in Table 3.5 indicated that the addition of sugar to *D. eriantha* medium quality grass, when ensiling, favoured the production of lactic acid, as the lactic acid concentration of both D+S and W+S silages where higher than that of the acetic acid concentrations. However, this effect was more pronounced for the D+S silage. In keeping with this statement, there was a significant decrease in the acetic acid concentration of the D+S and W+S silages in comparison with the D and W treatments, with sugar addition. This implies that sugar addition to *D. eriantha* improved silage quality. McDonald *et al.*, (1991) stated that a lactic acid fermentation process is preferred above acetic acid production, which would be reflected by a higher lactic acid concentration and a lower acetic acid concentration in the silage. The reason for this is that lactic acid is a stronger acid than acetic acid and this would lead to a faster drop in the pH value of the silage and thus better stability (Woolford, 1998).

A decrease in the rate of fermentation occurs when plant material is wilted before ensiling, leading to a decrease in the concentration of lactic and/or acetic acid being produced. This will be reflected in a higher pH value compared to direct ensiled plant material (McDonald *et al.*, 1991). This statement holds true for the present study as the lactic acid concentration of the W and W+S silages are lower than those of the D and D+S silages and the pH values are higher on day 120.

3.2.3 Nitrogen and ammonia-nitrogen

During the initial ensiling phase, after harvesting, rapid proteolysis takes place. The extent of true protein degradation varies with plant species, rate and extent of pH changes, DM concentration and temperature. The extent of protein degradation can be as much as 50-60%, even in well-preserved silage (McDonald *et al.*, 1990; McDonald *et al.*, 1991).

The initial N concentration of the D and D + S silages (31.82 g/kg DM) was significantly lower than that of the W and W + S silages (36.88 g/kg DM) (Table 3.6). The significant increase in N concentration of the wilted samples (W and W+S) on day 0 cannot totally be explained. All treatments were randomly selected from the same batch of *D. eriantha* grass. During wilting losses of non-structural carbohydrates and protein can occur (Marsh, 1979). The only explanation for an increase in N concentration would be that the non-structural carbohydrate loss exceeded the N loss during wilting, hence a small increase in N concentration.

The N concentration of the silages decreased as the ensiled period progressed, with only the N concentration of the D silage not being significantly lower on day 120 than day 0. The significant decrease in N concentration between day 0 and day 7 was more pronounced in the W and W + S silages compared to the D and D + S silages (Table 3.6). There were no significant differences in the N concentration between the four treatments on day 120. The decreases in N concentration between day 21 and day 120 were non-significant within the four treatments.

Table 3.6 The N and NH_3-N concentrations of *D. eriantha* grass ensiled at a medium growth stage

Parameter	Day	D	D+S	W	W+S
N (g/kg DM)	0	31.82^{1}_{b} ±0.30	31.82^{1}_{b} ±0.30	36.88^{1}_{a} ±0.10	36.88^{1}_{a} ±0.10
	7	28.85^{2}_{bc} ±0.56	27.17^{2}_{c} ±1.30	31.02^{2}_{ab} ±0.98	32.56^{2}_{a} ±0.53
	21	$29.89^{1,2}_{a}$ ±0.93	28.85^{2}_{a} ±0.39	$29.09^{2,3}_{a}$ ±0.94	29.04^{3}_{a} ±0.61
	120	$29.72^{1,2}_{a}$ ±0.95	27.81^{2}_{a} ±0.46	28.30^{3}_{a} ±1.16	27.66^{3}_{a} ±0.97
NH_3-N (g/kg N)	0	44.94^{2}_{a} ±6.94	44.94^{1}_{a} ±6.94	34.97^{1}_{a} ±0.39	$34.97^{1,2}_{a}$ ±0.39
	7	59.71^{2}_{a} ±3.87	$30.30^{1,2}_{b}$ ±0.10	43.67^{1}_{b} ±1.02	$31.60^{1,2}_{b}$ ±0.77
	21	59.09^{2}_{a} ±1.43	27.76^{2}_{b} ±6.81	41.01^{1}_{b} ±0.96	42.28^{1}_{b} ±2.61
	120	87.31^{1}_{a} ±2.05	27.12^{2}_{b} ±0.65	39.31^{1}_{b} ±0.97	26.32^{2}_{b} ±0.66

The NH_3-N concentration of the D silage, as a proportion of total N increased non-significantly as the ensiled period progressed from on day 0 to day 7. A significant increase occurred between day 21 and day 120. The D silage can be classified as 'well-fermented', according to the NH_3-N value on day 120, which is lower than the 110 g/kg N used as an index by McDonald *et al.*(1991), and lies between 50 and 100 g/kg N (or less) used by Wilkinson (1988) and Orr & Treacher (1990) "for a well-fermented" silage. Meeske *et al.* (1999) reported a NH_3-N g/kg TN of 50.3 after ensiling *D. eriantha* for 44 days. This is lower than the value obtained for the D silage.

In contrast to the D silage, the NH_3-N concentration of the D+S silage decreased as the ensiled period progressed. There was a non-significant decrease in the NH_3-N values of the D+S silage between day 0 and day 7. A further non significant decrease occurred between day 21, and 120. These values were significantly lower than the value on day 0 (Table 3.6.). The NH_3-N value of the D+S silage on day 120 (27.12 g/kg N) was

significantly lower than that of the D silage (87.31 g/kg N), implying a decrease in protein degradation when sugar was added to the *D. eriantha*, thus a protein saving effect. According to Ohshima & McDonald (1978), the wilting of crops prior to ensiling does not appear to inhibit plant protease activity, although in wilted silages, clostridial activity can be inhibited (higher osmotic pressure and lower moisture concentration). As a result, NH_3-N / kg N levels can be lower in wilted silages compared to unwilted silages.

The NH_3-N concentration of the W silage increased statistically between day 0 and day 7, whereafter it remained fairly constant. The NH_3-N values for the W silage is significantly lower than that for the D silage, but does not differ significantly from the D+S silage. The NH_3-N concentration of the W+S silage fluctuated as the ensiled period progressed. The final value on day 120 (26.32 g/kg N) was not significantly lower than the initial value of 34.97 g/kg N on day 0. The NH_3-N /kg N concentration of the W+S silage was significantly lower than that of the D silage on day 7, 21 and 120, but did not differ significantly from that of the D+S and W silages. According to Wilkinson (1988) and Orr & Treacher (1990), the D, D+S, W and W+S silages can be classified as 'well-fermented' (50 to 100 g/kg N, or less) silages.

The fact that the higher N degradation found for the W silages compared to the D silages, cannot be explained. In the case of D+S and W+S silages, the lower NH_3-N values can be due to the fact that sugar addition supported an increased lactic acid bacteria population and a decrease in the numbers of clostridia. This could have lead to a higher proportion of the protein, which had been degraded, being incorporated into the microbial protein mass and less being broken down to NH_3-N. This speculation is supported by the fact that sugar addition reduced the silage pH, increased lactic acid concentration and decreased acetic acid concentration for both the D+S and W+S silages.

The data presented on the medium growth stage *D. eriantha* silage, indicated that the addition of sugar to the silages, both D and W, resulted in lower pH and NH_3-N values. The implication of this was an improvement in silage quality (De Figueiredo, 1987; Offer & Al-Rwidah, 1989; McDonald *et al.*, 1991; Moore & Kennedy, 1994; Rouzbehan,

Galbraith, Topps & Rooke, 1996). Wilting of the medium growth *D. eriantha* grass, before ensiling resulted in a less drastic decrease in pH compared to the D silage when sugar was added. The latter being due to the fact that wilting decreases the fermentation processes in the silage (Marsh, 1979; McDonald *et al.*, 1991). This is supported by the lower lactic and acetic acid concentrations of the wilted silages compared to the direct silages in this study. The addition of sugar resulted in higher lactic acid production especially for the direct ensiled material, and lower acetic acid productions.

In summary, the data indicated that medium growth stage *D. eriantha* grass can be ensiled successfully. The quality of the silage made can be improved if sugar (molasses) was added to the grass material before ensiling. Despite the medium growth stage having a relatively low DM concentration, wilting before ensiling did not improve the silage quality in terms of a lower pH value. If the grass were wilted before ensiling, sugar addition would still improve the silage quality.

3.3 *D. eriantha* grass silage ensiled at a late growth stage

The average DM concentration of the unwilted plant material was 25%, whilst that of the wilted material had a higher DM concentration of 30%.

3.3.1 Silage pH and buffering capacity

The buffering capacity values of the silages, for the four different treatments did not differ significantly on day 0 (Table 3.7).

There was a significant decrease in the pH of the *D. eriantha*, late growth stage, ensiled directly (D), between day 0 and day 7, whereafter it remained fairly constant until day 21. A significant increase in pH occurred between day 21 and day 120, with the final value being significantly higher than the values on day 0 and day 7 (Table 3.7). The final pH value of 5.12 is almost 1 pH unit higher than the value of 4.20 recommended by McDonald *et al.* (1991) for a well-preserved silage.

The pH of the *D. eriantha* late direct silage, ensiled with sugar addition (D+S), decreased significantly between day 0 (4.70) and day 7 (4.08), whereafter it remained fairly constant until day 21. Between day 21 and day 120 the pH increased significantly to a value of 4.62 (day 120). This value was not significantly lower than the initial value on day 0.

Table 3.7 The pH and buffering capacity values of *D. eriantha* grass ensiled at a late growth stage

Parameter	Day	D	D+S	W	W+S
pH	0	4.70^{2}_{a} ±0.03	4.70^{1}_{a} ±0.03	4.79^{1}_{a} ±0.02	4.79^{2}_{a} ±0.02
	7	4.30^{3}_{c} ±0.01	4.08^{2}_{d} ±0.02	4.51^{2}_{b} ±0.02	5.17^{1}_{a} ±0.23
	21	4.40^{3}_{a} ±0.01	3.81^{2}_{b} ±0.03	4.31^{3}_{a} ±0.01	4.32^{4}_{a} ±0.01
	120	5.12^{1}_{a} ±0.11	4.62^{1}_{b} ±0.04	4.32^{3}_{c} ±0.02	4.61^{3}_{b} ±0.02
Buffering Capacity (meq/100g DM)	0	44.69_{a} ±0.26	44.69_{a} ±0.26	44.89_{a} ±0.83	44.89_{a} ±0.83

The pH of the D+S silage showed a similar decreasing and then increasing tendency as that of the D silage. The values on day 7, 21 and 120 were significantly lower for the D+S silage compared with the D silage. This would indicate that sugar addition resulted in a lower pH, but the final pH of the D+S silage on day 120 was still well above the 4.20 recommended by De Figueiredo (1987) and McDonald *et al.* (1991).

There was a significant decrease in the pH value of the *D. eriantha* late growth stage silage, which was wilted prior to ensiling (W), between day 0 and day 7, with a further significant decrease until day 21, whereafter it remained constant. This tendency was different compared to that of the D and D+S silages, as these silages showed a significant increase in pH between day 21 and 120. The value of the W silage on day 120 (4.32) is

higher than the 4.20 recommended by McDonald *et al.* (1991), but these authors stated that with wilted silages, the pH values could be higher.

The pH of the W+S silage was very unstable as it increased significantly between day 0 and day 7, whereafter it decreased significantly until day 21. Finally, between day 21 and 120 there was a significant increase. The value on day 120 was significantly lower than that of the D silage.

3.3.2 Lactic and acetic acid

The lactic acid concentration of the four silages did not differ significantly on day 0 (Table 3.8). The lactic acid concentrations of the D, D+S and W silages increased significantly between day 0 and day 7, while that of the W+S silage increased non-significantly. The value for the D+S silage was significantly higher than that of the W, D and the W+S silages. The values for the D and W+S silages did not differ significantly from each other, but they did differ significantly from that of the W silage.

A further significant increase occurred in the lactic acid concentrations of the silages between day 7 and day 21. There were no significant differences in the lactic acid concentrations of the D+S silage and the W, but they did differ significantly from the D and the W+S silages. The D and W+S silages also differed significantly. The lactic acid concentrations of the W and W+S silages remained relatively constant between day 21 and 120, while that of the D and D+S silages underwent a pronounced significant decrease. There were no significant differences between the D and D+S silages, while they differed significantly from the W and the W+S silages, with the W and W+S silages also being significantly different from one another on day 120.

There was no significant difference in the acetic acid concentrations of the four silages on day 0 (Table 3.8). The acetic acid concentration of the D silage increased significantly between day 0 and day 7, whereafter it increased non-significantly until its final value on day 120. The D+S silage showed an increase in acetic acid concentration between day 0,

day 7, day 21 and day 120, with only day 120 and day 21 differing significantly from day 0.

Table 3.8 The lactic and acetic acid concentrations of *D. eriantha* grass ensiled at a late growth stage

Parameter	Day	D	D+S	W	W+S
Lactic acid (g/kg DM)	0	2.27^{3}_{a} ±1.07	2.27^{3}_{a} ±1.07	2.82^{3}_{a} ±0.07	2.82^{2}_{a} ±0.07
	7	9.80^{2}_{c} ±1.61	31.85^{2}_{a} ±2.38	24.76^{2}_{b} ±1.49	7.48^{2}_{c} ±0.36
	21	26.98^{1}_{b} ±3.17	37.29^{1}_{a} ±1.33	35.64^{1}_{a} ±6.94	17.40^{1}_{c} ±0.45
	120	2.31^{3}_{c} ±0.06	2.37^{3}_{c} ±0.06	37.26^{1}_{a} ±4.00	18.53^{1}_{b} ±2.73
Acetic acid (g/kg DM)	0	2.78^{2}_{a} ±1.83	2.78^{2}_{a} ±1.83	5.18^{3}_{a} ±0.13	$5.18^{2,3}_{a}$ ±0.13
	7	8.76^{1}_{a} ±0.22	$6.32^{1,2}_{ab}$ ±0.11	4.87^{3}_{b} ±1.59	3.41^{3}_{b} ±0.98
	21	12.22^{1}_{a} ±0.69	6.72^{1}_{b} ±1.23	9.36^{2}_{ab} ±3.20	$8.33^{1,2}_{b}$ ±2.26
	120	12.52^{1}_{b} ±0.70	8.24^{1}_{c} ±0.07	16.74^{1}_{a} ±8.91	9.11^{1}_{bc} ±1.04

The acetic acid concentration of the W silage decreased non significantly between day 0 and day 7, whereafter it increased significantly until day 21. The final value on day 120 was significantly higher than that on day 0, 7 and 21. The acetic acid concentration of the W+S silage also showed a non significant decrease between day 0 and day 7, whereafter it increased significantly from day 7 to day 21. The final value on day 120 was not significantly higher than that on day 21, but was significantly higher than the values on day 0 and day 7.

On day 120 the acetic acid concentration for W silage was significantly higher than that of the D, D+S and W+S silages. The D silage had a significantly higher acetic acid concentration than that of the D+S silage, while the W+S silage did not differ

significantly from the D and D+S silages. The lactic and acetic acid concentrations of the W silage are higher than those obtained by Meeske *et al.* (1999) for the control and inoculated silage made from *D. eriantha*. The silage DM concentration (38.8%) in the study by Meeske *et al.* (1999) was higher than the DM concentration of the W silage in the present study (30%).

The W silage had the highest lactic and acetic acid concentrations on day 120 compared to the other silages and the W silage had the lowest pH values of the four silages on day 120. In the D and D+S silages there was a significant increase in the pH values between day 21 and day 120 and this was reflected in a dramatic decrease in the lactic acid concentrations of these silages between day 21 and day 120.

3.3.3 Nitrogen and ammonia-nitrogen

There were no significant differences in the N concentration of the four silages on day 0 (Table 3.9). On day 120 the D+S silage had a significantly higher N concentration than that of the D silage and the W silage. The N concentration of the W+S silage was significantly higher than that of the D silage, but did not differ significantly from the other silages.

The NH_3-N concentration as a proportion of total N for the four silages did not differ significantly on day 0 (Table 3.9). The D silage showed a significant increase in NH_3-N concentration between day 0 and day 7, whereafter it decreased non significantly until day 21. The NH_3-N values of the D+S and W silages did not differ significantly from day 0 to day 7 to day 21. The W+S silage showed a significant increase in NH_3-N concentration between day 0 and day 21, with day 7 not significantly different from day 0 and day 21.

The D, D+S and W silages showed significant increases in NH_3-N concentration between day 21 and day 120, while the NH_3-N concentration of the W+S silage remained constant. The NH_3-N concentration of the D silage (211.25 g/kg N) was significantly higher than that of the D+S silage (108.93). These two silages had significantly higher

values than that of the W (74.21 g/kg N) and W+S (60.13 g/kg N) silages. The values of the W and W+S silages did not differ significantly. In the study conducted by Steen *et al.* (1995) the NH_3-N values varied between 45 and 385 g/kg N, with the mean value being 123 g/kg N. The values in the current study were in most instances below the mean value obtained in the study by Steen *et al.* (1995) indicating that protein breakdown was not excessive.

Table 3.9 The N and NH_3-N concentrations of *D. eriantha* grass ensiled at a late growth stage

Parameter	Day	D	D+S	W	W+S
Nitrogen (g/kg DM)	0	25.16^{1}_{a} ±0.13	25.16^{1}_{a} ±0.13	25.23^{1}_{a} ±1.24	25.23^{1}_{a} ±1.24
	7	17.19^{3}_{b} ±0.64	20.79^{2}_{a} ±2.87	21.10^{2}_{a} ±1.29	19.20^{3}_{ab} ±2.68
	21	20.54^{2}_{a} ±1.21	$22.66^{1,2}_{a}$ ±1.76	20.66^{2}_{a} ±0.17	$21.63^{2,3}_{a}$ ±1.31
	120	17.95^{3}_{c} ±1.01	24.23^{1}_{a} ±0.72	19.66^{2}_{bc} ±1.21	21.83^{2}_{ab} ±4.52
NH_3-N (g/kg N)	0	35.57^{3}_{a} ±0.88	35.57^{2}_{a} ±0.88	43.53^{2}_{a} ±5.72	43.53^{2}_{a} ±5.72
	7	51.56^{2}_{a} ±1.29	45.77^{2}_{ab} ±1.07	35.67^{2}_{b} ±0.08	$48.44^{1,2}_{ab}$ ±15.01
	21	$42.19^{2,3}_{b}$ ±0.99	38.41^{2}_{b} ±0.90	49.33^{2}_{ab} ±1.15	62.05^{1}_{a} ±4.14
	120	211.25^{1}_{a} ±4.07	108.93^{1}_{b} ±11.88	74.21^{1}_{c} ±1.77	60.13^{1}_{c} ±1.42

When crop DM is in excess of 30%, clostridial fermentation is suppressed through a lack of moisture availability. Up to this level, a combination of acid, pH and moisture availability will inhibit clostridia (Woolford 1998). The DM% of the D and D+S silages was lower than this 30% value. This could lead to high levels of clostridial bacteria being present in these silages. If large numbers of clostridia are present, they will ferment lactic acid and sugars to butyric acid and ferment proteins to end products, including ammonia. This counteracts the positive effects of the lactic acid bacteria (Woolford, 1998). As the

ensiled period progresses and more protein is broken down to NH_3-N by clostridia, this leads to the formation of salts and these salts need to be neutralized. For this process lactic and acetic acid is used. As more lactic and acetic acid is being used, the pH of the silage increases. This will further favour microbial growth and lead to a further decrease in silage quality (McDonald *et al.*, 1991).

The decrease in lactic acid concentration, increase in NH_3-N concentration and an increase in pH of the D and D+S silages suggested that the above mentioned scenario occurred. The NH_3-N concentration of the D silage (211.25 g/kg N) was also far above the index for NH_3-N concentration for good quality silage (Wilkinson, 1988; Orr & Treacher, 1990; McDonald *et al.*, 1991). The NH_3-N concentration of the D+S silage was close to the value, which divides a well-fermented silage from a satisfactorily fermented silage, according to Orr & Treacher (1990).

From the data obtained for the D, D+S and W+S silages it seemed that ensiling the late growth stage, *D. eriantha* grasses may result in a very unstable silage. Another fact to take into account is that between day 21 and day 120 some sampling errors may have occurred.

It can be concluded from the above results that the preferred method of ensilage for *D. eriantha* cut at the late growth stage was wilting before ensiling. This silage had the lowest pH value, a slightly higher NH_3-N concentration than that of the W+S silage, but had the highest lactic and acetic acid concentrations. It would seem that the direct ensilage of *D. eriantha* at a late growth stage with or without sugar addition lead to an unstable silage, which deteriorated between day 21 and 120.

3.4 *P. maximum* grass silage ensiled at an early growth stage

The average DM concentration of the unwilted plant material was 21%, while that of the wilted material had a higher DM concentration of 31%.

3.4.1 Silage pH and buffering capacity

The pH of the D+S silage decreased significantly between day 0 (5.02) and day 7 (3.97), with a further non-significant decrease until day 120 (Table 3.10). According to Woolford (1998), a silage with a DM of 20% will reach stability at a pH of about 4.0 and thus the D+S silage can be classified as such. The pH value of the W+S silage decreased significantly between day 0 and day 7, and between day 7 and day 21. The final value on day 120 was not significantly different from the value on day 21.

The pH value of the D+S silage was significantly lower than that of the W+S silage on day 0, day 7, day 21 and day 120. Both silages had a final pH value on day 120 which was lower than the recommended value given by McDonald *et al.* (1991), of 4.20, for a good quality unwilted silage.

Table 3.10 The pH and buffering capacity values of *P. maximum* grass ensiled at an early growth stage

Parameter	Day	D+S	W+S
pH	0	5.02^{1}_{b} ±0.03	5.22^{1}_{a} ±0.03
	7	3.97^{2}_{b} ±0.01	4.42^{2}_{a} ±0.02
	21	3.94^{2}_{b} ±0.03	4.13^{3}_{a} ±0.04
	120	3.93^{2}_{b} ±0.02	4.16^{3}_{a} ±0.03
Buffering capacity (meq/100g DM)	0	63.34_{b} ±1.93	72.88_{a} ±0.91

The buffering capacity value of the D+S silage was significantly lower than that for the W+S silage on day 0. These values are lower than those obtained by Cushnahan, Mayne and Unsworth (1995), of 80.1 meq/100g DM for *Lolium perenne* with a DM concentration of 15.4%. In a study by Keady and Steen (1995), buffering capacity values

of ± 54 meq/ 100g DM, were reported for *Lolium perenne* with a DM concentration of ± 15%. Thus buffering capacity can vary drastically according to different situations and is not a fixed value in relation to DM concentration.

3.4.2 Lactic acid and acetic acid

The lactic acid concentration of the D+S silage increased significantly between day 0 and day 7 (Table 3.11) and from day 7 to day 21 there was a non-significant increase. Between day 21 and day 120 the lactic acid concentration increased significantly.

Table 3.11 The lactic and acetic acid concentrations of *P. maximum* grass ensiled at an early growth stage

Parameter	Day	D+S	W+S
Lactic acid (g/kg DM)	0	3.12^{3}_{a} ±1.27	2.62^{4}_{a} ±1.27
	7	29.61^{2}_{a} ±1.82	19.03^{3}_{b} ±1.85
	21	30.61^{2}_{a} ±0.20	25.04^{2}_{b} ±4.07
	120	37.79^{1}_{a} ±3.20	31.37^{1}_{b} ±5.02
Acetic acid (g/kg DM)	0	2.79^{2}_{a} ±1.77	2.73^{3}_{a} ±0.85
	7	4.71^{2}_{a} ±0.71	$5.37^{2,3}_{a}$ ±0.06
	21	9.90^{1}_{a} ±0.12	6.48^{2}_{b} ±0.23
	120	10.15^{1}_{a} ±0.12	12.25^{1}_{a} ±2.55

The lactic acid concentration of the W+S silage increased significantly as the ensiled period progressed from 2.62 on day 0 to 19.03 (day 7), to 25.04 (day 21), and a final value of 31.37 g/kg DM on day 120. There was no significant difference in the lactic acid concentrations of the D+S and W+S silages on day 0. On day 7, day 21 and day 120 the

D+S silage had a significantly higher lactic acid concentration compared to the W+S silage.

The acetic acid concentration of the D+S silage increased as the ensiled period progressed, with a significant increase occurring between day 7 and day 21 (Table 3.11). A significant increase occurred in the acetic acid concentration of the W+S silage between day 0 and day 21, with the value on day 7 not being significantly different from the values on day 0 and day 21. A further significant increase occurred between day 21 and day 120.

There were no significant differences in the acetic acid concentrations of the D+S and W+S silages on day 0, day 7 and day 120. On day 21 the D+S silage had a significantly higher acetic acid concentration compared to the W+S silage. The W+S silage had a higher non-significant acetic acid concentration on day 120 compared to the D+S silage.

According to McDonald *et al.* (1991), a fermentation process favouring the production of lactic acid is preferred. From the data present it can be seen that these silages had a predominant production of lactic acid, as the lactic acid concentration was higher than the acetic acid concentration. The significant increase in lactic acid concentration between day 0 and day 7, did result in a faster decrease in the pH of the silage (McDonald *et al.*, 1991; Woolford 1998). It must be remembered that it is of little consequence how low the final pH maybe, but it matters how quickly this final pH is achieved (Woolford, 1998).

In a study conducted by Petit and Flipot (1992), with Timothy silages, the lactic (13 and 19 g/kg DM) and acetic acid (4.2 and 2.2 g/kg DM) concentration for silage 1 and silage 2 respectively, were lower than those obtained in the current study for the D+S and W+S silages on day 120. The pH values for the Timothy silage were also higher than the pH values for the D+S and W+S silages on day 120. The DM concentration for silage 1 (23.6%) and silage 2 (27.5%) were between the DM concentration values for the D+S and W+S silages in the current study.

3.4.3 Nitrogen and ammonia-nitrogen

The N concentration of D+S silage decreased as the ensiled period progressed. The value on day 120 was significantly lower than the values on day 0 and day 21, but did not differ significantly from the value on day 7 (Table 3.12). The N concentration of the W+S silage decreased as the ensiled period progressed, but was not significantly lower on day 120 compared to the day 0, 7 and 120. There were no significant differences in the N concentrations of the D+S and W+S silages as the ensiled period progressed.

Table 3.12 The N and NH_3-N concentrations of *P. maximum* grass ensiled at an early growth stage

Parameter	Day	D+S	W+S
N (g/kg DM)	0	23.58^{1}_{a} ±0.20	21.97^{1}_{a} ±0.26
	7	$21.40^{1,2}_{a}$ ±0.57	20.54^{1}_{a} ±0.73
	21	22.74^{1}_{a} ±0.60	21.12^{1}_{a} ±1.04
	120	19.69^{2}_{a} ±1.53	20.82^{1}_{a} ±0.37
NH_3-N (g/kg N)	0	44.35^{3}_{a} ±5.95	27.29^{3}_{b} ±7.59
	7	52.31^{3}_{a} ±1.27	51.49^{2}_{a} ±0.52
	21	70.81^{2}_{a} ±12.67	72.18^{1}_{a} ±9.91
	120	98.71^{1}_{a} ±28.30	86.95^{1}_{a} ±1.94

The NH_3-N concentration of the D+S silage increased non-significantly between day 0 and day 7. A significant increase occurred between day 7 and day 21 and between day 21 and 120. The NH_3-N concentration of the W+S silage increased significantly between day 0, day 7 and day 21, with a non-significant increase occurring between day 21 and day 120.

The NH_3-N concentration of the D+S silage was significantly higher than that of the W+S silage on day 0, 44.35 and 27.29 g/kg N respectively. There were no significant differences in the NH_3-N concentration of the D+S and W+S silages on day 7, day 21 and day 120. The NH_3-N concentration, of the D+S and W+S silages are within the range given by Wilkinson (1988) and Orr & Treacher (1990) (50 to 100 g/kg N or less) and McDonald *et al.* (1991) (less than 110 g/kg N), for well-fermented silages.

A possible explanation for the increase in the NH_3-N concentration of the D+S silage as the ensiled period progressed is the low initial DM concentration of the plant material, which could have lead to clostridia growth, resulting in breakdown of protein (McDonald *et al.*, 1991). The increase in the NH_3-N concentration of the W+S silage could possible be a result of the initial pH value on day 0 which was above 5.00. This could have lead to clostridia growth, resulting in protein breakdown (Woolford, 1998).

In conclusion, the ensiling of *P. maximum* grass at an early growth stage with the addition of sugar, either ensiled directly or wilted, resulted in a good quality silage. The silages had a low pH, high lactic acid concentrations and an acceptable NH_3-N concentration.

3.5 *P. maximum* grass silage ensiled at a medium growth stage

The average DM concentration of the initial plant material was 20% for the *P. maximum* medium growth stage ensiled without prior wilting and for the wilted grass it was 24%.

3.5.1 Silage pH and buffering capacity

The pH of the *P. maximum* medium direct (D) silage decreased significantly between day 0 and day 7, followed by a significant increase to 4.94 on day 21 and then remained constant until day 120 (Table 3.13). The values on day 21 and 120 were not significantly different from the initial value on day 0. The pH of the *P. maximum* medium direct + sugar (D+S) silage decreased significantly between day 0 (4.98) and day 7 (3.94), and

then decreased non-significantly until day 120. The pH of the D+S silage was significantly lower than that of the D silage on day 120.

Between day 0 and day 7 the pH of the *P. maximum* medium wilted (W) silage remained constant, whereafter it increased significantly to a value of 5.28 on day 21 and 5.44 on day 120. The pH values of the W silage are significantly higher than that of the D and D+S silages. The pH of the *P. maximum* medium wilted + sugar (W+S) silage decreased significantly between day 0 (4.71) and day 7 (4.04), whereafter it increased significantly until a final value of 4.52 on day 120. The value on day 120 is significantly higher than that of the D+S silage and significantly lower than the D and W silages.

Table 3.13 The pH and buffering capacity values of *P. maximum* grass ensiled at a medium growth stage

Parameter	Day	D	D+S	W	W+S
pH	0	4.98^{1}_{a} ±0.05	4.98^{1}_{a} ±0.05	4.71^{3}_{b} ±0.02	4.71^{1}_{b} ±0.02
	7	4.61^{2}_{b} ±0.02	3.94^{2}_{c} ±0.03	4.76^{3}_{a} ±0.01	4.04^{4}_{c} ±0.05
	21	4.94^{1}_{b} ±0.03	3.89^{2}_{d} ±0.02	5.28^{2}_{a} ±0.25	4.17^{3}_{c} ±0.04
	120	4.96^{1}_{b} ±0.03	3.83^{2}_{d} ±0.04	5.44^{1}_{a} ±0.04	4.52^{2}_{c} ±0.02
Buffering capacity (meq/100g DM)	0	55.48_{b} ±0.55	55.48_{b} ±0.55	68.59_{a} ±1.06	68.59_{a} ±1.06

The addition of sugar to the *P. maximum* medium grass at ensiling (directly or wilted) resulted in a lower pH and a faster drop in pH. This increase in the rate of pH drop between day 0 and day 7 will result in a better quality silage (Woolford, 1998).

The buffering capacity of the D and D+S silages were significantly lower than that of the W and W+S silages on day 0. The difference of the buffering capacity values between the

D and W silages, are smaller than the differences between grasses and legumes (McDonald *et al.*, 1991).

3.5.2 Lactic and acetic acid

There were no significant differences in the lactic acid concentrations of the four treatments on day 0 (Table 3.14). There was a significant increase in the lactic acid concentration of the D silage between day 0 and day 7, with a further significant increase until day 21, after which it remained constant.

Table 3.14 The lactic and acetic acid concentrations of *P. maximum* grass ensiled at a medium growth stage

Parameter	Day	D	D+S	W	W+S
Lactic acid (g/kg DM)	0	3.54^{3}_{a} ±0.09	3.54^{3}_{a} ±0.09	$3.67^{1,2}_{a}$ ±1.60	3.67^{3}_{a} ±1.60
	7	14.97^{2}_{c} ±1.43	40.54^{2}_{a} ±7.36	8.06^{1}_{d} ±0.30	28.03^{1}_{b} ±3.44
	21	20.64^{1}_{b} ±2.44	48.47^{1}_{a} ±4.21	$3.11^{1,2}_{c}$ ±1.56	19.88^{2}_{b} ±2.83
	120	21.16^{1}_{b} ±2.50	49.03^{1}_{a} ±1.23	2.29^{2}_{c} ±0.31	21.46^{2}_{b} ±8.30
Acetic acid (g/kg DM)	0	5.98^{3}_{a} ±0.15	5.98^{1}_{a} ±0.15	3.71^{3}_{a} ±0.74	3.71^{2}_{a} ±0.74
	7	14.07^{2}_{a} ±0.32	7.71^{1}_{b} ±1.57	8.05^{2}_{b} ±4.71	5.00^{2}_{b} ±0.58
	21	23.06^{1}_{a} ±1.43	9.53^{1}_{c} ±1.61	16.22^{1}_{b} ±0.07	$5.43^{1,2}_{d}$ ±0.58
	120	26.49^{1}_{a} ±5.55	7.87^{1}_{c} ±0.42	16.63^{1}_{b} ±0.07	9.18^{1}_{c} ±0.23

The lactic acid concentration of the D+S silage increased significantly between day 0 and day 7. This was followed by a significant increase to 48.47 g/kg DM on day 21 and then the value remained constant until day 120. The values on day 7, 21 and 120 of the D+S silage were significantly higher than that of the D silage.

The lactic acid concentration of the W silage increased non-significantly between day 0 and day 7, followed by a non-significant decrease until day 21 and then remained fairly constant until day 120. The value on day 120 was significantly lower than the value on day 7. There was a significant increase in the lactic acid concentration of the W+S silage between day 0 and day 7, with a significant decrease between day 7 and day 21. From day 21 to day 120 the value remained fairly constant. The lactic acid concentration, on day 120, of the D+S silage was significantly higher than that of the other three silages. The D and W+S silages had similar values, which were significantly higher than that of the W silage.

The acetic acid concentration of the four silages did not differ significantly on day 0 (Table 3.14). There was a significant increase in the acetic acid concentration of the D silage between day 0, day 7 and day 21. This was followed by a slight increase in the value to 21.16 g/kg DM on day 120. The acetic acid concentration of the D+S silage increased as the ensiled period progressed, but there were no significant differences between the time periods. The final value on day 120 was slightly higher than the value on day 0.

There was a significant increase in the acetic acid concentration of the W silage between day 0, day 7 and day 21, followed by a fairly constant value until day 120. The acetic acid concentration of the W+S silage increased non-significantly between day 0, day 7 and day 21. The final value of 9.18 g/kg DM on day 120 was non significantly higher than that on day 21 and significantly higher than the values on day 7 and day 0.

On day 120 the D silage had a significantly higher acetic acid concentration than that of the other silages, with that of the W silage being significantly higher than that of the D+S and W+S silages. The acetic acid concentration of the D+S and W+S silages did not differ significantly on day 120.

The addition of sugar to the *P. maximum* medium grass ensiled (directly or wilted), resulted in a higher production of lactic acid. Where sugar was not added, the silages had

higher levels of acetic acid. This may explain the higher pH values for the D and W silages on day 120 (McDonald *et al.*, 1991; Woolford, 1998).

3.5.3 Nitrogen and ammonia-nitrogen

There were no significant differences in the N concentration of the four treatments on day 0 (Table 3.15). In general the N concentration of the four treatments decreased as the ensiling process progressed, with the D and D+S silages having significantly lower values on day 120 compared to day 0. The decrease in the N concentration of the W and W+S silages over the ensiled period was non-significant, except for the W silage where the N concentration on day 21 was significantly lower than that on day 0. The values for D and D+S silages were significantly lower than that of the W and W+S silages on day 120.

The NH_3-N concentration of the direct silages was significantly higher than that of the wilted silages on day 0 (Table 3.15). The NH_3-N concentration of the D silage remained constant between day 0 and day 7, increased slightly between day 7 and day 21, followed by a significant increase to 94.41 g/kg N on day 120. The low DM concentration (20%) of the D silage and the high pH of the silage could have resulted in clostridia growth (Woolford, 1998), which resulted in the increase in protein degradation, leading to an increase in the NH_3-N concentration of the D silage.

The NH_3-N concentration of the D+S silage decreased non significantly between day 0 and day 7, with a significant increase between day 7 and day 21, followed by a decrease from 54.4 to 47.54 g/kg N ($P > 0.05$) on day 120.

There was a non significant increase in the NH_3-N concentration of the W silage between day 0 and day 7, followed by a significant increase occurring between day 7 and day 21, with a non significant decrease to 54.98 g/kg N on day 120. The NH_3-N concentration of the W+S silage increased non significantly between day 0 and day 7, and then remained constant until day 21. There was a significant increase between day 21 and day 120, to a final value of 113.32 g/kg N. This value is just above that given as the upper limit by

Wilkinson (1988), Orr & Treacher (1990) (50 to 100 g/kg N) and McDonald *et al.*, (1991) (less than 110 g/kg N), for well-fermented silages. This silage can be classified as "satisfactorily fermented", according to Wilkinson (1988) and Orr & Treacher (1990).

Table 3.15 The N and NH_3-N concentrations of *P. maximum* grass ensiled at a medium growth stage

Parameter	Day	D	D+S	W	W+S
N (g/kg DM)	0	25.26^{1}_{a} ±0.04	25.26^{1}_{a} ±0.04	27.43^{1}_{a} ±0.23	27.43^{1}_{a} ±0.23
	7	24.29^{1}_{bc} ±2.90	$22.87^{1,2}_{c}$ ±0.98	$26.14^{1,2}_{ab}$ ±1.32	27.18^{1}_{a} ±1.45
	21	22.70^{2}_{b} ±1.48	$23.91^{1,2}_{b}$ ±0.59	24.37^{2}_{ab} ±0.69	26.65^{1}_{a} ±2.31
	120	20.60^{2}_{b} ±0.43	21.53^{2}_{b} ±0.52	$25.82^{1,2}_{a}$ ±3.92	25.53^{1}_{a} ±0.95
NH_3-N (g/kg N)	0	40.84^{2}_{a} ±1.01	$40.84^{1,2}_{a}$ ±1.01	21.77^{2}_{b} ±1.29	21.77^{2}_{b} ±1.29
	7	41.30^{2}_{a} ±2.42	29.12^{2}_{a} ±6.90	34.49^{2}_{a} ±4.51	30.98^{2}_{a} ±0.40
	21	46.55^{2}_{a} ±1.10	54.43^{1}_{a} ±1.26	60.90^{1}_{a} ±0.15	30.27^{2}_{b} ±0.79
	120	94.41^{1}_{b} ±2.19	47.54^{1}_{c} ±1.16	54.98^{1}_{c} ±1.33	113.32^{1}_{a} ±17.00

An explanation for the increase in the NH_3-N concentration of the W+S silage between day 21 and day 120, could be due to large numbers of clostridia being present. If large numbers of clostridia are present, they will ferment the lactic acid and sugars to butyric acid and ferment proteins to end products, including ammonia (Woolford, 1998). Thus as more protein is broken down to NH_3-N, this would lead to the formation of salts and these salts need to be neutralized and for this process, lactic and acetic acid is used. As more lactic and acetic acid is being used, the pH of the silage increases. This will further favour microbial growth and lead to a further decrease in silage quality (McDonald *et al.*, 1991). This could also explain the increase in the pH and decrease in the lactic acid concentration of the W+S between day 7 and day 120.

The W+S silage had a significantly higher NH_3-N concentration compared to that of the other silages, with the D silage having a significantly higher value than that of the D+S and W silages on day 120. The D+S and W silages did not differ significantly in their NH_3-N concentrations on day 120.

From the above results, the best silage produced from the *P. maximum* medium growth stage was the silage been made directly with the addition of sugar (D+S silage). This silage had the lowest pH, buffering capacity, NH_3-N concentration and acetic acid concentration. It also had the highest lactic acid concentration, which gives an indication of a lactic acid fermentation process. The next best silage is the W+S silage, except for its high NH_3-N concentration.

The improvement in the quality of the D+S silage, compared to the other three silages, corresponds with the improvement in silage quality obtained by Meeske *et al.* (1999), when comparing a control silage with an inoculated silage made from *D. eriantha*. This related to a lower pH, higher lactic acid concentration, lower acetic acid and NH_3-N concentration in the D+S and inoculated silages. Data published by Pieper (1996), showed that the addition of sugar (molasses) to alfalfa silage, resulted in lower pH values, higher lactic acid concentrations and lower NH_3-N concentrations. Thus it can be concluded that sugar addition had a beneficial effect on silage made from *P. maximum* medium growth stage plant material.

3.6 *P. maximum* grass silage ensiled at a late growth stage

The average DM concentration of the initial plant material was 23% for the *P. maximum* late growth stage ensiled without prior wilting and for the wilted material it was higher (26%).

3.6.1 Silage pH and buffering capacity

The buffering capacity of the direct cut silages (63.21 meq/ 100g DM) were significantly lower than that of the wilted silages (72.93 meq/ 100g DM) on day 0. The higher the

buffering capacity the more fermentation acids (lactic and acetic acid) need to be produced to decrease the pH of the silage (McDonald *et al.*, 1991).

Table 3.16 The pH and buffering capacity values of *P. maximum* grass ensiled at a late growth stage

Parameter	Day	D	D+S	W	W+S
pH	0	4.81^{1}_{a} ±0.03	4.81^{2}_{a} ±0.03	4.67^{2}_{b} ±0.01	4.67^{1}_{b} ±0.01
	7	4.30^{3}_{c} ±0.01	3.95^{3}_{d} ±0.02	4.65^{2}_{a} ±0.03	4.42^{2}_{b} ±0.03
	21	4.61^{2}_{a} ±0.02	3.95^{3}_{c} ±0.04	4.61^{2}_{a} ±0.02	4.32^{2}_{b} ±0.02
	120	4.22^{3}_{b} ±0.02	5.04^{1}_{a} ±0.05	5.00^{1}_{a} ±0.01	4.33^{2}_{b} ±0.05
Buffering capacity (meq/100g DM)	0	63.21_{b} ±1.78	63.21_{b} ±1.78	72.93_{a} ±0.05	72.93_{a} ±0.05

The pH of the direct and wilted silages differed significantly on day 0 (Table 3.16), with the direct cut silages having a higher value than the wilted silages. The pH of the *P. maximum* late direct (D) silage decreased significantly between day 0 and day 7, whereafter it increased significantly to 4.61 on day 21. There was a further significant decrease between day 21 and day 120. The pH of the *P. maximum* late direct + sugar (D+S) silage decreased significantly between day 0 from 4.81 to 3.95 on day 7, where it remained constant until day 21, followed by a significant increase until day 120 (5.04).

The pH of the *P. maximum* late wilted (W) silage remained fairly constant between day 0, day 7 and day 21, followed by a significant increase to 5.00 on day 120. The pH of the *P. maximum* late wilted + sugar (W+S) silage decreased significantly between day 0 and day 7, followed by a non significant decease until its final value of 4.33 on day 120.

The pH of the D and W+S silages did not differ significantly from each other on day 120, but these where significantly lower than the values of the D+S and W silages. There were no significant differences in the pH values of the D+S and W silages on day 120.

3.6.2 Lactic and acetic acid

There were no significant differences in the lactic acid concentrations of the direct and wilted silages on day 0 (Table 3.17). The lactic acid concentration of the D silage increased significantly between day 0 and day 7, remained constant until day 21 and then increased significantly to 26.95 g/kg DM on day 120. The lactic acid concentration of the D+S silage increased significantly between day 0 and day 7, with a further significant increase until day 21, followed by a significant decrease between day 21 and day 120.

Table 3.17 The lactic and acetic acid concentrations of *P. maximum* grass ensiled at a late growth stage

Parameter	Day	D	D+S	W	W+S
Lactic acid (g/kg DM)	0	2.44^{3}_{a} ±0.84	2.44^{4}_{a} ±0.84	2.38^{2}_{a} ±0.82	2.38^{3}_{a} ±0.82
	7	17.14^{2}_{b} ±0.42	37.75^{2}_{a} ±4.36	8.06^{1}_{c} ±1.04	16.32^{2}_{b} ±0.40
	21	18.94^{2}_{c} ±1.18	56.60^{1}_{a} ±1.42	$6.65^{1,2}_{d}$ ±0.25	26.02^{1}_{b} ±3.02
	120	26.95^{1}_{a} ±5.46	20.70^{3}_{b} ±0.20	$6.49^{1,2}_{c}$ ±0.24	25.41^{1}_{ab} ±1.42
Acetic acid (g/kg DM)	0	3.71^{3}_{a} ±0.20	3.71^{2}_{a} ±0.20	2.66^{2}_{a} ±1.26	2.66^{2}_{a} ±1.26
	7	$6.60^{2,3}_{a}$ ±0.26	6.17^{2}_{a} ±1.64	3.46^{2}_{a} ±0.79	$5.57^{1,2}_{a}$ ±0.83
	21	8.82^{2}_{b} ±0.22	13.13^{1}_{a} ±0.78	3.34^{2}_{c} ±0.70	7.84^{1}_{b} ±0.97
	120	13.08^{1}_{a} ±2.81	14.69^{1}_{a} ±0.37	7.54^{1}_{b} ±0.18	8.04^{1}_{b} ±0.99

The lactic acid concentration of the W silage increased significantly between day 0 and day 7, followed by a non significant decrease until day 120. The W+S silage had a

significant increase in lactic acid concentration between day 0 and day 7, followed by a further significant increase until day 21, whereafter it remained fairly constant until day 120.

The lactic acid concentration of the D silage was not significantly higher than that of the W+S silage, but was significantly higher than that of the D+S and W silages on day 120. The W+S silage did not differ significantly from the D+S silage, but was significantly higher than the W silage. The D+S silage had a significantly higher lactic acid concentration than the W silage.

The acetic acid concentration of the direct and wilted silages did not differ significantly on day 0 (Table 3.17). There was a significant increase in the acetic acid concentration of the D silage between day 0 and day 21, with a further significant increase between day 21 and day 120. The acetic acid concentration of the D+S silage increased non significantly between day 0 and day 7, with a significant increase between day 7 and day 21, with the value remaining fairly constant until day 120 (14.69 g/kg DM).

There was a non significant increase in the acetic acid concentration of the W silage between day 0, day 7 and day 21, followed by a significant increase until day 120 (7.54 g/kg DM). The acetic acid concentration of the W+S silage increased as the ensiled period progressed, with the final value on day 120 (8.04 g/kg DM) being significantly higher than that of the initial value on day 0. The acetic acid concentration of the D+S silage was not significantly higher than that of the D silage on day 120, but these values were significantly higher than that of the W and W+S silages. The latter two silages did not differ significantly in their acetic acid concentrations on day 120.

3.6.3 Nitrogen and ammonia-nitrogen

The N concentration of the direct cut silages was significantly higher than that of the wilted silages on day 0 (Table 3.18). There was a significant decrease in the N concentration of the D silage between day 0 and day 7, followed by a non significant increase until day 120. The N concentration of the D+S silage decreased significantly

between day 0 and day 21, with the value increasing to 19.22 g/kg DM on day 120. This value was not significantly different from the values on day 0, 7 or 21.

Table 3.18 The N and NH_3-N concentrations of *P. maximum* grass ensiled at a late growth stage

Parameter	Day	D	D+S	W	W+S
N (g/kg DM)	0	20.84^{1}_{a} ±0.07	20.84^{1}_{a} ±0.07	17.51^{1}_{b} ±1.36	$17.51^{1,2}_{b}$ ±1.36
	7	15.90^{2}_{b} ±0.62	$18.73^{1,2}_{a}$ ±1.12	16.08^{1}_{b} ±1.34	$18.36^{1,2}_{ab}$ ±0.87
	21	16.31^{2}_{bc} ±0.07	17.90^{2}_{ab} ±0.32	15.26^{1}_{c} ±0.94	19.41^{1}_{a} ±0.49
	120	16.25^{2}_{b} ±0.76	$19.22^{1,2}_{a}$ ±2.70	16.35^{1}_{b} ±0.27	16.55^{2}_{b} ±0.57
NH_3-N (g/kg N)	0	45.42^{1}_{a} ±1.86	45.42^{2}_{a} ±1.86	46.44^{2}_{a} ±2.44	46.44^{1}_{a} ±2.44
	7	56.47^{1}_{a} ±4.83	34.60^{2}_{b} ±2.10	38.64^{2}_{b} ±4.19	44.22^{1}_{ab} ±2.33
	21	47.88^{1}_{a} ±2.86	32.81^{2}_{a} ±0.80	44.37^{2}_{a} ±6.71	34.64^{1}_{a} ±2.07
	120	60.07^{1}_{b} ±1.38	91.75^{1}_{a} ±50.13	92.24^{1}_{a} ±2.09	34.53^{1}_{c} ±0.82

There was a decrease in the N concentration of the W silage as the ensiled period progressed to a value of 16.35 g/kg DM on day 120. This value was not significantly different from the values on day 0, 7 and 21. The N concentration of the W+S silage increased non significantly to a value of 19.41 g/kg DM on day 21 and then decreased significantly until day 120. The value on day 120 was not significantly different from the values on day 0 and day 7. The N concentration of the D, W and W+S silage on day 120 did not differ significantly, but these values were significantly lower than that of the D+S silage.

There were no significant differences in the NH_3-N concentrations of the direct and wilted silages on day 0 (Table 3.18). The NH_3-N concentration of the D silage increased

non significantly over the ensiled period with a final value of 60.07 g/kg N on day 120. There was a non significant decrease in the NH_3-N concentration of the D+S silage until day 21, followed by a significant increase to a value of 91.75 g/kg N on day 120.

The NH_3-N concentration of the W silage remained constant between day 0 and day 21, followed by a significant increase until day 120 (92.24 g/kg N). There was a non significant decrease in the NH_3-N concentration of the W+S silage to a value of 34.53 g/kg N on day 120. The NH_3-N concentration of the D+S and W silages did not differ significantly from each other as the ensiled period progressed. These two silages had a significantly higher NH_3-N concentration on day 120, than the D and W+S silages, with the W+S silage having a significantly lower value than the D silage on day 120.

The low DM concentration of the D+S silage could have favoured the growth of clostridia (Woolford, 1998). The clostridia will degrade protein resulting in the formation of salts. For these salts to be neutralized, lactic and acetic acids are used. This leads to an increase in the pH of the silage and this phenomena is known as "secondary fermentation" (McDonald *et al.*, 1991). This could explain the sudden decrease in the silage quality of the D+S silage between day 21 and 120. The other possibility is an error in sealing and sampling of the silage.

The W silage did not undergo any proper form of fermentation as the pH increased as the ensiling period progressed. This was reflected by low lactic and acetic acid concentrations, as well as a high NH_3-N concentration on day 120. The quality of this silage is not good. The best silages were obtained with the D and the W+S treatments. There is not much difference in their pH values, as well as in both N and lactic acid concentrations. The D silage did have a higher NH_3-N and acetic acid concentration compared to the W+S silage.

CHAPTER 4

Results and Discussion

Specific treatment differences across the three growth stages within a grass species

In this chapter, the results obtained when comparing specific treatments across the three growth stages, within a specific grass specie, will be discussed. The treatments which will be discussed are those where the plant material was ensiled directly with sugar addition (D+S) and where the material was ensiled with prior wilting and sugar addition (W+S). From the data obtained in Chapter 3, these treatments gave the better results in most circumstances, except where possible secondary fermentation occurred.

The three growth stages, of each specie, will be referred to as follows:

1: SE – *D. eriantha* grass ensiled at an early growth stage

2: SM – *D. eriantha* grass ensiled at a medium growth stage

3: SL – *D. eriantha* grass ensiled at a late growth stage

4: PE – *P. maximum* grass ensiled at an early growth stage

5: PM – *P. maximum* grass ensiled at a medium growth stage

6: PL – *P. maximum* grass ensiled at a late growth stage

4.1 Results and discussion of *D. eriantha* grass ensiled directly, with sugar addition

The average DM concentration of *D. eriantha* grass ensiled at an early growth stage was 23.2%, that of the medium growth stage was 19.2% and that of the late growth stage was 25%.

4.1.1 Silage pH and buffering capacity

The pH values of the three silages differed significantly on day 0, with the SL silage having the lowest value and the SM silage having the highest value (Table 4.1).

Table 4.1 The pH and buffering capacity values of *D. eriantha* grass ensiled directly, at three growth stages, with sugar addition

Parameter	Day	SE D+S	SM D+S	SL D+S
pH	0	4.98^{1}_{b} ±0.01	5.17^{1}_{a} ±0.21	4.70^{1}_{c} ±0.03
	7	4.11^{3}_{a} ±0.02	4.02^{2}_{a} ±0.02	4.08^{2}_{a} ±0.02
	21	4.08^{3}_{a} ±0.02	$3.99^{2,3}_{b}$ ±0.01	3.81^{2}_{c} ±0.03
	120	4.25^{2}_{b} ±0.04	3.90^{3}_{c} ±0.02	4.62^{1}_{a} ±0.04
Buffering capacity (meq/100g DM)	0	49.42_{a} ±0.77	43.80_{b} ±0.02	44.69_{b} ±0.26

The following information will apply to all the tables in Chapter 4.
1: Values with different superscripts (1,2,3,4) down a column differ significantly at $P \leq 0.05$.
2: Values with different subscripts (a,b,c,d) across a row differ significantly at $P \leq 0.05$.
3: Values after the ± sign are standard deviation values.
4: Three observations per treatment.

There were no significant differences in the pH values of the three silages on day 7. On day 21 the SL silage had a significantly lower pH value compared to the SM and SE silages. The SM and SE silages also differed significantly from each other. On day 120 the pH values of the three silages differed significantly, with the SM silage having the lowest value and the SL silage having the highest value.

The SE silage had a significantly higher buffering capacity value on day 0 compared to the other two silages. This could lead to a slower decrease in the pH value of the SE silage compared to the SM and SL silages, leading to lower production of fermentation acids (lactic and acetic acid). The production of lactic and acetic acid was lower in the SE silage on day 21 compared to the other two silages (Table 4.2). The values for the other two silages did not differ significantly.

4.1.2 Lactic and acetic acid

The lactic acid concentration of the three silages did not differ significantly on day 0 (Table 4.2). On day 7 and day 21 the lactic acid concentration of the three silages differed significantly, with the SM silage having the highest value and the SE silage the lowest value. The lactic acid concentration of the three silages differed significantly on day 120, with the SM silage having the highest value and the SL silage the lowest value. The latter phenomena was most probably due to a significant decrease in the lactic acid concentration of the SL silage between day 21 and day 120.

Table 4.2 The lactic and acetic acid concentrations of *D. eriantha* grass ensiled directly, at three growth stages, with sugar addition

Parameter	Day	SE D+S	SM D+S	SL D+S
Lactic acid (g/kg DM)	0	3.86^{3}_{a} ±0.71	1.41^{4}_{a} ±0.05	2.27^{3}_{a} ±1.07
	7	15.39^{2}_{c} ±1.24	53.47^{3}_{a} ±5.66	31.85^{2}_{b} ±2.38
	21	22.73^{1}_{c} ±1.63	64.24^{1}_{a} ±2.85	37.29^{1}_{b} ±1.33
	120	27.68^{1}_{b} ±1.81	58.97^{2}_{a} ±1.52	2.37^{3}_{c} ±0.06
Acetic acid (g/kg DM)	0	4.23^{1}_{a} ±0.71	5.28^{2}_{a} ±1.01	2.78^{2}_{a} ±1.83
	7	4.12^{1}_{b} ±0.60	10.31^{1}_{a} ±1.72	$6.32^{1,2}_{b}$ ±0.11
	21	4.06^{1}_{b} ±0.46	11.89^{1}_{a} ±3.42	6.72^{1}_{b} ±1.23
	120	3.95^{1}_{c} ±0.55	11.58^{1}_{a} ±1.52	8.24^{1}_{b} ±0.07

There were no significant differences in the acetic acid concentration of the three silages on day 0. On day 7 and day 21 the SM silage had a significantly higher acetic acid concentration compared to the other two silages, which did not differ significantly from each other. The acetic acid concentration of the three silages differed significantly from

each other on day 120, with the SM silage having the highest value and the SE silage the lowest value.

4.1.3 Nitrogen and ammonia-nitrogen

The N concentration of the three silages differed significantly on day 0, day 7 and day 21 (Table 4.3). The N concentration of the SM silage was significantly higher than that of the SE and SL silages on day 120, but the N concentration of the SE and SL silages did not differ significantly on day 120.

Table 4.3 The N and NH_3-N concentrations of *D. eriantha* grass ensiled directly, at three growth stages, with sugar addition

Parameter	Day	SE D+S	SM D+S	SL D+S
N (g/kg DM)	0	29.73^{1}_{b} ±0.13	31.82^{1}_{a} ±0.30	25.16^{1}_{c} ±0.13
	7	29.47^{1}_{a} ±1.82	27.17^{2}_{b} ±1.30	20.79^{2}_{c} ±2.87
	21	25.75^{2}_{b} ±1.28	28.85^{2}_{a} ±0.39	$22.66^{1,2}_{c}$ ±1.76
	120	24.74^{2}_{b} ±1.59	27.81^{2}_{a} ±0.46	24.23^{1}_{b} ±0.72
NH_3-N (g/kg N)	0	$46.71^{1,2}_{a}$ ±4.91	44.94^{1}_{a} ±6.94	35.57^{2}_{a} ±0.88
	7	35.42^{2}_{ab} ±1.50	$30.30^{1,2}_{b}$ ±0.10	45.77^{2}_{a} ±1.07
	21	55.27^{1}_{a} ±0.82	27.76^{2}_{b} ±6.81	38.41^{2}_{b} ±0.90
	120	56.52^{1}_{b} ±12.20	27.12^{2}_{c} ±0.65	108.93^{1}_{a} ±11.88

On day 0 there were no significant differences in the NH_3-N concentration of the three silages, but the SM silage differed significantly from the SL silage on day 7. The SE silage did not differ significantly from the other two silages on day 7. There were no significant differences in the NH_3-N concentration of the SM and SL silages on day

21.The SE silage had a significantly higher NH_3-N concentration compared to the other two silages on day 21.

The NH_3-N concentration of the three silages differed significantly from each other on day 120, with the SM silage (27.12 g/kg N) having the lowest value and the SL silage (108.93 g/kg N) having the highest value. The NH_3-N concentration of the SE and SM silages were in the range described for "well-fermented" silages and the NH_3-N concentration of the SL silage places this silage in the category of a "satisfactorily fermented" silage according to Wilkinson (1988) and Orr & Treacher (1990). Meeske *et al.* (1999) reported a NH_3-N g/kg TN of 50.3 after ensiling *D. eriantha* for 44 days, this is lower than the value obtained for the SL silage, higher than that for the SM silage and equal to the value for the SE silage, obtained in the present study.

According to Woolford (1998), it is essential that the decline in pH is as rapid as possible and such a rapid reduction is critical in the first two to three days of ensilage to stem proteolysis. The rate of pH decline between day 0 and day 7 was more rapid for the SM silage (1.15), compared to the SE (0.82) and SL (0.62) silages. The efficiency of fermentation refers to the efficiency with which sugars are fermented to and how one can realize an optimum value from the sugar in terms of acid production to prevent the spoilage activities of clostridia (Woolford, 1998). The production of lactic acid between day 0 and day 7 was much higher for the SM silage compared to the SE and SL silages.

If large numbers of clostridia are present, they will ferment lactic acid and sugars to butyric acid and ferment proteins to end products, including ammonia (Woolford, 1998). As more NH_3-N is produced more salts will be produced. These salts need to be neutralized and for this acetic and lactic acid is used. This leads to a decrease in the lactic and acetic concentrations and an increase in the pH of the silage (McDonald *et al.*, 1991). Due to the slower rate of pH decline in the SL silage between day 0 and day 7 compared to the SM and SE silages, clostridia growth could have occurred. This could have lead to an increase in protein breakdown, thus higher NH_3-N concentration and more salts. These salts need to be neutralized by lactic acid. This would have lead to lactic and acetic acid

being utilized to neutralize the salts formed, resulting in an increase in the pH of the silage, as was observed between day 21 and day 120.

The scenario described above could explain the data obtained for the SL silage. The rate of pH decline between day 0 and day 7, was slower in the SL silage compared to the SM and SE silages. Between day 21 and day 120 there was an increase in the NH_3-N concentration of the SL silage and a decrease in the lactic acid concentration, resulting in the increase in the pH value of the SL silage as was observed between day 21 and day 120.

In a study conducted by Jaakkola and Huhtanen (1993), the quality of the silage made from Timothy grass (*Phleum pratense*) was comparable to the SE and SM silages in the current study. The SM silage was of a slightly better quality, having a lower pH, higher lactic acid and a lower NH_3-N concentration compared to the Timothy grass silage. While the SE silage was of a slightly poorer quality, having a higher pH value and a lower lactic acid concentration compared to the Timothy grass silage. The values for the Timothy grass silage were as follows, DM concentration (23.9%), pH value (4.04), lactic acid concentration (42 g/kg DM), acetic acid concentration (12 g/kg DM), N concentration (27.4 g/kg DM) and the NH_3-N concentration (55 g/kg N) (Jaakkola and Huhtanen, 1993).

It can be concluded that of the three silages, the quality of the SM silage was the best, followed by the SE and then the SL silage. This is due to the SM silage having the lowest pH, lowest NH_3-N concentration and the highest lactic acid concentration on day 120. The SM silage also had the highest N concentration of the three silages on day 120. According to Woolford (1998), generally, at a DM of 20% silage, stability is achieved at a pH of about 4.0. The pH of the SM silage on day 120 was 3.90.

4.2 Results and discussion of *D. eriantha* grass ensiled with prior wilting and sugar addition

The average DM concentration of the *D. eriantha* grass ensiled at an early growth stage was 30.7%, that of the medium growth stage was 29.5% and that of the late growth stage was 29.6%. The DM concentrations of the three growth stages are within 1.2% points from each other. When the crop DM is in excess of 30%, clostridial fermentation is suppressed through a lack of moisture availability (Woolford, 1998). The DM concentrations of these silages are close to this value of 30%.

4.2.1 Silage pH and buffering capacity

The pH value of the SL silage was significantly lower than that of the SM silage on day 0 and the SE silage did not differ significantly from the other two silages on day 0 (Table 4.4). The pH value of the SM silage was significantly lower compared to the other two silages on day 7, with no significant difference in the pH values on day 21. The pH value of the SL silage increased between day 21 and day 120 and was significantly higher than that of the other two silages.

Table 4.4 The pH and buffering capacity values of *D. eriantha* grass ensiled at three growth stages, after wilting and sugar addition

Parameter	Day	SE W+S	SM W+S	SL W+S
pH	0	4.82^{1}_{ab} ±0.03	4.91^{1}_{a} ±0.04	4.79^{2}_{b} ±0.02
	7	4.70^{2}_{b} ±0.02	4.33^{2}_{c} ±0.02	5.17^{1}_{a} ±0.23
	21	4.24^{3}_{a} ±0.03	4.31^{2}_{a} ±0.03	4.32^{4}_{a} ±0.01
	120	4.32^{3}_{b} ±0.03	4.33^{2}_{b} ±0.03	4.61^{3}_{a} ±0.02
Buffering capacity (meq/100g DM)	0	56.55_{a} ±0.49	32.05_{c} ±0.12	44.89_{b} ±0.83

The SL silage was a very unstable silage, as the pH increased between day 0 and day 7, then decreased between day 7 and day 21, followed by an increase between day 21 and day 120. The other possible explanation for this variation in the pH values was that an experimental error could have occurred. This could have resulted in some bottles not being properly sealed, leading to variation in silage quality on certain days.

The buffering capacity of the three silages differed significantly from each other on day 0, with the SE silage (56.55) having the highest value and the SM silage (32.05) having the lowest value. Due to the higher buffering capacity of the SE silage the pH decline between day 0 and day 7 was slower than that compared to the SM silage.

4.2.2 Lactic and acetic acid

There was no significant difference in the lactic acid concentration of the three silages on day 0 (Table 4.5). On day 7 the lactic acid concentrations of the three silages differed significantly. The rate of production in lactic acid was faster in the SM silage compared to the other two silages between day 0 and day 7.

On day 21 the lactic acid concentration of the SM silage was significantly higher than that of the SL silage, with the SE silage not differing significantly from the other two silages. There was a significant difference in the lactic acid concentration of the three silages on day 120, with the SE silage having the highest value, followed by the SM silage, and then the SL silage.

There were no significant differences in the acetic acid concentrations of the three silages on day 0 and day 7. On day 21 and day 120 the SM silage had significantly lower acetic acid concentrations compared to the SE and SL silages, with no significant differences between the SE and SL silages on day 21 and day 120.

Table 4.5 The lactic and acetic acid concentrations of *D. eriantha* grass ensiled at three growth stages, after wilting and sugar addition

Parameter	Day	SE W+S	SM W+S	SL W+S
Lactic acid (g/kg DM)	0	2.95^{4}_{a} ±0.07	1.37^{2}_{a} ±0.05	2.82^{2}_{a} ±0.07
	7	13.58^{3}_{b} ±1.33	24.08^{1}_{a} ±7.78	7.48^{2}_{c} ±0.36
	21	21.52^{2}_{ab} ±0.53	22.86^{1}_{a} ±3.94	17.40^{1}_{b} ±0.45
	120	32.14^{1}_{a} ±1.30	24.14^{1}_{b} ±0.62	18.53^{1}_{c} ±2.73
Acetic acid (g/kg DM)	0	4.09^{2}_{a} ±1.49	6.19^{1}_{a} ±1.33	$5.18^{2,3}_{a}$ ±0.13
	7	3.34^{2}_{a} ±0.65	6.35^{1}_{a} ±1.37	3.41^{3}_{a} ±0.98
	21	8.53^{1}_{a} ±0.84	5.08^{1}_{b} ±0.56	$8.33^{1,2}_{a}$ ±2.26
	120	9.07^{1}_{a} ±1.89	5.34^{1}_{b} ±2.44	9.11^{1}_{a} ±1.04

4.2.3 Nitrogen and ammonia-nitrogen

On day 0 and day 7 the N concentration of the three silages differed significantly, with the SL silage having the lowest and the SM silage the highest values (Table 4.6). The N concentration of the SM silage was non significantly higher than the SE silage, but these two values were significantly higher than that of the SL silage on day 21. On day 120 the N concentration of the SE and SM silages were significantly higher than that of the SL silage.

The NH_3-N concentration of the SM silage did not differ significantly from the SE and SL silages on day 0, but the SE (25.67 g/kg N) and SL (43.53 g/kg N) silages did differ significantly. On day 7, the NH_3-N concentration of the SE silage did not differ significantly from the SM and SL silages, but the SM and SL silages differed significantly. The only significant difference in the NH_3-N concentration on day 21, was that the SL silage had a significantly higher value than the SM and SE silages. On day

120 the three silages differed significantly in their NH_3-N concentrations, with the SM silage having the lowest value and the SL silage having the highest value.

The rate of pH decrease in the SE silage is much slower than that in the SM silage. This could lead to the growth of clostridia in the SE silage compared to the SM silage. The higher number of clostridia would lead to an increase in protein breakdown in the silage (McDonald *et al.*, 1991; Meeske, 1998; Woolford, 1998), as was observed in the increase in the NH_3-N concentration of the SE silage between day 21 and day 120. The NH_3-N concentration of the SE silage on day 120 is within the range given by Wilkinson (1988) and Orr & Treacher (1990), for "well-fermented" silages.

Table 4.6 The N and NH_3-N concentrations of *D. eriantha* grass ensiled at three growth stages, after wilting and sugar addition

Parameter	Day	SE W+S	SM W+S	SL W+S
N (g/kg DM)	0	31.81^{1}_{b} ±0.07	36.88^{1}_{a} ±0.10	25.23^{1}_{c} ±1.24
	7	28.77^{2}_{b} ±2.11	32.56^{2}_{a} ±0.53	19.20^{3}_{c} ±2.68
	21	27.61^{2}_{a} ±1.06	29.04^{3}_{a} ±0.61	$21.63^{2,3}_{b}$ ±1.31
	120	28.42^{2}_{a} ±1.25	27.66^{3}_{a} ±0.97	21.83^{2}_{b} ±4.52
NH_3-N (g/kg N)	0	25.67^{2}_{b} ±3.90	$34.97^{1,2}_{ab}$ ±0.39	43.53^{2}_{a} ±5.72
	7	36.72^{2}_{ab} ±5.41	$31.60^{1,2}_{b}$ ±0.77	$48.44^{1,2}_{a}$ ±15.01
	21	36.26^{2}_{b} ±0.81	42.28^{1}_{b} ±2.61	62.05^{1}_{a} ±4.14
	120	98.79^{1}_{a} ±5.93	26.32^{2}_{c} ±0.66	60.13^{1}_{b} ±1.42

The results obtained for the SE and SM silages are comparable to data obtained for ryegrass silage in the UK by Williams, Hoxey and Lowe (1997). One has to keep in mind that such comparisons of data obtained from bunker and laboratory studies are not always

directly comparable. The silages in the UK study had the following values for bunker 1 and bunker 2 respectively, DM concentration (28.5% vs 32.2 %), pH value (4.32 vs 4.32), lactic acid concentration (72.4 vs 69.8 g/kg DM), acetic acid concentration (28.1 vs 13.0 g/kg DM) and NH_3-N concentration (66 vs 73 g/kg N). The SE and SM silages in the current study had the same pH value and the NH_3-N concentration of the SE silage was higher and that of the SM silage lower compared to the UK study. The biggest difference was that the ryegrass silages had higher lactic and acetic acid concentrations compared to the SE and SM silages. This indicated that a temperate grass with a higher WSC concentration leads to more fermentation acids (lactic and acetic acid) being produced (McDonald *et al.*, 1991).

It can be concluded that the quality of the SM silage was the best, due to a low pH value, low NH_3-N value and a high lactic acid concentration. The quality of the SE silage was also acceptable, except for having a higher NH_3-N concentration compared to the SM silage. The SL silage was unstable due to the variation in pH values.

4.3 Results and discussion of *P. maximum* grass ensiled directly with sugar addition

The average DM concentration of the *P. maximum* grass ensiled at an early growth stage was 20.6%, that of the medium growth stage was 19.9% and that of the late growth stage was 23.2%.

4.3.1 Silage pH and buffering capacity

The pH value of the PL silage was significantly lower than that of the PE and PM silages on day 0, with no significant differences in the pH values of the three silages on day 7 and day 21 (Table 4.7).

There was a significant difference in the pH values of the three silages on day 120, with the PM silage (3.83) having the lowest pH value and the PL silage (5.04) having the highest pH value.

There were no significant differences in the buffering capacity of the PE (63.34) and PL (63.21) silages on day 0, but these values were significantly higher than that for the PM silage (55.48). The difference between the silages were smaller than the differences found between legumes and grasses (McDonald *et al.*, 1991).

Table 4.7 The pH and buffering capacity values of *P. maximum* grass ensiled directly, at three growth stages, with sugar addition

Parameter	Day	PE D+S	PM D+S	PL D+S
pH	0	5.02^{1}_{a} ±0.03	4.98^{1}_{a} ±0.05	4.81^{2}_{b} ±0.03
	7	3.97^{2}_{a} ±0.01	3.94^{2}_{a} ±0.03	3.95^{3}_{a} ±0.02
	21	3.94^{2}_{a} ±0.03	3.89^{2}_{a} ±0.02	3.95^{3}_{a} ±0.04
	120	3.93^{2}_{b} ±0.02	3.83^{2}_{c} ±0.04	5.04^{1}_{a} ±0.05
Buffering capacity (meq/100g DM)	0	63.34_{a} ±1.93	55.48_{b} ±0.55	63.21_{a} ±1.78

4.3.2 Lactic and acetic acid

There were no significant differences in the lactic acid concentration of the three silages on day 0 (Table 4.8). On day 7 the PE silage had a significantly lower lactic acid concentration compared to the PM and PL silages, with no significant differences between the PM and PL silages. The lactic acid concentrations of the three silages differed significantly on day 21, and day 120, but there was a significant drop in the lactic acid concentration of the PL silage between day 21 and day 120.

The PM silage had a significantly higher acetic acid concentration on day 0 compared to the PE silage, with the value of the PL silage not differing significantly from the other two. On day 7 there were no significant differences in the acetic acid concentrations of

the three silages. On day 21 the PE and PM silages had a significantly lower acetic acid concentration compared to that of the PL silage. The PL silage had a significantly higher acetic acid concentration on day 120 compared to the PE and the PM silages.

The rate of pH drop between the PE (1.05 units) and PM (1.04 units) silages between day 0 and day 7 were similar. The PL (0.86 units) silage had a slower rate of pH drop than the other two silages. The production of lactic acid was higher in the PM silage compared to the PL and PE silages between day 0 and day 7. The PL silage produced more lactic acid between day 0 and day 7 compared to the PE silage. The rate of pH drop is almost more important than the final pH value (Woolford, 1998), as the faster the pH drops, the quicker the clostridia can be inhibited, thus resulting in a reduction in protein breakdown to NH_3-N.

Table 4.8 The lactic and acetic acid concentrations of *P. maximum* grass ensiled directly, at three growth stages, with sugar addition

Parameter	Day	PE D+S	PM D+S	PL D+S
Lactic acid (g/kg DM)	0	3.12^{3}_{a} ±1.27	3.54^{3}_{a} ±0.09	2.44^{4}_{a} ±0.84
	7	29.61^{2}_{b} ±1.82	40.54^{2}_{a} ±7.36	37.75^{2}_{a} ±4.36
	21	30.61^{2}_{c} ±0.20	48.47^{1}_{b} ±4.21	56.60^{1}_{a} ±1.42
	120	37.79^{1}_{b} ±3.20	49.03^{1}_{a} ±1.23	20.70^{3}_{c} ±0.20
Acetic acid (g/kg DM)	0	2.79^{2}_{b} ±1.77	5.98^{1}_{a} ±0.15	3.71^{2}_{ab} ±0.20
	7	4.71^{2}_{a} ±0.71	7.71^{1}_{a} ±1.57	6.17^{2}_{a} ±1.64
	21	9.90^{1}_{b} ±0.12	9.53^{1}_{b} ±1.61	13.13^{1}_{a} ±0.78
	120	10.15^{1}_{b} ±0.12	7.87^{1}_{b} ±0.42	14.69^{1}_{a} ±0.37

4.3.3 Nitrogen and ammonia-nitrogen

On day 0, day 7 and day 21 the PE and PM silages had significantly higher N concentration values compared to the PL silages, with the PE and PM silage not differing significantly (Table 4.9). The PM silage had a significantly higher N concentration compared to that of the PL silage on day 120. The value for the PE silage did not differ significantly from the PL and PM silages on day 120.

There were no significant differences in the NH_3-N concentrations of the three silages on day 0. On day 7 the PE silage had a significantly higher NH_3-N concentration compared to that of the PM and PL silages and the PM and PL silages did not differ significantly from each other. There was a significant difference in the NH_3-N values for the three silages on day 21, with the PE silage having the highest value and the PL silage the lowest value. On day 120 the PE (98.71 g/kg N) and the PL (91.75 g/kg N) silages had significantly higher NH_3-N values compared to that of the PM (47.54 g/kg N) silage.

Table 4.9 The N and NH_3-N concentrations of *P. maximum* grass ensiled directly, at three growth stages, with sugar addition

Parameter	Day	PE D+S	PM D+S	PL D+S
N (g/kg DM)	0	23.58^{1}_{a} ±0.20	25.26^{1}_{a} ±0.04	20.84^{1}_{b} ±0.07
	7	$21.40^{1,2}_{a}$ ±0.57	$22.87^{1,2}_{a}$ ±0.98	$18.73^{1,2}_{b}$ ±1.12
	21	22.74^{1}_{a} ±0.60	$23.91^{1,2}_{a}$ ±0.59	17.90^{2}_{b} ±0.32
	120	19.69^{2}_{ab} ±1.53	21.53^{2}_{a} ±0.52	$19.22^{1,2}_{b}$ ±2.70
NH_3-N (g/kg N)	0	44.35^{3}_{a} ±5.95	$40.84^{1,2}_{a}$ ±1.01	45.42^{2}_{a} ±1.86
	7	52.31^{3}_{a} ±1.27	29.19^{2}_{b} ±6.90	34.60^{2}_{b} ±2.10
	21	70.81^{2}_{a} ±12.67	54.43^{1}_{b} ±1.26	32.81^{2}_{c} ±0.80
	120	98.71^{1}_{a} ±28.30	47.54^{1}_{b} ±1.16	91.75^{1}_{a} ±50.13

According to Woolford (1998), when crop DM is in excess of 30%, clostridial fermentation is suppressed through lack of moisture availability. Up to this level a combination of acid, pH and moisture availability will inhibit clostridia. Generally, at a DM of 20% stability is achieved at a pH of about 4.0. Both the PE and PM silages had pH values below 4.0 on day 120, thus according to McDonald *et al.* (1991), these silages can be described as being stable. The lower production in lactic acid in the PE silage, with its lower DM concentration compared to the PL silage, could have resulted in the growth of some clostridia. Clostridia would ferment protein to NH_3-N (McDonald *et al.*, 1991; Woolford, 1998), thus explaining the increase in NH_3-N concentration of the PE silage as the ensiled period progressed.

The increase in the pH, NH_3-N concentration and the decrease in the lactic acid concentration of the PL silage between day 21 and day 120, could have been a result of secondary fermentation. Another possibility is that an experimental fault could have led to these values being obtained. The NH_3-N concentration of the PL and PE silages are within the range for "well-fermented" silages, as suggested by Wilkinson (1988) and Orr & Treacher (1990). The NH_3-N concentration of the PM silage is below the above mentioned range and is thus classified as a better quality compared to the other two silages.

The data obtained in the current study for the PE and PM silages compared to data obtained by Rinne, Huhtanen and Jaakkola (1997) for silage made from a mixed sward of Timothy and Fescue grass. In the study by Rinne *et al.* (1997), the pH values of the grasses were comparable to that of the PE and PM silages in this study. The Timothy/Fescue silages had higher lactic and acetic acid concentrations compared to the PE and PM silages. The NH_3-N concentrations of the Timothy/Fescue silages were higher than that of the PM silage and lower than that of the PE silage.

It can be concluded from the results that the PM silage had a lower pH, lower NH_3-N concentration and a higher lactic acid concentration compared to the other two silages on day 120. The PE silage has a better quality than the PL silage. The decrease in silage

quality between day 21 and day 120 in the PL silage could be a result of possible experimental errors in the processing of the samples or secondary fermentation.

4.4 Results and discussion of *P. maximum* grass ensiled with prior wilting and sugar addition

The average DM concentration of the *P. maximum* grass ensiled at an early growth stage was 30.1%, that of the medium growth stage was 24.4% and that of the late growth stage was 25.6%.

4.4.1 Silage pH and buffering capacity

The pH value of the PE silage was significantly higher than that of the PM and PL silages on day 0 (Table 4.10). On day 7 the pH value of the PM silage was significantly lower than that of the PE and PL silages. The PL silage had a significantly higher pH value compared to the PE and PM silages on day 21. On day 120 the pH values of the three silages differed significantly from each other.

Table 4.10 The pH and buffering capacity values of *P. maximum* grass ensiled at three growth stages, with prior wilting and sugar addition

Parameter	Day	PE W+S	PM W+S	PL W+S
pH	0	5.22^{1}_{a} ±0.03	4.71^{1}_{b} ±0.02	4.67^{1}_{b} ±0.01
	7	4.42^{2}_{a} ±0.02	4.04^{4}_{b} ±0.05	4.42^{2}_{a} ±0.03
	21	4.13^{3}_{b} ±0.04	4.17^{3}_{b} ±0.04	4.32^{2}_{a} ±0.02
	120	4.16^{3}_{c} ±0.03	4.52^{2}_{a} ±0.02	4.33^{2}_{b} ±0.05
Buffering capacity (meq/100g DM)	0	72.88_{a} ±0.91	68.59_{b} ±1.06	72.93_{a} ±0.05

The PM silage (68.59) had a significantly lower buffering capacity value compared to that of the PE (72.88) and PL (72.93) silages. Due to the lower buffering capacity value of the PM silage the rate of the pH drop in the PM silage was fast between day 0 and day 7. The PE and PL silages did not differ significantly from each other.

4.4.2 Lactic and acetic acid

There were no significant differences in the lactic acid concentration of the three silages on day 0 (Table 4.11). The PM silage had a significantly higher lactic acid concentration compared to the PE and PL silages on day 7. On day 21 the PL and PE silages had a significantly higher lactic acid concentration compared to the PM silage. The PE silage had a significantly higher lactic acid concentration compared to the PM and PL silages on day 120. There was no significant difference in the lactic acid concentration of the PM and PL silages on day 120.

Table 4.11 The lactic and acetic acid concentrations of *P. maximum* grass ensiled at three growth stages, with prior wilting and sugar addition

Parameter	Day	PE W+S	PM W+S	PL W+S
Lactic acid (g/kg DM)	0	2.62^{4}_{a} ±1.27	3.67^{3}_{a} ±1.60	2.38^{3}_{a} ±0.82
	7	19.03^{3}_{b} ±1.85	28.03^{1}_{a} ±3.44	16.32^{2}_{b} ±0.40
	21	25.04^{2}_{a} ±4.07	19.88^{2}_{b} ±2.83	26.02^{1}_{a} ±3.02
	120	31.37^{1}_{a} ±5.02	21.46^{2}_{b} ±8.30	25.41^{1}_{b} ±1.42
Acetic acid (g/kg DM)	0	2.73^{3}_{a} ±0.85	3.71^{2}_{a} ±0.74	2.66^{2}_{a} ±1.26
	7	$5.37^{2,3}_{a}$ ±0.06	5.00^{2}_{a} ±0.58	$5.57^{1,2}_{a}$ ±0.83
	21	6.48^{2}_{a} ±0.23	$5.43^{1,2}_{a}$ ±0.58	7.84^{1}_{a} ±0.97
	120	12.25^{1}_{a} ±2.55	9.18^{1}_{ab} ±0.23	8.04^{1}_{b} ±0.99

There were no significant differences in the acetic acid concentrations of the three silages on day 0, day 7 and day 21. On day 120 the PE silage (12.25 g/kg DM) had a significantly higher acetic acid concentration compared to the PL silage (8.04 g/kg DM). The acetic acid concentration of the PM silage (9.18 g/kg DM) did not differ significantly from the PE or PL silages on day 120.

4.4.3 Nitrogen and ammonia-nitrogen

The N concentration of the three silages differed significantly from each other on day 0 and day 7, with the PM silage having the highest value and the PL silage having the lowest value (Table 4.12.). On day 21 the PM silage had a significantly higher N concentration compared to the PE and PL silages, with the PE and PL silages not being significantly different from each other. The N concentrations of the three silages differed significantly from each other on day 120.

Table. 4.12 The N and NH_3-N concentrations of *P. maximum* grass ensiled at three growth stages, with prior wilting and sugar addition

Parameter	Day	PE W+S	PM W+S	PL W+S
N (g/kg DM)	0	21.97^{1}_{b} ±0.26	27.43^{1}_{a} ±0.23	$17.51^{1,2}_{c}$ ±1.36
	7	20.54^{1}_{b} ±0.73	27.18^{1}_{a} ±1.45	$18.36^{1,2}_{c}$ ±0.87
	21	21.12^{1}_{b} ±1.04	26.65^{1}_{a} ±2.31	19.41^{1}_{b} ±0.49
	120	20.82^{1}_{b} ±0.37	25.53^{1}_{a} ±0.95	16.55^{2}_{c} ±0.57
NH_3-N (g/kg N)	0	27.29^{3}_{b} ±7.59	21.77^{2}_{b} ±1.29	46.44^{1}_{a} ±2.44
	7	51.49^{2}_{a} ±0.52	30.98^{2}_{b} ±0.40	44.22^{1}_{a} ±2.33
	21	72.18^{1}_{a} ±9.91	30.27^{2}_{b} ±0.79	34.64^{1}_{b} ±2.07
	120	86.95^{1}_{b} ±1.94	113.32^{1}_{a} ±17.00	34.53^{1}_{c} ±0.82

The NH_3-N concentration of the PE and PM silages on day 0 was significantly lower than that of the PL silage. On day 7 the PM silage had a significantly lower NH_3-N value compared to that of the PE and PL silages, with the PE and PL silages not being significantly different from each other.

The PE silage had a significantly higher NH_3-N concentration on day 21 compared to that of the PM and PL silages. The NH_3-N concentrations of the three silages differed significantly on day 120. The values were 113.32 g/kg N, 86.95 g/kg N and 34.53 g/kg N respectively for the PM, PE and PL silages. The NH_3-N concentration of the PM silage is higher than the value of 110 g/kg N given by McDonald *et al.* (1991) for a well-fermented silage and lies within the range of 100 to 150 g/kg N used by Wilkinson (1988) and Orr & Treacher (1990), to classify it as a "satisfactorily fermented" silage.

The higher DM concentration of the PE silage, would inhibit some clostridia growth, compared to the PM and PL silages. This combined with the higher rate of pH drop and increased production of lactic and acetic acid between day 0 and day 7, should have a negative effect on clostridia growth (McDonald *et al.*, 1991; Woolford, 1998). This should have resulted in a lower NH_3-N concentration in the PE silage, than was found. According to Jonsson (1991), the growth of most acid-tolerant clostridia is inhibited by a pH just below 5.0 although there is evidence that *Clostridia tyrobutyricum* is able to grow at a pH 4.4 to 4.9. With the initial pH of the PE silage being above 5.0, this could have resulted in more growth of clostridia compared to the other two silages. This could thus explain why more protein breakdown was detected in the PE silage compared to what would have been expected.

The lower DM concentration of the PM silage on day 0 could have resulted in more growth of clostridia (Woolford, 1998). This would explain the change due to secondary fermentation, in the pH, lactic acid concentration and NH_3-N concentration of the PM silage from day 7 to day 21. During this period, the pH increased the lactic acid concentration decreased and the NH_3-N concentration increased.

According to McDonald *et al.* (1991), the higher osmotic pressure associated in general with wilting can inhibit clostridial growth, so that a stable pH can be achieved at pH values above 5.0. The rate of pH drop in the PL silage was slower than that for the PE and PM silages. The concentration of lactic acid produced was less in the PL silage between day 0 and day 7, compared to the PE and PM silages. The NH_3-N concentration on day 120 was much lower in the PL silage compared to the PE and PM silages. Thus, the wilting of the *P. maximum* grass at a late growth stage could have inhibited clostridia growth, resulting in a stable silage. In summary, the PE and PL silages are of better quality than the PM silage.

CHAPTER 5

Results and Discussion
Specific treatment differences across *D. eriantha* and *P. maximum*, within a growth stage.

According to Tainton (1988), grass species such as *Panicum maximum*, *Digitaria eriantha*, *Eragrostis curvula* and *Cenchrus ciliaris* are classified as Tropical / Subtropical species. Ryegrass is classified as a temperate specie. The WSC concentration of subtropical grasses is generally lower than that of temperate species (McDonald *et al.*, 1991). If there are insufficient sugar, acidification may not be of sufficient magnitude to prevent an unwanted fermentation during ensiling (Woolford, 1998). The type of fermentation pattern aimed for is that where more lactic acid is produced than acetic acid. This leads to a fast decrease in the pH value of the silage and silage stability is thus achieved (McDonald *et al.*, 1991).

In this chapter the data obtained between *D. eriantha* and *P. maximum* grass ensiled will be compared and discussed. Data comparison will be within a specific growth stage and for a specific treatment. The treatments, which will be used for the comparisons, are the D+S and W+S treatments, as discussed in Chapter 4. Comparisons will be made within all three growth stages.

5.1 *D. eriantha* vs *P. maximum* ensiled directly, at an early growth stage with sugar addition

The average DM concentration of *D. eriantha* grass ensiled at an early growth stage was 23.2 % compared to that of *P. maximum* grass ensiled at an early stage, which had an average DM concentration of 20.6%.

5.1.1 Silage pH and buffering capacity

There was no significant difference in the pH values of the SE and PE silages on day 0 (Table 5.1). On day 7, day 21 and day 120 the PE silage had significantly lower pH values compared to the SE silage. This would indicate a better rate of fermentation leading to a faster decrease in pH of the PE silage compared to the SE silage.

Table 5.1 The pH and buffering capacity values of *D. eriantha* and *P. maximum* grass ensiled directly at an early growth stage, with sugar addition

Parameter	Day	SE D+S		PE D+S	
pH	0	4.98^{1}_{a}	±0.01	5.02^{1}_{a}	±0.03
	7	4.11^{3}_{a}	±0.02	3.97^{2}_{b}	±0.01
	21	4.08^{3}_{a}	±0.02	3.94^{2}_{b}	±0.03
	120	4.25^{2}_{a}	±0.04	3.93^{2}_{b}	±0.02
Buffering capacity (meq/100g DM)	0	49.42_{b}	±0.77	63.34_{a}	±1.93

The following information will apply to all the tables in Chapter 5.
1: Values with different superscripts (1,2,3,4) down a column differ significantly at $P \leq 0.05$.
2: Values with different subscripts (a,b,c,d) across a row differ significantly at $P \leq 0.05$.
3: Values after the ± sign are standard deviation values.
4: Three observations per treatment.

The SE silage had a significantly lower buffering capacity value compared to the PE silage on day 0. In a study conducted in Northern Ireland with one hundred and thirty six silages the buffering capacity varied between 53.4 and 255.4 meq/ 100g DM (Steen, Gordon, Dawson, Park, Mayne, Agnew, Kilpatrick and Porter, 1998). The difference in the buffering capacity of the SE and PE silages are smaller than the variation found in that study.

5.1.2 Lactic acid and acetic acid

The lactic acid concentration of the SE and PE silages on day 0 did not differ significantly from each other (Table 5.2). The PE silage had significantly higher lactic acid concentrations on day 7, day 21 and day 120, compared to the SE silage. There were no significant differences in the acetic acid concentrations of the SE and PE silages on day 0 and day 7. On day 21 and day 120, the PE silage had significantly higher acetic acid concentrations compared to the SE silages. One of the possible reasons for the higher production of lactic acid in the PE silage compared to the SE silage, could have been the higher buffering capacity of the PE silage.

Table 5.2 The lactic and acetic acid concentrations of *D. eriantha* and *P. maximum* grass ensiled directly at an early growth stage with sugar addition

Parameter	Day	SE D+S	PE D+S
Lactic acid (g/kg DM)	0	3.86^{3}_{a} ±0.71	3.12^{3}_{a} ±1.27
	7	15.39^{2}_{b} ±1.24	29.61^{2}_{a} ±1.82
	21	22.73^{1}_{b} ±1.63	30.61^{2}_{a} ±0.20
	120	27.68^{1}_{b} ±1.81	37.39^{1}_{a} ±3.20
Acetic acid (g/kg DM)	0	4.23^{1}_{a} ±0.71	2.79^{2}_{a} ±1.77
	7	4.12^{1}_{a} ±0.60	4.71^{2}_{a} ±0.71
	21	4.06^{1}_{b} ±0.46	9.90^{1}_{a} ±0.12
	120	3.95^{1}_{b} ±0.55	10.15^{1}_{a} ±0.12

5.1.3 Nitrogen and ammonia-nitrogen

The SE silages had significantly higher N concentration values, on day 0, day 7, day 21 and day 120, compared to the PE silages (Table 5.3).

There were no significant differences in the NH_3-N concentrations of the SE and PE silages on day 0. On day 7, day 21 and day 120 the PE silages had significantly higher NH_3-N values compared to that of the SE silages.

Table 5.3 The N and NH_3-N concentrations of *D. eriantha* and *P. maximum* grass ensiled directly at an early growth stage with sugar addition

Parameter	Day	SE D+S	PE D+S
N (g/kg DM)	0	29.73^{1}_{a} ±0.13	23.58^{1}_{b} ±0.20
	7	29.47^{1}_{a} ±1.82	$21.40^{1,2}_{b}$ ±0.57
	21	25.75^{2}_{a} ±1.28	22.74^{1}_{b} ±0.60
	120	24.74^{2}_{a} ±1.59	19.69^{2}_{b} ±1.53
NH_3-N (g/kg N)	0	$46.71^{1,2}_{a}$ ±4.91	44.35^{3}_{a} ±5.95
	7	35.42^{2}_{b} ±1.50	52.31^{3}_{a} ±1.27
	21	55.27^{1}_{b} ±0.82	70.81^{2}_{a} ±12.67
	120	56.52^{1}_{b} ±12.20	98.71^{1}_{a} ±28.30

The quality of both the SE and PE silages are good on day 120. The pH of the SE (4.25) silage is just above the value of 4.20 given by McDonald *et al.* (1991), for a good quality unwilted silage. Woolford (1998), stated that for a silage with a DM of 20%, generally, a stable silage is obtained at a pH of 4.0. The pH of the PE silage is just below this value. The lactic acid concentration and acetic acid concentrations of the SE and PE silages on day 120 are similar to those obtained by Meeske *et al.* (1999), when ensiling *D. eriantha* with a bacterial inoculant. The NH_3-N concentration of the SE and PE silages on day 120, are within the range of 50 to 100 g/kg N, given by Wilkinson (1988) and Orr & Treacher (1990), for "well-fermented" silages. The NH_3-N values are below the 110 g/kg N used by McDonald *et al.* (1991), for well-fermented silages. Meeske *et al.* (1999) reported a

NH_3-N g/kg TN of 50.3 after ensiling *D. eriantha* for 44 days, this is lower than the value obtained for the PE silage and in line with the value obtained for the SE silage.

The PE silage had a lower pH and a higher lactic acid concentration compared to that of the SE silage on day 120. It would thus be expected that the PE silage (98.71 g/kg N) would have had a lower NH_3-N concentration compared to the SE silage (56.52 g/kg N) on day 120. This could possibly be explained, due to the initial pH of the PE silage being above 5.0 and that the DM concentration was 20.6%. This could have lead to the growth of clostridia bacteria (Jonsson, 1991; McDonald *et al.*, 1991; Woolford, 1998), resulting in the breakdown of protein to NH_3-N in the PE silage. The quality of the SE and PE silages are good and are better than that which was found by Meeske (1998), for *Eragrostis curvula* silage.

5.2 *D. eriantha* vs *P. maximum*, ensiled at an early growth stage, with prior wilting and sugar addition

The average DM concentration of the *D. eriantha* grass ensiled at an early growth stage was 30.7% compared to that of 30.1% for the *P. maximum* grass cut at the same growth stage.

5.2.1 Silage pH and buffering capacity

The pH value of the SE silage (4.82) was significantly lower than that of the PE silage (5.22) on day 0 (Table 5.4). On day 7, day 21 and day 120 the PE silages had significantly lower pH values compared to the SE silages.

The SE silage had a significantly lower buffering capacity value compared to that of the PE silage on day 0.

Table 5.4 The pH and buffering capacity values of *D. eriantha* and *P. maximum* grass ensiled at an early growth stage, with prior wilting and sugar addition

Parameter	Day	SE W+S	PE W+S
pH	0	4.82^{1}_{b} ±0.03	5.22^{1}_{a} ±0.03
	7	4.70^{2}_{a} ±0.02	4.42^{2}_{b} ±0.02
	21	4.24^{3}_{a} ±0.03	4.13^{3}_{b} ±0.04
	120	4.32^{3}_{a} ±0.03	4.16^{3}_{b} ±0.03
Buffering capacity (meq/100g DM)	0	56.55_{b} ±0.49	72.88_{a} ±0.91

5.2.2 Lactic acid and acetic acid

On day 0 there were no significant differences in the lactic acid concentrations of the SE and PE silages (Table 5.5). On day 7, the PE silage had a significantly higher lactic acid concentration compared to that of the SE silage. There was no significant difference in the lactic acid concentrations of the SE and PE silages on day 21 and day 120.

On day 0, day 7 and day 21, there were no significant differences in the acetic acid concentrations of the SE and PE silages. On day 120 the PE silage (12.25 g/kg DM) had a significantly higher acetic acid concentration compared to that of the SE silage (9.07 g/kg DM).

The lactic acid concentration of the SE and PE silages on day 120 were comparable, while the acetic acid concentration and pH values of the SE and PE silages were higher than that which was obtained by Meeske *et al.* (1999), for *D. eriantha* silage which was inoculated with Sil-All®. The control silage in the study by Meeske *et al.* (1999) had a

lower lactic acid concentration and a higher pH value than that of the PE and SE silages on day 120, in this study.

Table 5.5 The lactic and acetic acid concentrations of *D. eriantha* and *P. maximum* grass ensiled at an early growth stage, with prior wilting and sugar addition

Parameter	Day	SE W+S	PE W+S
Lactic acid (g/kg DM)	0	2.95^{4}_{a} ±0.07	2.62^{4}_{a} ±1.27
	7	13.58^{3}_{b} ±1.33	19.03^{3}_{a} ±1.85
	21	21.52^{2}_{a} ±0.53	25.04^{2}_{a} ±4.07
	120	32.14^{1}_{a} ±1.30	31.37^{1}_{a} ±5.02
Acetic acid (g/kg DM)	0	4.09^{2}_{a} ±1.49	2.73^{3}_{a} ±0.85
	7	3.34^{2}_{a} ±0.65	$5.37^{2,3}_{a}$ ±0.06
	21	8.53^{1}_{a} ±0.84	6.48^{2}_{a} ±0.23
	120	9.07^{1}_{b} ±1.89	12.25^{1}_{a} ±2.55

5.2.3 Nitrogen and ammonia-nitrogen

There was a significant difference in the N concentration values on day 0, day 7, day 21 and day 120, with the SE silages having higher values than the PE silages (Table 5.6).

On day 0, there were no significant differences in the NH_3-N concentrations of the SE and PE silages. On day 7 and day 21 the PE silages had significantly higher NH_3-N concentrations compared to the SE silages. On day 120 there was no significant difference in the NH_3-N concentrations of the SE and PE silages.

Table 5.6 **The N and NH_3-N concentrations of *D. eriantha* and *P. maximum* grass ensiled at an early growth stage, with prior wilting and sugar addition**

Parameter	Day	SE W+S	PE W+S
N (g/kg DM)	0	31.81^{1}_{a} ±0.07	21.97^{1}_{b} ±0.26
	7	28.77^{2}_{a} ±2.11	20.54^{1}_{b} ±0.73
	21	27.61^{2}_{a} ±1.06	21.12^{1}_{b} ±1.04
	120	28.42^{2}_{a} ±1.25	20.82^{1}_{b} ±0.37
NH_3-N (g/kg N)	0	25.67^{2}_{a} ±3.90	27.29^{3}_{a} ±7.59
	7	36.72^{2}_{b} ±5.41	51.49^{2}_{a} ±0.52
	21	36.26^{2}_{b} ±0.81	72.18^{1}_{a} ±9.91
	120	98.79^{1}_{a} ±5.93	86.95^{1}_{a} ±1.94

The quality of the SE and PE silages can be described as good, having a low pH value, high lactic acid concentration, low acetic acid concentration and a low NH_3-N concentration (McDonald *et al.*, 1991). The significant increase in the NH_3-N concentration of the SE silage between day 21 and day 120, could be a result of secondary fermentation due to clostridia. The slow decrease in the pH of the SE silage between day 0 and day 21, could have resulted in the growth of clostridia. This would lead to the breakdown of protein to NH_3-N (Woolford, 1998). The significant increase in the NH_3-N concentration of the PE silage can also be attributed to clostridia growth, due to the initial high pH (5.22) of the silage on day 0, thus resulting in protein breakdown to NH_3-N (Jonsson, 1991; McDonald *et al.*, 1991; Woolford, 1998). The NH_3-N concentration of the SE and PE silages are within the range given by Wilkinson (1988) and Orr & Treacher (1990), for "well-fermented" silages.

5.3 *D. eriantha* vs *P. maximum*, ensiled directly at a medium growth stage, with sugar addition

The *D. eriantha* grass ensiled at a medium growth stage had an average DM concentration of 19.2% compared to the 19.9% for the *P. maximum* grass ensiled at a medium growth stage.

5.3.1 Silage pH and buffering capacity

On day 0 the SM silage had a significantly higher pH value compared to the PM silage (Table 5.7). On day 7 the PM silage (3.94) had a lower, but non-significantly different, pH value compared to the SM silage (4.02). On day 21 the PM silage had a significantly lower pH value compared to the SM silage as well as on day 120 although non significantly.

Table 5.7 The pH and buffering capacity values of *D. eriantha* and *P. maximum* grass ensiled directly at a medium growth stage, with sugar addition

Parameter	Day	SM D+S	PM D+S
pH	0	5.17^{1}_{a} ±0.21	4.98^{1}_{b} ±0.05
	7	4.02^{2}_{a} ±0.02	3.94^{2}_{a} ±0.03
	21	$3.99^{2,3}_{a}$ ±0.01	3.89^{2}_{b} ±0.02
	120	3.90^{3}_{a} ±0.02	3.83^{2}_{a} ±0.04
Buffering capacity (meq/100g DM)	0	43.80_{b} ±0.02	55.48_{a} ±0.55

The SM silage had a significantly lower buffering capacity value on day 0 compared to the PM silage. The difference in the buffering capacity values of the SM and PM silage is smaller than that found between grasses and legumes (McDonald *et al.*, 1991).

5.3.2 Lactic acid and acetic acid

On day 0 there were no significant differences in the lactic acid concentration of the SM and PM silages (Table 5.8). The SM silages had significantly higher lactic acid concentrations on day 7, day 21 and 120 compared to the PM silages.

On day 0, day 7 and day 21 there were no significant differences in the acetic acid concentrations of the SM and PM silages. On day 120, the SM silage had a significantly higher acetic acid concentration compared to the PM silage.

Table 5.8 The lactic and acetic acid concentrations of *D. eriantha* and *P. maximum* grass ensiled directly at a medium growth stage, with sugar addition

Parameter	Day	SM D+S	PM D+S
Lactic acid (g/kg DM)	0	1.41^{4}_{a} ±0.05	3.54^{3}_{a} ±0.09
	7	53.47^{3}_{a} ±5.66	40.54^{2}_{b} ±7.36
	21	64.24^{1}_{a} ±2.85	48.47^{1}_{b} ±4.21
	120	58.97^{2}_{a} ±1.52	49.03^{1}_{b} ±1.23
Acetic acid (g/kg DM)	0	5.28^{2}_{a} ±1.01	5.98^{1}_{a} ±0.15
	7	10.31^{1}_{a} ±1.72	7.71^{1}_{a} ±1.57
	21	11.89^{1}_{a} ±3.42	9.53^{1}_{a} ±1.61
	120	11.58^{1}_{a} ±1.52	7.87^{1}_{b} ±0.42

5.3.3 Nitrogen and ammonia-nitrogen

The SM silages had a significantly higher N concentration compared to the PM silages on day 0, day 7, day 21 and day 120 (Table 5.9).

Table 5.9 The N and NH_3-N concentrations of *D. eriantha* and *P. maximum* grass ensiled directly at a medium growth stage, with sugar addition

Parameter	Day	SM D+S	PM D+S
N (g/kg DM)	0	31.82^{1}_{a} ±0.30	25.26^{1}_{b} ±0.04
	7	27.17^{2}_{a} ±1.30	$22.87^{1,2}_{b}$ ±0.98
	21	28.85^{2}_{a} ±0.39	$23.91^{1,2}_{b}$ ±0.59
	120	27.81^{2}_{a} ±0.46	21.53^{2}_{b} ±0.52
NH_3-N (g/kg N)	0	44.94^{1}_{a} ±6.94	$40.84^{1,2}_{a}$ ±1.01
	7	$30.30^{1,2}_{a}$ ±0.10	29.12^{2}_{a} ±6.90
	21	27.76^{2}_{b} ±6.81	54.43^{1}_{a} ±1.26
	120	27.12^{2}_{b} ±0.65	47.54^{1}_{a} ±1.16

On day 0 and day 7 there were no significant differences in the NH_3-N concentrations of the SM and PM silages. On day 21 and day 120 the SM silages had significantly lower NH_3-N values compared to the PM silages.

There is very little difference in the silage quality of the SM and PM silages on day 120. Both the silages have a pH value below 4.0, which is acceptable according to Woolford (1998), for silages with a DM of 20%. They have high lactic acid concentrations compared to their acetic acid concentrations, as was found by Meeske *et al.* (1999) and the NH_3-N concentrations are below the 50 to 100 g/kg TN given by Wilkinson (1988) and Orr & Treacher (1990), for "well-fermented" silages. The SM silage had possibly a better quality, as its NH_3-N concentration on day 120 is lower than that of the PM silage.

5.4 *D. eriantha* vs *P. maximum*, ensiled at a medium growth stage, with prior wilting and sugar addition

The average DM concentration for the *D. eriantha* grass ensiled at a medium growth stage with prior wilting was 29.5% compared with a value of 24.4% for the *P. maximum* grass. The DM concentration of the SM silage is close to the 30% quoted by Woolford (1998), where moisture availability could be the major factor inhibiting the growth of clostridia in the silage.

5.4.1 Silage pH and buffering capacity

The SM silage had a significantly higher pH value compared to the PM silage on day 0 (Table 5.10).

Table 5.10 The pH and buffering capacity values of *D. eriantha* and *P. maximum* grass ensiled at a medium growth stage, with prior wilting and sugar addition

Parameter	Day	SM W+S		PM W+S	
pH	0	$4.91^{1}{}_{a}$	±0.04	$4.71^{1}{}_{b}$	±0.02
	7	$4.33^{2}{}_{a}$	±0.02	$4.04^{4}{}_{b}$	±0.05
	21	$4.31^{2}{}_{a}$	±0.03	$4.17^{3}{}_{b}$	±0.04
	120	$4.33^{2}{}_{b}$	±0.03	$4.52^{2}{}_{a}$	±0.02
Buffering capacity (meq/100g DM)	0	32.05_{b}	±0.12	68.59_{a}	±1.06

On day 7 and day 21, the PM silages had a significantly lower pH value compared to the SM silages. On day 120 the pH value of the SM silage was significantly lower than that of the PM silage. The SM silage had a significantly lower buffering capacity value on day

0, compared to that of the PM silage. The buffering capacity value of the SM silage was twice as high as that of the PM silage.

5.4.2 Lactic acid and acetic acid

There were no significant differences in the lactic acid concentrations of the SM and PM silages on day 0, day 7, day 21 and day 120 (Table 5.11). On day 0, day 7 and day 21, there were no significant differences in the acetic acid concentrations of the SM and PM silages. On day 120, the PM silage (9.18 g/kg DM) had a significantly higher acetic acid concentration compared to the SM silage (5.34 g/kg DM).

Table 5.11 The lactic and acetic acid concentrations of *D. eriantha* and *P. maximum* grass ensiled at a medium growth, with prior wilting and sugar addition

Parameter	Day	SM W+S	PM W+S
Lactic acid (g/kg DM)	0	1.37^{2}_{a} ±0.05	3.67^{3}_{a} ±1.60
	7	24.08^{1}_{a} ±7.78	28.03^{1}_{a} ±3.44
	21	22.86^{1}_{a} ±3.94	19.88^{2}_{a} ±2.83
	120	24.14^{1}_{a} ±0.62	21.46^{2}_{a} ±8.30
Acetic acid (g/kg DM)	0	6.19^{1}_{a} ±1.33	3.71^{2}_{a} ±0.74
	7	6.35^{1}_{a} ±1.37	5.00^{2}_{a} ±0.58
	21	5.08^{1}_{a} ±0.56	$5.43^{1,2}_{a}$ ±0.58
	120	5.34^{1}_{b} ±2.44	9.18^{1}_{a} ±0.23

5.4.3 Nitrogen and ammonia-nitrogen

The SM silages had a significantly higher N concentration compared to the PM silages on day 0, day 7, day 21 and day 120 (Table 5.12).

On day 0, the SM silage had a significantly higher NH_3-N value compared to the PM silage. On day 7 and day 21 there were no significant differences in the NH_3-N concentrations of the SM and PM silages. On day 120 the PM silage (113.32 g/kg N) had a significantly higher NH_3-N value compared to the SM silage (26.32 g/kg N).

Table 5.12 The N and NH_3-N concentrations of *D. eriantha* and *P. maximum* grass ensiled at a medium growth stage, with prior wilting and sugar addition

Parameter	Day	SM W+S	PM W+S
N (g/kg DM)	0	$36.88^{1}{}_{a}$ ±0.10	$27.43^{1}{}_{b}$ ±0.23
	7	$32.56^{2}{}_{a}$ ±0.53	$27.18^{1}{}_{b}$ ±1.45
	21	$29.04^{3}{}_{a}$ ±0.61	$26.65^{1}{}_{b}$ ±2.31
	120	$27.66^{3}{}_{a}$ ±0.97	$25.53^{1}{}_{b}$ ±0.95
NH_3-N (g/kg N)	0	$34.97^{1,2}{}_{a}$ ±0.39	$21.77^{2}{}_{b}$ ±1.29
	7	$31.60^{1,2}{}_{a}$ ±0.77	$30.98^{2}{}_{a}$ ±0.40
	21	$42.28^{1}{}_{a}$ ±2.61	$30.27^{2}{}_{a}$ ±0.79
	120	$26.32^{2}{}_{b}$ ±0.66	$113.32^{1}{}_{a}$ ±17.00

The PM silage underwent secondary fermentation between day 21 and day 120, as the pH value of the silage increased between day 7 and day 21, and between day 21 and day 120. This secondary fermentation could be result of clostridia growth as the initial DM concentration of the PM silage was below the 30% as suggested by Woolford (1998) where clostridial growth could be inhibited due to a lack of moisture. This resulted in a decrease in the lactic acid concentration of the silage between day 7 and day 120, and a dramatic increase in the NH_3-N concentration between day 21 and day 120. The NH_3-N concentration of the PM silage on day 120 (113.32 g/kg N) was higher than the

recommended value of 110 g/kg N by McDonald *et al.* (1991) and is in the range given by Wilkinson (1988) and Orr & Treacher (1990) for "satisfactorily fermented" silages. Thus, the SM silage was of better quality, having a lower pH value, higher lactic acid concentration and a lower NH_3-N concentration compared to PM silage on day 120.

5.5 *D. eriantha* vs *P. maximum*, ensiled directly at a late growth stage, with sugar addition

The average DM concentration of the *D. eriantha* grass ensiled at a late growth stage was 25.0% compared to 23.2% for the *P. maximum* grass ensiled at a late growth stage. These values are below the 30% DM concentration, where clostridia growth would be inhibited by moisture availability (Woolford, 1998).

5.5.1 Silage pH and buffering capacity

On day 0, the SL silage had a significantly lower pH value compared to the PL silage (Table 5.13). On day 7, the SL silage had a significantly higher pH value compared to the PL silage.

Table 5.13 The pH and buffering capacity values of *D. eriantha* and *P. maximum* grass ensiled directly at a late growth stage, with sugar addition

Parameter	Day	SL D+S		PL D+S	
pH	0	$4.70^{1}{}_{b}$	±0.03	$4.81^{2}{}_{a}$	±0.03
	7	$4.08^{2}{}_{a}$	±0.02	$3.95^{3}{}_{b}$	±0.02
	21	$3.81^{2}{}_{b}$	±0.03	$3.95^{3}{}_{a}$	±0.04
	120	$4.62^{1}{}_{b}$	±0.04	$5.04^{1}{}_{a}$	±0.05
Buffering capacity (meq/100g DM)	0	44.69_{b}	±0.26	63.21_{a}	±1.78

On day 21, the SL silage had a significantly lower pH value compared to that of the PL silage. Between day 21 and 120 the pH values of the SL and PL silages increased significantly. The pH value of the PL silage was significantly higher than that of the SL silage on day 120.

The buffering capacity value of the SL silage (44.69) was significantly lower than that of the PL silage (63.21) on day 0. Due to the higher buffering capacity of the PL silage, more lactic and acetic acid molecules, would have to be produced to bring the silage pH down and preserve the plant material (McDonald *et al.*, 1991).

5.5.2 Lactic acid and acetic acid

There were no significant differences in the lactic acid concentrations of the SL and PL silages on day 0 (Table 5.14).

Table 5.14 The lactic and acetic acid concentrations of *D. eriantha* and *P. maximum* grass ensiled directly at a late growth stage, with sugar addition

Parameter	Day	SL D+S	PL D+S
Lactic acid (g/kg DM)	0	2.27^{3}_{a} ±1.07	2.44^{4}_{a} ±0.84
	7	31.85^{2}_{b} ±2.38	37.75^{2}_{a} ±4.36
	21	37.29^{1}_{b} ±1.33	56.60^{1}_{a} ±1.42
	120	2.37^{3}_{b} ±0.06	20.70^{3}_{a} ±0.20
Acetic acid (g/kg DM)	0	2.78^{2}_{a} ±1.83	3.71^{2}_{a} ±0.20
	7	$6.32^{1,2}_{a}$ ±0.11	6.17^{2}_{a} ±1.64
	21	6.72^{1}_{b} ±1.23	13.13^{1}_{a} ±0.78
	120	8.24^{1}_{b} ±0.07	14.69^{1}_{a} ±0.37

On day 7 and day 21 the PL silages had significantly higher lactic acid concentrations compared to the SL silages. There was a significant decrease in the lactic acid concentrations of the SL and PL silages between day 21 and day 120. The PL silage had a significantly higher lactic acid concentration on day 120 compared to the SL silage.

On day 0 and day 7 there were no significant differences in the acetic acid concentration of the SL and PL silages. On day 21 and day 120 the PL silages had a significantly higher acetic acid concentration compared to the SL silages.

5.5.3 Nitrogen and ammonia-nitrogen

On day 0, day 21 and day 120 the SL silages had a significantly higher N concentration compared to the PL silages (Table 5.15). On day 7 there was a non significant difference in the N concentration of the SL and PL silages.

Table 5.15 The N and NH_3-N concentrations of *D. eriantha* and *P. maximum* grass ensiled directly at a late growth stage, with sugar addition

Parameter	Day	SL D+S	PL D+S
N (g/kg DM)	0	25.16^{1}_{a} ±0.13	20.84^{1}_{b} ±0.07
	7	20.79^{2}_{a} ±2.87	$18.73^{1,2}_{a}$ ±1.12
	21	$22.66^{1,2}_{a}$ ±1.76	17.90^{2}_{b} ±0.32
	120	24.23^{1}_{a} ±0.72	$19.22^{1,2}_{b}$ ±2.70
NH_3-N (g/kg N)	0	35.57^{2}_{a} ±0.88	45.42^{2}_{a} ±1.86
	7	45.77^{2}_{a} ±1.07	34.60^{2}_{a} ±2.10
	21	38.41^{2}_{a} ±0.90	32.81^{2}_{a} ±0.80
	120	108.93^{1}_{a} ±11.88	91.75^{1}_{b} ±50.13

On day 0, day 7 and day 21 there were no significant differences in the NH_3-N concentration of the SL and PL silages. On day 120, the SL silage (108.93 g/kg N) had a significantly higher NH_3-N concentration compared to the PL silage (91.75 g/kg N). In this regard Wilkinson (1988) and Orr & Treacher (1990) reported that the SL silage could be classified as a "satisfactorily fermented" silage, while the PL silage could be classified as a "well-fermented" silage. The NH_3-N values obtained for the SL and PL silages are higher than the value reported by Meeske *et al.* (1999) of 50.3 NH_3-N g/kg TN after ensiling *D. eriantha* for 44 days.

The pH of the SL and PL silages, were below the 4.2, used by McDonald *et al.* (1991) as an indication of a good quality silage. This decrease in the pH occurred within the first seven days of ensiling, thus the rate of pH drop was fast (Woolford, 1998). In both the SL and PL silages, there was a high production of lactic acid within the first seven days. The lactic acid concentrations of both the silages were above 30 g/kg DM on day 7, which was comparable to those obtained by Meeske *et al.* (1999) for inoculated silage.

In the SL and PL silages, a significant change in quality occurred between day 21 and day 120 of ensiling. There was an increase in the silage pH, a decrease in the lactic acid concentration and an increase in the NH_3-N concentration of the two silages. According to the literature (McDonald *et al.*, 1991; Woolford, 1998), the fermentation process, which occurred between day 0 and day 7, should have resulted in a stable silage, which was not the case in both the SL and PL silages.

According to Woolford (1998), in most circumstances, clostridia growth is inhibited at a pH below 5.0. But according to Jonsson (1991), a certain clostridia species can still grow within a pH range of 4.4 to 4.9. The other factor that could lead to clostridia growth, is that the DM concentration of these silages were below 30%, quoted by Woolford (1998), as being the value above which clostridia growth could be inhibited by the moisture concentration of the plant material. These factors could thus have lead to some clostridia growth in the early stages of ensiling, leading to a secondary fermentation occurring in the latter stages of ensiling. This could be an explanation for the dramatic decrease in

silage quality of the SL and PL silages between day 21 and day 120. The other possible explanation is that due to an experimental fault, the bottles were not properly sealed, leading to infiltration of oxygen, which could also have caused this decrease in silage quality.

5.6 *D. eriantha* vs *P. maximum*, ensiled at a late growth stage, with prior wilting and sugar addition

The average DM concentration of the *D. eriantha* grass ensiled at a late growth stage with prior wilting was 29.6%, while that of the *P. maximum* grass ensiled at a late growth stage was 25.6%.

5.6.1 Silage pH and buffering capacity

On day 0 and day 7, the pH values of the SL silages were significantly higher than that of the PL silages (Table 5.16).

Table 5.16 The pH and buffering capacity values of *D. eriantha* and *P. maximum* grass ensiled at a late growth stage, with prior wilting and sugar addition

Parameter	Day	SL W+S		PL W+S	
pH	0	4.79^{2}_{a}	±0.02	4.67^{1}_{b}	±0.01
	7	5.17^{1}_{a}	±0.23	4.42^{2}_{b}	±0.03
	21	4.32^{4}_{a}	±0.01	4.32^{2}_{a}	±0.02
	120	4.61^{3}_{a}	±0.02	4.33^{2}_{b}	±0.05
Buffering capacity (meq/100g DM)	0	44.89_{b}	±0.83	72.93_{a}	±0.05

On day 21 the SL silage and PL silage had the same pH value. On day 120 the pH value of the SL silage was significantly higher than that of the PL silage. On day 0, the SL silage had a significantly lower buffering capacity value than the PL silage.

5.6.2 Lactic acid and acetic acid

There were no significant differences in the lactic acid concentration of the SL and PL silages on day 0 (Table 5.17). On day 7, day 21 and day 120 the PL silages had significantly higher lactic acid concentrations compared to the SL silages. There were no significant differences between the acetic acid concentrations of the SL and PL silages on day 0, day 7, day 21 and day 120.

Table 5.17 The lactic and acetic acid concentrations of *D. eriantha* and *P. maximum* grass ensiled at a late growth stage, with prior wilting and sugar addition

Parameter	Day	SL W+S	PL W+S
Lactic acid (g/kg DM)	0	2.82^{2}_{a} ±0.07	2.38^{3}_{a} ±0.82
	7	7.48^{2}_{b} ±0.36	16.32^{2}_{a} ±0.40
	21	17.40^{1}_{b} ±0.45	26.02^{1}_{a} ±3.02
	120	18.53^{1}_{b} ±2.73	25.41^{1}_{a} ±1.42
Acetic acid (g/kg DM)	0	$5.18^{2,3}_{a}$ ±0.13	2.66^{2}_{a} ±1.26
	7	3.41^{3}_{a} ±0.98	$5.57^{1,2}_{a}$ ±0.83
	21	$8.33^{1,2}_{a}$ ±2.26	7.84^{1}_{a} ±0.97
	120	9.11^{1}_{a} ±1.04	8.04^{1}_{a} ±0.99

The lactic acid concentration of the SL and PL silages on day 120 are lower than those of the inoculated and higher than that for the control *D. eriantha* silages obtained by Meeske

et al. (1999). The acetic acid concentration of the SL and PL silages on day 120 are comparable to those obtained by Meeske *et al.* (1999), for the inoculated and control *D. eriantha* silages.

5.6.3 Nitrogen and ammonia-nitrogen

On day 0, day 21 and day 120 the N concentration of the SL silages were significantly higher than that of the PL silages (Table 5.18). There was no significant difference in the N concentration of the SL and PL silages on day 7.

Table 5.18 The N and NH_3-N concentrations of *D. eriantha* and *P. maximum* grass ensiled at a late growth stage, with prior wilting and sugar addition

Parameter	Day	SL W+S	PL W+S
N (g/kg DM)	0	25.23^{1}_{a} ±1.24	$17.51^{1,2}_{b}$ ±1.36
	7	19.20^{3}_{a} ±2.68	$18.36^{1,2}_{a}$ ±0.87
	21	$21.63^{2,3}_{a}$ ±1.31	19.41^{1}_{b} ±0.49
	120	21.83^{2}_{a} ±4.52	16.55^{2}_{b} ±0.57
NH_3-N (g/kg N)	0	43.53^{2}_{a} ±5.72	46.44^{1}_{a} ±2.44
	7	$48.44^{1,2}_{a}$ ±15.01	44.22^{1}_{a} ±2.33
	21	62.05^{1}_{a} ±4.14	34.64^{1}_{b} ±2.07
	120	60.13^{1}_{a} ±1.42	34.53^{1}_{b} ±0.82

There were no significant differences in the NH_3-N values of the SL and PL silages on day 0 and day 7. On day 21 and day 120, the SL silages had significantly higher NH_3-N values compared to the PL silages. According to the NH_3-N concentration of the SL and PL silages on day 120, they can be classified as "well-fermented' silages according to Wilkinson (1988) and Orr & Treacher (1990).

The pH of the SL silage was unstable as it increased between day 0 and day 7, decreased between day 7 and day 21 and then increased between day 21 and day 120. The rate of pH drop in the PL silage was slow, but the production of lactic acid in this silage was faster than in the SL silage, between day 0 and day 7. The DM concentration of the SL silage was higher than that of the PL silage and this could have inhibited some clostridia growth (Woolford, 1998). In contrast, due to the higher pH value of the SL silage clostridia growth could have been stimulated (McDonald *et al.*, 1991; Woolford, 1998). This could have lead to some clostridia growth occurring, leading to a degree of secondary fermentation taking place. This could have resulted in a higher NH_3-N concentration in the SL silage compared to the PL silage as this was observed on day 120 after ensiling.

To conclude the PL silage can be classified as a better quality, due to a lower pH value and a lower NH_3-N concentration and a higher lactic acid concentration on day 120, compared to the SL silage.

CHAPTER 6

Results and discussion

Partial digestibility trial

Silage was made from the medium and late growth stages of the *P. maximum* and *D. eriantha* grasses. The plant material was wilted before ensiling and sugar (molasses) was added during ensiling. The material was wilted to a DM concentration of ±30%. According to Woolford (1998), clostridia growth can be inhibited, due to moisture availability, if the DM concentration of the plant material is above 30%. Comparison was made between the two growth stages and the two plant species by means of a partial digestibility study.

The treatments were as follows,

PM - *P. maximum*, medium growth stage
PL - *P. maximum*, late growth stage
SM - *D. eriantha*, medium growth stage
SL - *D. eriantha*, late growth stage

6.1 Silage quality parameters

In Table 6.1 the quality of the *P. maximum* and *D. eriantha* silages fed to the fistulated sheep, during the partial digestibility study, is presented. There was a significant difference in the DM concentration of the silages, except for the PM and SL silages which did not differ significantly.

The DM concentration of the forage at feeding plays a key role, both in intake and production, although the direct effect of DM on intake in grass silages seems small. Zimmer & Wilkens (1984) observed no effect of DM concentration on intake when silages were finely chopped and preserved. Maximum DM intake occurred at a DM concentration of about 500 g/kg and a DM concentration below 250 g/kg DM resulted in changes in silage fermentation characteristics (Ingvartsen, 1992). The DM concentrations

in the present study are in line according to the literature and most probably did not have any negative effects on intake and silage quality.

Table 6.1 The quality of the *P. maximum* and *D. eriantha* silages fed to sheep

Parameter		PM	PL	SM	SL	SE_m
DM	(g/kg)	362.5^{a}	350.8^{b}	299.8^{c}	360.4^{a}	2.8
OM	(g/kg DM)	853.4^{c}	839.2^{d}	879.1^{b}	892.1^{a}	2.7
N	(g/kg DM)	18.6^{b}	24.0^{a}	23.9^{a}	22.3^{a}	1.3
pH		4.5^{ab}	4.7^{a}	4.2^{c}	4.4^{bc}	0.4
Ammonia-N	(g/kg N)	38.1^{a}	49.6^{a}	46.8^{a}	36.2^{a}	4.3
Lactic acid	(g/kg DM)	16.2^{a}	12.6^{a}	13.0^{a}	24.1^{a}	3.4
Acetic acid	(g/kg DM)	7.0^{a}	13.7^{a}	2.6^{a}	9.3^{a}	2.5
Propionic acid	(g/kg DM)	0.14^{b}	0.23^{ab}	0.08^{b}	0.38^{a}	0.4
N Butyric acid	(g/kg DM)	0.20^{a}	0.06^{a}	0.07^{a}	0.23^{a}	0.4
Valeric acid	(g/kg DM)	0.19^{a}	0.23^{a}	0.04^{a}	0.32^{a}	0.5
Tot VFA	(g/kg DM)	7.5^{a}	14.2^{a}	2.8^{a}	10.3^{a}	2.5

The following information will apply to all the tables in Chapter 6.
1: Values with different subscripts (a,b,c,d) across a row differ significantly at $P \leq 0.05$.
2: SE_m – Standard error of the mean.

The OM concentration of the four silages differed significantly from each other, with the SL silage having the highest OM concentration and the PL silage the lowest. There was no significant difference in the N concentration of the PL, SM and SL silages. These silages had a significantly higher N concentration compared to the PM silage.

The PL silage had a significantly higher pH value compared to the SM and SL silages. The pH value of the PM silage did not differ significantly from that of the PL and SL silages, but was significantly higher than that of the SM silage. The pH value of the SM and SL silage did not differ significantly. These pH values are higher than the recommended 4.20 for good quality unwilted silage (McDonald *et al.*, 1991). However,

the higher osmotic pressure associated in general with wilting can inhibit clostridial growth, so that a stable wilted silage can be achieved at pH values of 5 and above (McDonald *et al.*, 1991). This does not, however, mean that pH values of wilted silages are always high (Marsh, 1979). The pH values of the four silages are thus acceptable, according to the literature.

There were no significant differences in the NH_3-N concentration of the four silages, with the PL silage having the highest concentration and the SL silage having the lowest concentration. According to the NH_3-N concentration of the silages they can be classified as "well-fermented' silages, with NH_3-N values less than the 50 to 100 g/kg N as quoted by Wilkinson (1988) and Orr & Treacher (1990) for "well-fermented" silages. The NH_3-N values obtained for the PM, PL, SM and SL silages are lower than the value reported by Meeske *et al.* (1999) of 50.3 NH_3-N g/kg TN for *D. eriantha* after 44 days of ensiling.

There were no significant differences in the lactic, acetic, N-butyric and valeric acid concentrations of the silages fed to the different treatment groups of animals. The PM, SM and SL silages had higher lactic acid concentrations compared to acetic acid concentrations, while the PL silage had a higher acetic acid concentration than a lactic acid concentration. The higher acetic acid concentration of the PL silage indicated a fermentation process favouring the production of acetic acid (McDonald *et al.*, 1991; Woolford, 1998), and could explain the higher pH value of this silage compared to the other silages, as acetic acid is a weaker acid than lactic acid.

There was a significant difference in the propionic acid concentration of the four silages, with the SL silage having a significantly higher propionic acid concentration compared to the PM and SM silages. There was no significant differences in the propionic acid concentration of the PM, PL and SM silages. The total VFA concentration of the four silages did not differ significantly.

6.2 Rumen parameters

The rumen pH values varied between 6.27 and 6.81 (Table 6.2). The sheep fed the *P. maximum* silages had significantly higher rumen pH values compared to those animals on

the *D. eriantha* silages. The optimal pH for proteolytic activity in the rumen is suggested to be between 6 and 7 (Tamminga, 1979). According to Theron, Kistner & Kornelius (1982), pH levels less than 6.0 had an adverse effect on microbial growth. The production of VFA's was unaffected at a pH of between 6.2 and 6.8, but acetate production was inhibited below a pH of 6.2 due to a decrease in the activity of cellulolytic bacteria (Esdale & Satter, 1972). The rumen pH levels of this study were thus in the range of a normal rumen environment.

Table 6.2 Rumen parameters of sheep fed the *P. maximum* and *D. eriantha* silages

Parameter	PM	PL	SM	SL	SE_m
Rumen pH	6.66^{a}	6.81^{a}	6.27^{b}	6.35^{b}	0.4
Rumen NH_3-N (mg/100ml)	15.6^{c}	20.0^{a}	16.4^{bc}	19.5^{ab}	1.5
Acetic acid (mmol/100ml)	9.7^{ab}	9.6^{ab}	8.1^{b}	10.4^{a}	1.0
Propionic acid (mmol/100ml)	2.4^{ab}	2.4^{ab}	2.0^{b}	2.5^{a}	0.6
Butyric acid (mmol/100ml)	0.75^{b}	0.68^{b}	0.77^{b}	0.98^{a}	0.4
Total VFA (mmol/100ml)	13.1^{ab}	13.0^{ab}	11.2^{b}	14.2^{a}	1.2
*A / P	4.0^{a}	4.0^{a}	4.1^{a}	4.1^{a}	0.6

*A / P = acetic and propionic acid ratio

The rumen NH_3-N concentration of the animals on the PL and the SL silages were significantly higher than that of the animals on the PM silage, with no significant difference in the values for the animals on PL, SM and SL silages. The lower N concentration of the PM silage most probably resulted in a lower NH_3-N concentration in the rumen of the animals on this diet.

Minimum levels of NH_3-N in the rumen for maximum microbial activity have been estimated at values from 4.2 to 290 mg/l rumen fluid (Hespell, 1979). The wide range may be attributed to different methodologies, different substrates and different types of

microbes which may require different concentrations of NH_3-N to maximise microbial yield (Orskov, 1982). According to Satter & Roffler (1977), rumen activity can be inhibited if the rumen NH_3-N values drop below 5 mg/100 ml. If the rumen NH_3-N values are exceptionally high, above 20 mg/100 ml, a negative effect can be obtained with regards to animal performance, especially with fast growing animals if available energy is limited (Van Niekerk, 1997). The reason for this is that only the feed protein degraded in the rumen, which is incorporated into microbial protein and rumen undegraded protein, can be utilised in the lower digestive tract. The rest of the NH_3-N will be absorbed into the blood stream and converted to urea, which will be excreted. Significant amounts of energy, that could otherwise have been utilised for productive purposes, are lost during this process (Satter & Roffler, 1977).

According to Wallace (1979), most of the results indicated that rumen NH_3-N levels should lie between 2 and 20 mg/100 ml rumen fluid for normal rumen function. This can vary according to the microflora present in the rumen. This is in accordance with the values recommended by Losada *et. al.*, 1982, as quoted by Minson (1990). The rumen NH_3-N levels in this experiment were thus sufficient to support maximum microbial activity in the rumen.

Acetic, propionic and butyric acid are the main end products of anaerobic fermentation within the rumen. This is the main form in which energy from plant material (silages and pastures) can be absorbed. The rumen acetic acid concentration was significantly higher on the SL diet compared to the SM diet. The PM and PL diets did not differ significantly in rumen acetic acid concentrations compared to the SM and SL diets. The rumen propionic acid concentration of the animals on the SL diet was significantly higher than that of the animals on the SM diet, with the PM and PL diets not being significantly different from the SM and SL diets.

The animals on the SL diet had significantly higher rumen butyric acid concentrations compared to those on the SM, PM and PL diets, with the PM, PL and SM diets not significantly different from each other. The concentration of acetic and propionic acid in

the rumen was higher compared to the values obtained by Paulsmeier (1987) for *D. eriantha* grass and are similar to the values obtained by Van Niekerk (1997) for *D. eriantha* pastures.

The total production of VFA's, was significantly higher in the animals on the SL diet compared to those on the SM diet, with the PM and PL diets not being significantly different than those of the SM and SL diets. The VFA production in the present study was comparable to data obtained by Paulsmeier (1987) for *D. eriantha* pasture in spring. The values are higher than those obtained by Van Niekerk (1997) for foggage of *D. eriantha*, similar to that for *P. maximum* foggage and *D. eriantha* pastures. However, the values in the present study are lower than those obtained by De Bruyn (1995) for Bana Grass, Greengold and Pennaris, and Acheampong-Boateng (1991) for lucerne.

When comparing the values of the different VFA in the rumen it is important to look at the molar ratio between acetic acid, propionic acid and butyric acid. This will give an indication of possible differences in the production of a specific VFA compared to another. The molar ratio of acetic : propionic : butyric acid in the rumen were as follows for the different diets:

PM diet	0.75	:	0.19	:	0.06
PL diet	0.75	:	0.19	:	0.06
SM diet	0.75	:	0.18	:	0.07
SL diet	0.75	:	0.19	:	0.07

There were no major differences in the molar ratio between the three VFA. The above molar ratios were comparable to data presented by McDonald *et al.* (1990) for cattle feeding on grass silage. In studies where different proportions of these three VFA were used, a decrease was observed in the efficiency of metabolisable energy utilisation for animal performance as the proportion of propionic acid decreased (Hovell & Greenhalgh, 1978). The efficiency of VFA utilisation in this study could have been similar between the four treatments. There were no significant differences in the acetic: propionic acid ratio, on the different diets. The *P. maximum* diets tended to have lower values compared

to those on the *D. eriantha* diets. The lower this ratio, the more propionic acid is produced compared to acetic acid. If the proportion propionic acid decreases it could have a negative effect on the efficiency of ME utilisation (Hovell & Greenhalgh, 1978).

6.3 Intake, true digesta flow and partial digestibility of organic matter in the digestive tract

There was a significant difference in the organic matter intake (**OMI**) of the animals on the PM silages compared to those on the SL silages, with OMI on the PL and SM silages not being significantly different than that of the other two silages (Table 6.3). The OMI was higher on the late growth stage silages compared to the medium growth stage silages.

Table 6.3 Partial digestibility of organic matter of sheep fed the *P. maximum* and *D. eriantha* silages

Parameter	PM	PL	SM	SL	SE_m
OMI (g/d)	977^{b}	1086^{ab}	978^{ab}	1194^{a}	12.4
DOMI (g/kg $W^{0.75}$/d)	33.2^{a}	33.9^{a}	32.2^{a}	38.9^{a}	2.2
Digesta flow (l/d):					
Abomasum	24.3^{a}	19.2^{a}	19.4^{a}	19.1^{a}	2.2
Ileum	7.1^{a}	5.8^{a}	6.7^{a}	5.6^{a}	1.2
OM-disappearance					
1) Rumen (g/d)	475^{a}	599^{a}	499^{a}	615^{a}	11.6
1a) % of OMI	48^{a}	55^{a}	51^{a}	51^{a}	3.2
1b) % of OM-digested	65^{a}	78^{a}	69^{a}	68^{a}	3.6
2) Small Intestine (g/d)	107^{a}	158^{a}	107^{a}	204^{a}	9.1
2a) % of OMI	11^{a}	15^{a}	11^{a}	17^{a}	2.8
3) Total *GIT (g/d)	729^{b}	772^{ab}	715^{b}	895^{a}	10.6
3a) % of OMI	74^{a}	72^{a}	74^{a}	75^{a}	2.4

* GIT – Gastrointestinal tract

When the OMI was expressed as digestible organic matter intake (**DOMI**) as a proportion of body weight ($W^{0.75}$), no significant differences were found between the four diets. The DOMI (g/kg $W^{0.75}$/d) value for the SL silage was similar to the values obtained by Meeske *et al.* (1999) for silage made from *D. eriantha* grass. The values in the present study for *P. maximum* and *D. eriantha* were comparable to data obtained by Van Niekerk (1997) on pastures during the summer months, lower than that on pastures during the spring months, but are higher than the values obtained with foggages.

The generally lower voluntary intake of grass silages compared with hay prepared from the same fresh forage or with the fresh forage itself, is mainly attributed to fermentation end products present in the silage. This reduction in intake varies widely (ranging from 0 to 64%) and is related to the quantities of the fermentation products in the silage, which vary with the method of preservation (Donaldson & Edwards, 1976; Thiago *et al.*, 1992; Dermarquilly 1973 as cited by Van Niekerk, 1997). Rumen fill appears to be of minor importance in controlling silage intake, because rumen DM concentrations remains lower when the animals are fed on silages compared with hays prepared from the same original herbage (Thiago & Gill, 1986; Chiofalo, Dulphy & Baumont, 1992). Relationships between silage constituents and intake suggest that NH_3-N is one of the factors responsible for reduction in intake of poor-quality silages (Wilkins *et al.*, 1971; Dulphy & Michalet-Doreau, 1981 as cited by Van Niekerk, 1997). The low levels of NH_3-N in the present study could not have had a negative effect on normal rumen function, as these values lie within the range of 2 to 20 mg/ 100 ml (Wallace, 1979). Thus as normal rumen function was not impaired, rumen NH_3-N concentration could possibly not have had a negative effect on silage intake in this study.

The digesta flow in the abomasum and in the ileum of the animals did not differ significantly between the four treatments. The digesta flow of the animals on the PM diet, was numerically higher, compared to the animals on the other three diets. The digesta flow in the abomasum in the present study is higher than that which was obtained by Paulsmeier (1987), De Bruyn (1995) and Van Niekerk (1997). A factor that could have contributed to this is the fact that these studies were done on fresh material and not on

silages. The values for the digesta flow in the ileum were very similar between the present study and the above three mentioned studies.

There were no significant differences in the OM disappearance in the rumen between the four treatments. The animals on the late growth stage silage diets, tended to have a higher OM disappearance in the rumen compared to the animals on the medium growth stage silage diets. This is a result of the higher intake of OM on these diets. When the disappearance of OM was expressed as a percentage of OMI, and as a percentage of OM digested, no significant differences were found between the four diet treatments. The PL diet tended to result in a higher percentage value compared to the other diets. The values of OM disappearance as a percentage of OMI, obtained in this study were higher than those obtained by Van Niekerk (1997) and tended to be slightly higher than those obtained by De Bruyn (1995). When the disappearance of OM is expressed as a percentage of OM digested, the results of the present study are within the range obtained by Van Niekerk (1997).

The disappearance of OM in the small intestine (g/d) did not differ significantly between the four treatments. The animals on the late growth stage silage diets tended to have a higher OM disappearance (g/d) compared to the animals on the medium growth stage diets. If the OM disappearance is expressed as a percentage of OMI, no significant differences are observed between the four diet treatments. The late growth stage diets tend to have higher percentage values compared to the medium growth stage diets. The data obtained in the present study, for OM disappearance expressed as a percentage of OMI, falls within the range obtained by Van Niekerk (1997) for hays and growing pastures. The hays had lower values and the growing pastures had higher values compared to the present study.

There was a significant difference in the total OM disappearance (g/d) over the whole gastrointestinal tract (GIT tract). The animals on the SL silage had a significantly higher value compared to the animals on PM and SM silages, with the values for the animals on the PL silage not being significantly different from the animals on the other three silages.

This is a reflection of OM intake. When these values were expressed as a percentage of OMI, no significant differences were observed between the four diet treatments. The total OM disappearance across the GIT tract, expressed as a percentage of OMI, was higher in the present study compared to that, which was reported by Van Niekerk (1997), except for those of the *P. maximum* pasture during the spring period and similar to that obtained by De Bruyn (1995), for Bana, Greengold and Panaris grass.

6.4 Partial digestibility of nitrogen in the digestive tract

The N intake (g/d) was significantly higher in the animals on the PL and SL silages compared to the animals on the PM silage, with the values on the SM silage not being significantly different from that of the other three treatments (Table 6.4). The variation in N intake between the treatments is a reflection of the differences in OM intake and N concentration of the four silages.

In the abomasum, the total N-flow was significantly higher on the SL diet compared to that of the PM and PL diets. The total N-flow on SM diet did not differ significantly from the other three diets. The higher digesta flow in the abomasum and low N-intake on the PM diet resulted in a total N-flow in the abomasum, which was not significantly different from that of the PL and SM diets.

There were no significant differences in the NH_3-N-flow in the abomasum when comparing the four different diets. The medium diets tended to have lower NH_3-N-flows in the abomasum compared to the late diets. According to Corbett (1987), up to 20% of the N reaching the duodenum may consist of NH_3-N. In the present study the amount of N in the form of NH_3-N, as a proportion of N-intake is less than $\pm$10% for the four different diets. The rest of the N reaching the duodenum consists of protein and nucleic acids (Lindsay & Armstrong, 1982) and a small endogenous fraction (Corbett, 1987). The protein fraction comes from plant proteins and microbial protein synthesis (Buttery & Lewis, 1982),

Table 6.4 Partial digestibility of nitrogen of sheep fed the *P. maximum* and *D. eriantha* silages

Parameter	PM	PL	SM	SL	SE_m
N-intake (g/d)	20.0^{b}	28.1^{a}	25.5^{ab}	27.4^{a}	2.0
1. Abomasum:					
1a) Digesta flow (l/d)	24.3^{a}	19.2^{a}	19.4^{a}	19.1^{a}	2.2
1b) Total N-flow (g/d)	19.5^{b}	20.5^{b}	21.0^{ab}	27.5^{a}	2.2
1c) NH_3-N-flow (g/d)	2.1^{a}	1.9^{a}	2.3^{a}	1.9^{a}	0.8
1d) NAN-flow (g/d)	17.4^{b}	18.6^{b}	18.7^{ab}	25.6^{a}	2.2
1e) NAN-flow/N-intake	0.87^{ab}	0.66^{b}	0.74^{ab}	0.95^{a}	0.04
2. Ileum:					
2a) Digesta flow (l/d)	7.1^{a}	5.8^{a}	6.7^{a}	5.6^{a}	1.2
2b) Total N-flow (g/d)	8.5^{a}	7.7^{a}	8.9^{a}	10.2^{a}	1.5
2c) NH_3-N-flow (g/d)	0.11^{b}	0.10^{b}	0.22^{a}	0.10^{b}	0.2
2d) NAN-flow (g/d)	8.4^{a}	7.6^{a}	8.7^{a}	10.1^{a}	1.4
3. NAN-disappearance (g/d)	9.0^{b}	11.0^{b}	10.1^{b}	15.4^{a}	1.8
4. NAN-disappearance (% of N-intake)	45^{ab}	39^{b}	40^{b}	58^{a}	3.7
5. NAN-digestibility (%)	52^{a}	59^{a}	54^{a}	60^{a}	2.6
6. Faeces NDF-N (g/d)	2.51^{b}	3.05^{ab}	3.55^{ab}	3.74^{a}	0.9
7. NDF-N (% of N-intake)	19^{a}	19^{a}	20^{a}	19^{a}	2.1
9. Metabolic Faecal –N (g/d)	2.63^{b}	3.95^{ab}	2.64^{ab}	4.35^{a}	1.1
10. True N-digested (%)	88^{a}	89^{a}	86^{a}	86^{a}	1.6

The flow of non-ammonia nitrogen (NAN) in the abomasum was significantly higher on the SL diet compared to that on the PM and the PL diets. The flow of NAN in the abomasum on the SM diet did not differ significantly from the other three diet treatments. The higher NAN flow in the abomasum, would indicate a more efficient production of microbial protein and this could lead to an increase in intake, with a further positive

effect on animal performance (Van Niekerk, 1997). Site of cannulation could have played a role here – abomasal cannulas led to larger estimates of variation than use of duodenal cannulas. It is more difficult to obtain representative samples from a collection pool (abomasum) than from a partition with tubular flow (duodenum). This is applicable to all calculations (Titgemeyer, 1997).

When expressing the NAN flow in the abomasum as a proportion of N-intake, the following was observed. The value for the animals on the SL diet was significantly higher than that for the animals on the PL diet, with the values for the PM and SM diets not being significantly different from the other two diets. The higher this value, the more N is present in plant protein or a higher total microbial protein production and the lower the amount of NPN. A possible explanation for the low value on the PL diet could be as a result of the higher NH_3-N concentration (49.55 g/kg N) of the silage fed to the animals compared to the other diet (McDonald *et al.*, 1991).

There were no significant differences in the total N-flow in the ileum of the animals between the four diet treatments. The NH_3-N-flow in the ileum was significantly higher on the SM diet compared to that on the PM, PL and SL diets. There were no significant differences in the ileum NAN-flow between the four diet treatments, with the value for the SL diet being higher than that for the other diets.

The total disappearance of NAN through the whole GIT tract was significantly higher on the SL diet compared to the PM, PL and SM diets. The values for the PM, PL and SM diets did not differ significantly. The higher NAN disappearance observed on the SL diet could have a direct positive effect on animal performance, utilising this diet. This can lead to higher intakes due to an increase in amino acid absorption (Leng, 1981), as a possible result of an increase in growth hormone secretion (Oldham, 1980). This might further lead to an increase in the efficiency of utilisation of absorbed amino acids (Bines, Hart & Morant, 1980). Unfortunately, the SL diet did lead to a significantly higher intake of DOMI / $W^{0.75}$ compared to the other diets, but the OMI value of the SL diet was significantly higher than the other silages. Inadequate number of replications could have

affected these results. Flow studies may be ineffective unless large numbers of replications are used, even more so for N flow studies (Titgemeyer, 1997).

When these values were expressed as a percentage of N-intake, the value was significantly higher for the SL diet compared to the PL diet. These values indicate an improvement in silage nitrogen utilisation on the SL diet compared to the PL diet. The values for the PM and the SM diets did not differ significantly from those for the SL and PL diets. The NAN digestibility did not differ significantly between the four diet treatments, with the late growth stages having higher values than the medium growth stages. The biggest difference was between the SL and PM diets.

The N concentration of the faeces consists of two components, undigested N from the feed (NDF-N) and a metabolic component which embraces endogenous secretions from the gut in addition to varying amounts of microbial N from the large intestine. Mycopolysaccharides are secreted in various sites in the gut and reach the fermentation site. This material contains glucosamine, which liberates some ammonia upon fermentation and might contribute to the microbial component of metabolic faecal nitrogen (**MFN**). MFN is important in so far as it enables one to estimate the true digestibility of feed N (Paulsmeier, 1987).

The amount of N bound to the NDF fraction (**NDF-N**) in the faeces is significantly lower for the animals on the PM diet compared to the animals on the SL diet. The values for the PL and SM diets did not differ significantly from those of the PM and SL diets. The difference in these values, reflect differences in N concentration of the silages and the variation in OM intake between the different treatments. The *D. eriantha* silages tended to result in higher NDF-N concentrations in the faeces, compared to the *P. maximum* silages, this indicated that a portion of the N was bound in the lignin fraction, which is not available to the animal (McDonald *et al.*, 1990). Thus the N in the *P. maximum* silages could have been more available than the N in the *D. eriantha* silages. There is no significant difference in the NDF-N as a percentage of N-intake, between the four different diet treatments.

There was a significantly higher excretion of metabolic faecal N by the animals on the SL diet compared to the animals on the PM diet. The PL and SM diets did not differ significantly from the PM and SL diets. The late growth stage diets tended to lead to higher excretions of metabolic faecal N compared to the medium growth stage diets. If the metabolic faecal N was not taken into account the N digestibility of the four diets could possibly have been significantly different from each other. There was no significant difference in the true nitrogen digestibility between the four diet treatments. The values for the *P. maximum* silages tended to be higher than the values for the *D. eriantha* silages ($p>0.05$). Thus by taking the metabolic faecal N into account a true reflection was obtained for N digestibility as discussed by Paulsmeier (1987).

When comparing the four diets with regards to DOMI / $W^{0.75}$, OM-digestibility through the whole GIT and true N-digestibility, there are no significant differences. The PL and SL diets had tended to have higher DOMI / $W^{0.75}$ compared to the PM and SM diets ($P>0.05$). With regards to true N-digestibility, the *P. maximum* diets tended to have higher values than the *D. eriantha* diets ($P>0.05$). The animals on the SL diet tended to have higher N intake, total N flow, higher NAN flow, higher NAN flow / N intake and higher NAN disappearance in the small intestine, than the other treatments ($P>0.05$). This could indicate a better utilisation of silage N. A growth study would possibly be able to show if the differences observed in the partial digestibility of the silages could result in different growth rates of animals.

CHAPTER 7

Summary, recommendations and conclusions

7.1 Summary

The data obtained from the first part of this study indicated that the making of grass silage from *D. eriantha* and *P. maximum* is a viable option. A better quality silage could be described as having a low pH value (± 4.20), a low HN_3-N concentration (less 100 g/kg N) and a higher lactic acid concentration compared to acetic acid concentration. The latter is an indication of a fermentation pattern favouring lactic acid production and not acetic acid production.

1 In Chapter 3 the treatment differences within a specific growth stage and a specific grass specie was discussed. Silages were made from two grasses (*D. eriantha* and *P. maximum*), three growth stages (early, medium and late) and four treatments were applied at ensiling. The treatments were as follows, plant material ensiled directly (D), plant material ensiled directly with sugar addition (D+S), plant material ensiled with prior wilting (W) and plant material ensiled with prior wilting and sugar addition (W+S). The plant material of the early growth stages was only ensiled with sugar addition, either directly or with prior wilting of the plant material.

The D+S and W+S silages made from the *D. eriantha* grass ensiled at an early growth stage was classified as well-fermented silages, having low pH values, higher lactic acid concentrations compared to acetic acid concentrations and having NH_3-N concentrations less than 100 g/kg N.

The data obtained indicated that the medium growth stage *D. eriantha* grass could be ensiled successfully. The quality of the silage could be improved if sugar (molasses) was added to the grass material before ensiling. Despite the medium growth stages having a relatively low DM concentration, wilting before ensiling

did not improve the silage quality in terms of a lower pH value. If the grass were wilted before ensiling sugar addition would still improve the silage quality. The D+S and W+S silages were of better quality compared to the other two silages.

The data obtained for the grass silages made from the late growth stage of *D. eriantha* grass, indicated that a better quality silage was made from the W treatment. This silage had the lowest pH value, highest lactic and acetic acid concentrations of the four treatments. The NH_3-N concentration of the W silage was slightly higher than that of the W+S silage. It would seem that the direct ensilage of *D. eriantha* grass at a late growth stage with or without sugar addition lead to an unstable silage, which deteriorated between day 21 and day 120.

The ensiling of *P. maximum* grass at an early growth stage with the addition of sugar, either ensiled directly or wilted, resulted in better quality silage than the material that was ensiled without sugar addition. These silages had a low pH, high lactic acid concentrations and acceptable NH_3-N concentrations according to the literature.

The data obtained for the silage made from the medium growth stage of *P. maximum* grass, indicated that the D+S silage was of better quality compared to the other three silages. This silage had a lower pH value, higher lactic acid concentration and lower NH_3-N concentrations compared to the other three silages.

With the *P. maximum* grass ensiled at a late growth stage, the D and W+S silages were of better quality compared to that of the other two silages, having lower pH values, lower NH_3-N concentrations and higher lactic acid concentrations. The D+S and W silages were unstable and there was a decrease in silage quality between day 21 and day 120. This decrease in silage quality could also have been due to an experimental error occurring.

The data discussed in Chapter 3 indicated that the addition of sugar (molasses) to the plant material at ensiling does have a positive effect on the final silage quality. A positive effect, lower pH, lower NH_3-N concentration and higher lactic acid concentrations, were obtained with direct ensiling and with prior wilting of the plant material. The addition of sugar to the plant material ensiled at a late growth stage, tended to result in silages which became unstable between day 21 and day 120. A possible explanation for this could be that an experimental fault occurred in the sealing of the jars in which the plant material was ensiled. Using the data obtained in Chapter 3, it was decided to only use the D+S and W+S treatments for further comparisons between growth stages and plant species.

2 In Chapter 4 the treatment differences across the three growth stages (E: early, M: medium and L: late) and within a grass specie (S: *D. eriantha* and P: *P. maximum*) was discussed. Comparison was only made within the D+S and W+S treatments as these treatments gave better results in Chapter 3.

From the data obtained of the *D. eriantha* grass ensiled directly with sugar addition, it was concluded that the SM silage was of better quality compared to the other two silages. The SM silage had a lower pH value, lower NH_3-N concentration and a higher lactic acid concentration compared to the other silages.

With the ensiling of the *D. eriantha* grass ensiled with prior wilting and sugar addition, the SM silage was of better quality compared to the other two silages. The SE silage was also acceptable, it had a higher NH_3-N concentration compared to the SM silage. The SL silage was unstable due to a variation in pH values.

The data obtained with the direct ensiling of the *P. maximum* grass with sugar addition, indicated that the PM silage was of better quality, having lower pH values, lower NH_3-N concentration and higher lactic acid concentration compared to the other two silages. The PE silage had a better quality compared to the PL

silage. There was a decrease in the silage quality of the PL silage between day 21 and day 120, this could have been due to an experimental error occurring.

From the data obtained with the ensiling of *P. maximum* grass with prior wilting and sugar addition, indicated that the PE and PL silages were of better quality than that of the PM silage. The wilting of the *P. maximum* material could have inhibited clostridia growth resulting in stable silages.

The data discussed in Chapter 4 indicated that a better quality silage is obtained when ensiling the early and medium growth stages, compared to the late growth stage. Except for the PM W+S treatment, the medium growth stages gave the better silage quality.

3 In Chapter 5 specific treatment differences across *D. eriantha* and *P. maximum* within a growth stage was discussed and comparison was only made within the D+S and W+S treatments as these treatments gave better results in Chapter 3.

The silages made from the early growth stage material with sugar addition from *D. eriantha* and *P. maximum grass*, resulted in silages of good quality. The PE silage had a lower pH and higher lactic acid concentration compared to the SE silage. The SE silage had a lower NH_3-N concentration compared to the PE silage.

Both the SE and PE silages made from the *D. eriantha* and *P. maximum* grass ensiled at an early growth stage, with prior wilting and sugar addition, was of good quality. Both silages had low pH values, high lactic acid concentrations and low NH_3-N concentration, which were acceptable according to the literature.

There was very little difference in the silage quality of the SM and PM silages, made from *D. eriantha* and *P. maximum* grass ensiled directly at a medium growth stage with sugar addition. Both these silages had low pH values, high

lactic acid concentrations and a NH_3-N concentration less than 50 g/kg N. The SM silage was possibly of better quality as it had a lower NH_3-N concentration on day 120 compared to the PM silage.

The data obtained with the direct ensiling of *D. eriantha* and *P. maximum* grass at a late growth stage indicated that between day 0 and day 7, there was a significant decrease in the pH values of both the silages. But between day 21 and day 120 there was a significant increase in the pH values of both the silages leading to a decrease in silage quality. This decrease in silage quality could have been due to an experimental error occurring or due to secondary fermentation. These two silages were unstable and the silage quality on day 120 was not good.

The ensiling of *D. eriantha* and *P. maximum* grass at a late growth stage with prior wilting and sugar addition, resulted in the PL silage being of better quality than the SL silage. The PL silage had a lower pH value, lower NH_3-N concentration and a higher lactic acid concentration compared to the SL silage on day 120.

When comparing the silages made from *P. maximum* and *D. eriantha* grass, the data in Chapter 5, indicated that *P. maximum* tended to give a better quality silage compared to *D. eriantha*. This is not true for all the growth stages as the SM W+S and SL D+S treatments, produced a better quality silage than that of the PM W+S and PL D+S treatments. In general, the differences in silage quality between *P. maximum* and *D. eriantha* tend to be smaller than the differences between growth stages within a specific plant specie.

4 In the second part of this study, a partial digestibility study was done. The data obtained in this study showed that there are no major differences in the total digestibility of OM and N between the two plant species and the two growth stages used.

When comparing the four diets with regards to DOMI / $W^{0.75}$, OM-digestibility through the whole GIT and true N-digestibility, it can be observed that there are no significant differences. The PL and SL diets tended to have higher DOMI / $W^{0.75}$ compared to the PM and SM diets. There were significant differences in the partial digestibility of nitrogen, especially between the PM and SL diets. With regards to true N-digestibility, the *P. maximum* diets tended to have higher values than the *D. eriantha* diets (P>0.05). The animals on the SL diet tended to have higher N intake, total N flow, higher NAN flow, higher NAN flow / N intake and higher NAN disappearance in the small intestine, than the other treatments (P>0.05). This could indicate a better utilisation of silage N. An animal growth study / production study would be a viable option to determine if the differences observed in silage quality and partial digestibility are large enough to have a marked influence on animal performance. From a nutritional point of view, both these silages can be used as a roughage source in sheep diets.

7.2 Recommendations and conclusions

When ensiling these grasses it is important to note the following:

1 The DM concentration of the plant material at ensiling does have an effect on the silage quality obtained. The lower the DM concentration, the lower the final pH needs to be. To achieve a good quality silage a DM concentration of at least 30% is recommended, as below this value clostridia growth cannot be inhibited due to a lack of moisture. Clostridia bacteria can have a negative effect on silage quality due to secondary fermentation and the breakdown of protein.

2 The WSC concentration of *D. eriantha* and *P. maximum* can be limiting, resulting in a fermentation pattern favouring the production of acetic acid and not lactic acid. This will have a negative effect on silage quality and silage stability. The addition of sugar (molasses) at ensiling does have a positive effect on the fermentation process and the rate of fermentation in the silage. This was observed

where the addition of sugar lead to a decrease in the pH of the silage, an increase in lactic acid production and a decrease in the NH_3-N concentration of these silages. The application of molasses onto the plant material at ensiling does have a practical and cost implication. The amount needed to have a positive effect could be a limiting factor.

3 The maturity of the plant material at ensiling did have a marked effect on the fermentation process. The material ensiled at an early and medium growth stage resulted in a better quality silage, with lower pH values, lower NH_3-N concentration and higher lactic acid concentrations compared to acetic acid concentrations. It must be kept in mind that this material did have a lower DM concentration and that the lower the DM concentration the greater the chance that clostridia could grow, which could break down protein leading to a decrease in the protein quality of the silage.

4 The lower the DM concentration the more advisable it is to wilt the material prior to ensiling and the addition of molasses does have a further positive effect on the final silage quality obtained.

5 There were no major differences in the quality of the silages made from *D. eriantha* and *P. maximum* grass found in this experiment. There were also no major differences in the partial digestibility of OM and N between the four different silages. Thus, the grass giving a higher DM yield per ha, in a specific region will be the advisable option to ensile.

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