The influence of nitrogen fertilization, physiological stage and season on qualitative and quantitative characteristics of *Panicum maximum* cv Gatton for sheep.

by

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DECLARATION

I declare that this dissertation
for the degree MSc (Agric) at the University of Pretoria,
has not been submitted by me for a degree at any other University.

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SUMMARY

THE INFLUENCE OF NITROGEN FERTILIZATION, PHYSIOLOGICAL STAGE AND SEASON ON QUALITATIVE AND QUANTITATIVE CHARACTERISTICS OF *PANICUM MAXIMUM* CV GATTON FOR SHEEP.

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The aim of this study was to evaluate the nutritional value of *Panicum maximum* cv Gatton for sheep under different levels of nitrogen fertilization during different stages of maturity and in different seasons.

Four different experiments were conducted. The aim of the first experiment was to study the influence of nitrogen fertilization and stage of maturity on the dry matter yield and chemical composition of *Panicum maximum* cv Gatton. The experiment was conducted during autumn and repeated during the subsequent summer. The second part of the study was a partial digestibility study, aimed at the estimation of the influence of the fertilized grass at different stages of maturity on the animal. This experiment was conducted during autumn and repeated during the subsequent summer.

Seven levels of N were used, namely 0, 25, 50, 75, 100, 125, and 150 kg N/ha, and three stages of maturity namely vegetative stage, early bloom and full bloom. Measurements included dry matter (DM) yield, nitrogen (N) content, total nonstructural carbohydrates (TNC), neutral detergent fibre (NDF), acid detergent lignin (ADL) and *in vitro* digestibility of organic matter (IVDOM).

In this treatise no comparisons between seasons were made, only within seasons.

Dry matter yield increased linearly in both seasons with increased level of N fertilization and with maturity. Nitrogen fertilization increased the N content of grass in both seasons, while N concentration decreased as the grass grew older.

Total nonstructural carbohydrate content decreased as the N fertilization level increased, but as plants matured TNC showed, over all fertilization levels, a tendency to increase. Neutral detergent fibre and ADL seemed to decrease as N fertilization levels increased, but increased markedly with advancing stage of maturity. Organic matter digestibility showed no change with increasing levels of N fertilization, but decreased as grass matured.

In the partial digestibility study it was found that N fertilization did not have a statistically meaningful influence on rumen pH, while stage of maturity increased rumen pH significantly in both autumn and summer.

Nitrate nitrogen content in the rumen increased sharply shortly after N fertilization was applied to pastures, but it decreased as plants matured so that no differences could be observed between sheep grazing on pastures fertilized with 0 and 150 kg N/ha respectively.

Total volatile fatty acids (VFA) were increased by level of N fertilization during the vegetative stage in summer, while no change was seen during early bloom or full bloom stages. Total volatile fatty acids were, however, significantly increased during all stages in the autumn. As grass pastures matured, the VFA concentration decreased in both seasons.

Nitrogen fertilization did not appear to have a very strong influence on the flow of organic matter through the digestive tract of the sheep.

OPSOMMING

DIE INVLOED VAN STIKSTOF BEMESTING, FISIOLOGIESE STADIUM EN SEISOEN OP DIE KWALITATIEWE EN KWANTITATIEWE EIENSKAPPE VAN *PANICUM MAXIMUM* CV. GATTON VIR SKAPE

deur

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Die doel van hierdie studie was om die voedingswaarde van *Panicum maximum* cv Gatton onder verskillende vlakke van stikstof (N) bemesting, verskillende stadiums van volwassenheid en in verskillende seisoene, vir skape, te evalueer.

Vier verskillende eksperimente is uitgevoer. Die doel van die eerste eksperiment was om die invloed van N-bemesting en stadium van volwassenheid op die droë materiaal opbrengs en die chemiese samestelling van *P. maximum* cv Gatton te bestudeer. Die eksperiment is gedurende die herfs uitgevoer en gedurende die daaropvolgende somer herhaal. Die tweede eksperiment is gedoen om die invloed van die bemeste gras, by verskillende stadiums van volwassenheid, op die dier is te bepaal. Hierdie eksperiment is gedurende die herfs gedoen en weer gedurende die daaropvolgende somer herhaal.

Sewe stikstof peile naamlik 0, 25, 50, 75, 100, 125 en 150kg N/ha, en drie stadiums van volwassenheid, naamlik vegetatiewe, vroeë blom en laat blom stadiums is geëvalueer. Droë materiaal (DM) opbrengs, stikstof (N) inhoud, totale nie-strukturele koolhidrate (TNC), neutraal bestande vesel (NDF), suurbestande lignien (ADL), nitraat-stikstof (NO_3-N) en verteerbaarheid van organiese materiaal (IVOMD) is bepaal. In hierdie verhandeling is daar nie tussen seisoen vergelykings gedoen nie, maar wel binne seisoen vergelykings.

Die DM opbrengs het in beide seisoene liniêr toegeneem soos wat N-peile verhoog is, asook met toenemende veroudering van die weiding.

Stikstof bemesting het die N-inhoud van die gras in beide seisoene vehoog, terwyl N-inhoud verlaag het soos wat die gras verouder het.

Die TNC-inhoud van die gras is verlaag met 'n verhoging in N-vlakke, terwyl dit toegeneem het met veroudering van die gras. Die NDF- en ADL-inhoud van die gras is effens verlaag deur toenemende N-peile, maar met veroudering het dit tot 'n groot mate verhoog.

In die parsiële verteringstudie is gevind dat N-bemesting nie 'n betekenisvolle invloed op rumen pH gehad het nie, terwyl stadium van volwassenheid in beide die herfs en somer die rumen pH betekenisvol verhoog het.

Nitraat-stikstof is kort na die toediening van N-kunsmis in die rumen verhoog, maar het verlaag soos wat die gras verouder het, sodat daar later geen verskille was tussen die NO_3-N konsentrasie in die rumen van skape wat gras bewei het wat onderskeidelik met 0 en 150 kg N/ha bemes was nie.

Totale vlugtige vetsure (VVS) is gedurende die vegetatiewe stadium in die somer deur verhoogde peile van N-bemesting verhoog, terwyl geen verandering tydens vroeë blom of vol blom waargeneem was nie. Totale vlugtige vetsure is betekenisvol verhoog gedurende al die groeistadiums in die herfs. Soos plante ouer geword het, het die totale VVS konsentrasies in die rumen afgeneem.

Dit blyk dat N-bemesting nie 'n groot waarneembare invloed op die verdwyning van OM deur die spysverteringstelsel van die skape gehad het nie.

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CHAPTER 1

LITERATURE REVIEW
THE NEED FOR NITROGEN FERTILIZATION OF GRASS PASTURES

1.1 INTRODUCTION

With the increase in human population in South Africa, less natural pasture is available for animal production. As a result, there is a growing interest in intensive grassland production in order to sustain the same, or even better, level of animal production. In 1960, 92 million ha of land were available for animal production, which had decreased to only 84 million ha in 1988 (Whiteman, 1980).

Because of the declining contribution of range, planted pastures will become increasingly important for animal production in the tropics and subtropics. Planted pastures will enable more animals to be kept per ha and will also provide a better quality forage to the animals than natural grazing (McDowell, 1972).

Animal production in the tropics can be increased by increasing the output per animal, but also by increasing the productivity per unit land. An important factor in the increase of animal production is the improvement of animal feeding and the provision of feed to the animal, especially ruminants. Improvements in disease and parasite control, breeding and management are also important in the improvement of animal production, but an improvement in feeding status is the most important factor (McDowell, 1972).

Animal products are the most important source of protein for the fast growing human population and are important for a balanced diet. Ruminants play a very important role in the provision of this source of protein, since they can utilize cheap and low quality feeds to produce high quality animal protein (McDowell, 1972).

A large proportion of the South African veld consists of dry tropical bushveld and savannas where temperate grass production is often problematical. It is, therefore, often necessary to plant tropical grass pastures in these areas to improve animal production. Such tropical grasses often have a low feeding value and intake, but have a high dry matter yield (Whiteman, 1980). There is, therefore, much room for improvement. In the light of the above mentioned, it is necessary to distinguish between temperate (C3) and tropical (C4) plants and the difference between the photosynthetic pathways followed by these two groups of plants.

1.2 THE C3, C4 AND CAM PHOTOSINTHETIC PATHWAYS

On grounds of certain specific physiological, morphological and biochemical characteristics, we can distinguish between three different groups of plants, namely, C3, C4 and CAM plants (Taiz and Zeiger, 1991).

The C3 plants such as grains (barley, oats, rice, rye, wheat), peanuts, soybeans, cotton, sugar beet, tabacco and some evergreen and deciduous trees, fix carbon dioxide with a ribulose 1, 5- diphosphate to form two molecules of 3-phosphoglyceric acid (a three- carbon acid) via the Calvin – Benson pathway of photosynthesis. This reaction is catalyzed by the enzyme diphosphate carboxylase.

The C4 plants fix carbon during photosynthesis by reacting CO_2 with phosphoenolpyruvic acid in the presence of the enzyme phosphoenolpyruvate carboxylase to produce oxaloacetic acid (a four-carbon acid) via the Hatch-Slack pathway (Taiz and Zeiger, 1991). Plants in this group includes corn, sorghum, sugarcane, millet and different grass species, such as crabgrass, bermudagrass, *Amaranthus* and *Atriplex*.

The CAM type plants often display a diurnal pattern of organic formation and fix CO_2 in a modified C4 pathway called crassulacern acid metabolism (CAM). Some of these plants have large succulent leaf cells, with stomata that open at night, allowing carboxylase enzymes to fix CO_2 into organic C4 acids. This group

includes members of the cactus, orchid and pineapple families (Taiz and Zeiger, 1991).

The C3 and C4 pathways of photosynthesis which apply to most forage species will be discussed in more detail in the following discussion.

1.2.1 C3 Pathway

C3 plants have a single chloroplast type that performs all of the reactions that convert light energy into the chemical energy that is used to fix CO_2 and to synthesize the reduced carbon compounds upon which all life depends. Ribulose- 1,5-biphosphate carboxylase/oxygenase (Rubisco) catalyzes primary carbon fixation, in which a five-carbon sugar phosphate, ribulose-1, 5 biphosphate (RuBP), and CO_2 are converted to two molecules of the three-carbon compound 3-phosphoglycerate (hence the name C3). Phosphoglycerate is then phosphorylated and reduced by the reactions of the light reactions of photosynthesis (ATP and NADPH) to produce triose phosphate(TP). Triose phosphate can be exported from the chloroplast via the chloroplast envelope phosphate (Pi) transporter to the cytosol and used in the synthesis of sucrose, which is then translocated throughout the plant or it can be retained within the chloroplast for starch synthesias or recycling to RuBP. Rubisco also catalyzes the fixation of O_2 in a process known as photorespiration, which competes directly with fixation of CO_2. At air levels of CO_2, for every three CO_2 molecules fixed by Rubisco it formed 3-phosphoglycerate and 3-phosphoglycolate. Because 3-phosphoglycolate cannot be used in the photosynthetic carbon reduction (PCR) cycle, it must be recycled to phosphoglycerate via the photorespiratory pathway, expending ATP and NADPH (Figure 1). This competition between O_2 and CO_2 and the energy costs associated with recycling phosphoglycolate largely determine the efficiency of C3 photosynthesis in air (Furbank and Taylor, 1995).

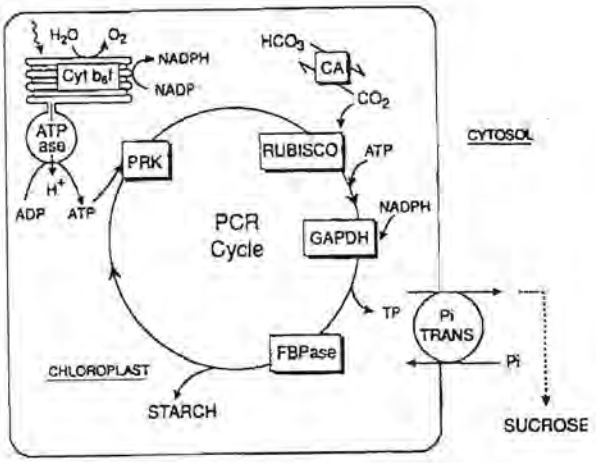


Figure 1 Simple schematic scheme of the C3 pathway (Furbank and Taylor, 1995)

1.2.2 C4 Pathway

The C4 pathway (Figure 2) is a complex adaptation of the C3 pathway that has evolved in both monocot and dicot families, eliminating the energy-wasting process of photorespiration that impedes C3 photosynthetic function (Nelson and Langdale, 1992). It is found in a diverse collection of species, many of which grow in hot climates with sporadic rainfall. The C4 pathway effectively suppresses photorespiration by elevating the CO_2 concentration at the site of Rubisco using a biochemical CO_2 pump. C4 plants have two chloroplast types, each found in a specialized cell type. Leaves of C4 plants show extensive vascularization, with a ring of bundle sheath (B) cells surrounding each vein and an outer ring of mesophyll (M) cells surrounding the bundle sheath. The development of this so-called Kranz anatomy and the cell-specific compartmentalization of C4 enzymes are important features of C4 photosynthesis. The CO_2 fixation in these plants is a two-step process. Atmospheric CO_2 is initially fixed in the cytosol of M cells by phosphoenolpyruvate carboxylase (PEPC) to form the four-carbon dicarboxylic acid oxaloacetate (therefore the name C4), which is converted to malate or aspartate. These C4 acids then diffuse into the inner ring of B cells, where they are decarboxylated in the chloroplasts. The CO_2 produced is then refixed by Rubisco. The mechanism of decarboxylation in B chloroplasts varies among the

three different C4 types. The key characteristics of C4 photosynthesis is the compartmentalization of activities into two specialized cell and chloroplast types. Rubisco and the C3 PCR cycle are found in the inner ring of B cells. These cells are separated from the mesophyll cells and from the air in the intercellular spaces by a lamella that is highly resistant to the diffusion of CO_2. Therefore, by virtue of this two-stage CO_2 fixation pathway, the mesophyll-located C4 cycle acts as a biochemical CO_2 pump to increase the concentration of CO_2 in the bundle sheath to about 10 times than in the atmosphere. The nett result is that the oxygenase activity of Rubisco is effectively suppressed and the PCR cycle operates more efficiently. C4 plants show higher rates of photosynthesis at high light intensities and high temperatures because of the increased efficiency of the PCR cycle.

In favourable environments, C4 plants do much better than C3 plants, making them the most productive crops and the worst weeds.

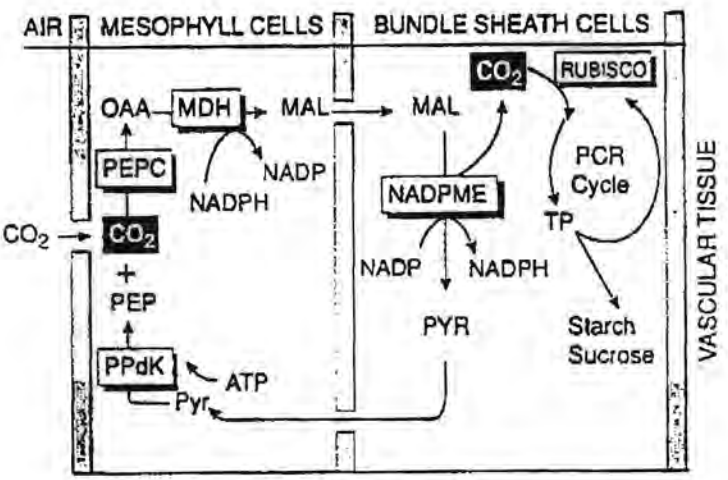
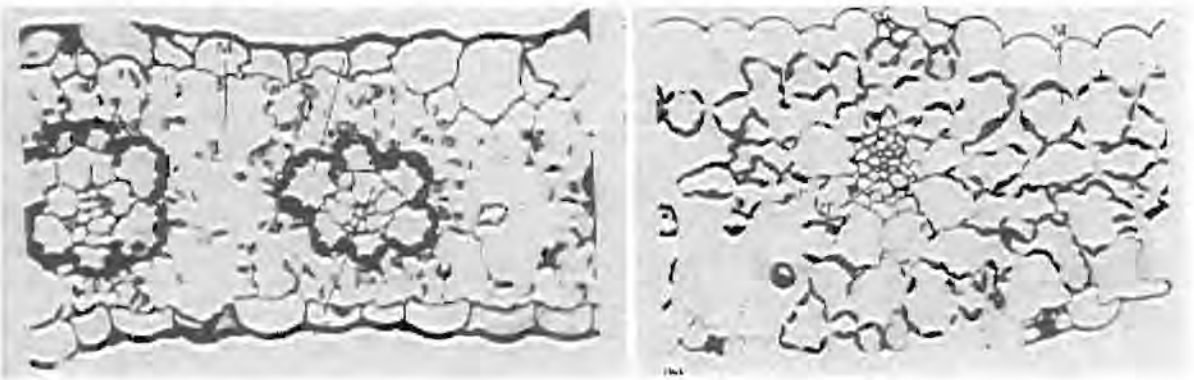


Figure 2 A Schematic scheme of the C4 photosynthetic assimilation cycle (Furbank and Taylor, 1995)

1.2.3. The differences between C3 and C4 plants

Except for the differences in photosynthetic pathways, there are a large number of other anatomical, morphological and chemical differences between C3 and C4

plants. When a cross section of a typical C3 leaf is examined, it reveals essentially one type of photosynthetic, chloroplast-containing cell, the mesophyll (Figure 3b). In contrast with this, a typical C4 leaf has two distinct chloroplast-containing cell types, the mesophyll and the bundle sheath cells (also called the Kranz cells, German for "wreath") (Figure 3a) (Hudson *et al.*, 1990). There is considerable anatomic variation in the arrangement of the bundle sheath cells with respect to the mesophyll and vascular tissues. However, the operation of the C4 PCA cycle requires the cooperative effort of both cell types and no mesophyll cell of a C4 plant is more than two or three cells distant from the nearest bundle sheath cell (Hudson *et al.*, 1990). An extensive network of plasmodesmata connects mesophyll and bundle sheath cells, providing a pathway for the flow of metabolites between the cells.



a b

Figure 3 **Cross section of leaves showing the anatomic difference between C3 and C4 plants: a) a C4 monocot, *Zea mays*; b) a C3 monocot, *Avena sativa* (Hudson *et al.*, 1990)**

A distinguishing feature of the anatomy of C4 plants is the sheath of specialized cells surrounding the vascular tissues. These cells usually have thick walls and are relatively resistant to degradation by rumen organisms (Wilson *et al.*, 1983). C4 grasses also have a high frequency of vascular bundles with few mesophyll

cells between the bundles. Leaves of C4 plants, therefore, appear to have, in general, a lower proportion of mesophyll than C3 plants, which is readily digested and a higher proportion of bundle sheath, epidermis, vascular and sclerenchyma tissues, which are either poorly digested or indigestible (Wilson *et al.*, 1983).

Akin *et al.* (1983) found that C3 plants were about 7 units higher in DM digestibility than C4 plants.

The genus *Panicum* is unusual because it contains species using the C3 photosynthesis path and species using the C4 photosynthesis path. Table 5 shows some *Panicum* species and the differences between digestibility and cell wall content.

Table 1 ***Panicum*** **species in order of dry matter digestibility and cell wall content (Wilson *et al.*, 1983)**

Panicum **species**	**Digestibility**		**Cell wall content**	
	Species code	**%**	**Species code**	**%**
tricantum	Tra-3	78.9	Bis-3	29.4
clandestinum	Cla-3	78.3	Tra-3	29.7
trichoides	Tri-3	76.4	Tri-3	32.4
hylaeicum	Hyl-3	75.0	Cla-3	33.6
bisulcatum	Bis-3	74.6	Lax-3	36.0
laevifolium	Lae-4	73.7	Hyl-3	37.4
virgatum	Vir-4	73.6	Tur-4	40.8
turgidum	Tur-4	73.5	Des-3/4	41.0
laxum	Lax-3	73.0	Mil-3/4	41.4
effusum	Eff-4	72.2	Sch-3/4	43.8
miliodes	Mil-3/4	72.0	Lae-4	43.8
buncei	Bun-4	71.4	Eff-4	44.0
bergii	Ber-4	70.4	Mon-4	46.2
monticola	Mon-4	70.3	Pri-4	47.3
decipiens	Dec-3/4	70.2	Ber-4	47.4
prolutum	Pro-4	69.7	Ant-4	47.5
amarum	Amr-4	69.5	Pro-4	47.6
schenckii	Sch-3/4	69.1	Qld-4	48.8
decompositum	dcm-4	68.5	Riv-3	49.0
queenslandicum	Qld-4	68.2	Col-4	49.3
hallii	Hal-4	68.0	Hal-4	49.3
maximum cv green panic	Max1-4	67.8	Bun-4	49.4
coloratum	Col-4	67.7	Vir-4	51.3
antidotale	Ant-4	67.5	Aml-4	52.2
maximum cv guinea	Max2-4	66.1	dcm-4	52.3
prionitis	Pri-4	64.7	Max1-4	57.0
rivulare	Riv-3	64.6	Amr-4	58.2
amarulum	Aml-4	63.5	Max2-4	58.2
LSD	$P < 0.05$	3.1		4.2

Species code indicates photosynthetic type

An important difference between C3 and C4 plants is their differential response in nett photosynthesis to various light intensities (Figure 4). C3 plants have low nett photosynthetic rates, high carbon dioxide compensation points (50 to 150 ppm CO_2), and high photorespiration rates. The C4 plants, on the other hand, have high nett photosynthetic rates, low carbon dioxide compensation points (0 to 10 ppm CO_2), and low photorespiration rates. The C4 plants are the more efficient users of CO_2. C4 plants have the additional advantage of greater water use efficiency than the C3 plants (Taiz and Zeiger, 1991).

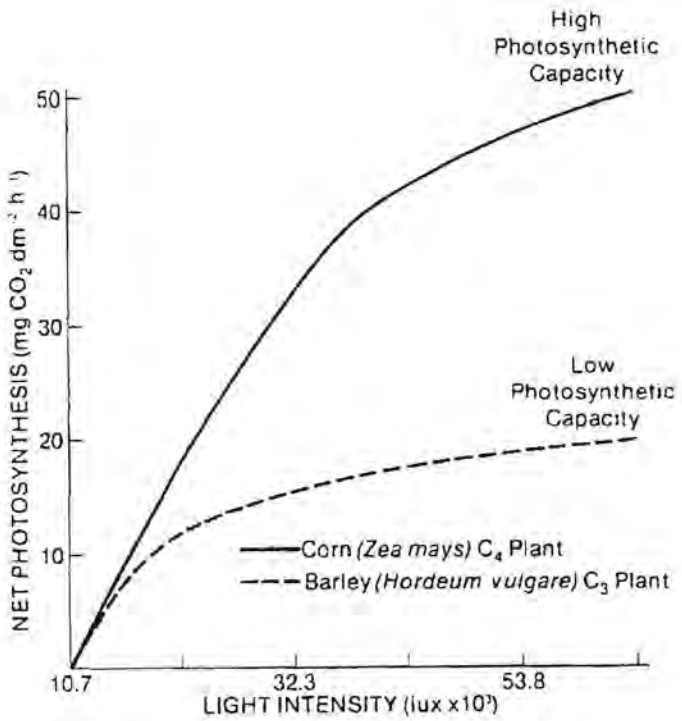


Figure 4 **Effect of light intensity on the nett photosynthetic rates of a C4 plant (corn) and a C3 plant (barley) (Taiz and Zeiger, 1991)**

Another characteristic difference between the C3 and C4 plants is that at the normal CO_2 concentration (0.03 percent), light saturation is difficult to attain for C4 plants, but is easily reached at low light intensities for C3 plants (11 000 to 43 000 lux or 1000 to 4000 ft-c).

The voluntary intake of tropical (C4) grasses is usually less than that of temperate (C3) grasses grown at the same time. This lower intake of tropical grasses applies at all stages of growth and is associated with a higher fibre content, lower DM digestibility, larger quantities of indigestible fibre and the longer time the fibre is retained in the reticulo-rumen (Minson, 1990). Minson and Milford (1967) found that with young temperate pastures, with a low fibre content, only 20 % of the daily intake of DM appeared in the faeces (80% DM digestibility), while with mature tropical pasture as much as 60% of the feed may be excreted in the faeces (40% DM digestibility).

Tropical grasses contain less protein than temperate grasses. Norton (1982) found that 53% of all tropical grasses contained less than 8% crude protein (CP), while only 32% of all temperate grasses contained less than 8% CP. Norton (1982) stated that a minimum of 15% CP is needed for lactation and growth. Most of the temperate grasses have adequate amounts of CP, while only 20% of the tropical grasses contained more than 15% protein. The relatively low CP content of tropical pastures places a limitation on the more intensive forms of animal production (Norton 1982). The C4 plants, however, use N more effectively than C3 plants (Humphreys, 1991). The higher effectiveness is also associated with lower tissue N content. It may be concluded that the low protein content found in many tropical grasses, even under N fertilization, is an inherent characteristic of C4 metabolism and is related to survival under conditions of low fertility (Humphreys, 1991).

Glucose, fructose, sucrose and the polisaccharides like starch and fructosan, are the main groups of soluble carbohydrates that can be found in plant cells (McDonald *et al.*, 1992).

Tropical grasses store mainly starch and sucrose, with high concentrations in their leaves. Temperate grasses on the other hand accumulate mostly sucrose and fructose and mostly in their stems (Humphreys, 1991). Where temperate grasses and legumes grow in the same temperate area as tropical grasses and

legumes, they have higher concentrations of soluble carbohydrates then tropical grasses and legumes. In warm climates, however, the soluble carbohydrates are low in all groups (Norton, 1982).

1.3 MANAGEMENT TECHNIQUES TO IMPROVE THE NUTRITIONAL VALUE OF A GRASS

Various management techniques are available to improve the productivity and quality of grassland. Three of these techniques are (Minson, 1990):

a) Species and cultivar selection,
b) The use of nitrogen (N) fertilization to improve the quality and quantity of grass produced,
c) The stage of maturity at which the grass is harvested, or used for grazing.

In this trial, the focus fell on N fertilization and stage of maturity.

A plant consists chemically of many different components which all react differently to different levels of nitrogen fertilization and stages of maturity. It is necessary to know the different chemical compositions in order to fully understand the changes that take place in the plant and how these influence the animal.

Chemically a plant consists of the following basic components (Table 2).

Table 2 Components of different fractions in the proximate analysis of foods (McDonald *et al.*, 1992)

Fraction	Components
Moisture	Water (and volatile acids and bases if present)
Ash	Major: Ca, K, Mg, Na, S, P, Cl Essential Elements { Trace: Fe, Mn, Cu, Co, I, Zn, Si, Mo, Se, Cr, F, V, Sn, As, Ni Non-essential elements: Ti, Al, B, Pb
Crude protein	Proteins, amino acids, amines, nitrates, nitrogenous glycosides, glycolipids, B-vitamins, nucleic acids
Ether extract	Fats, oils, waxes, organic acids, pigments, sterols, vitamins A, D, E, K
Crude fibre	Cellulose, hemicellulose, lignin
Nitrogen free extractives	Cellulose, hemicellulose, lignin, sugars, fructans, starch, pectins, organic acids, resins, tannins, pigments, water-soluble vitamins

These components are essential for the maintenance and production requirements of animals (Steenekamp, 1995). These components can all be altered to a greater or lesser extent by the above mentioned management techniques, namely N fertilization and stage of maturity at which the grass is cut or grazed.

With the use of N fertilization the DM production can be increased and the level of protein, P and K can be altered (Steenekamp, 1995). By altering the time of utilization, DM production, protein content, lignin and structural and nonstructural carbohydrate levels can be changed.

With all the changes that occur with N fertilization and with different stages of maturity, it is important to know what influence different levels of N fertilization and different stages of maturity will have on the DM production, protein and nonstructural carbohydrate content, but also on the more undesirable components such as lignin, cellulose and hemicellulose. It is also important to determine the influence of N fertilization and stage of maturity on the digestibility of the grass.

1.4 INFLUENCE OF STAGE OF MATURITY ON DIFFERENT PARAMETERS.

1.4.1 Nutritional value of maturing grass

The nutrient value of a pasture depends on its nutrient content and on the extent to which the pasture is voluntary eaten by the animal (Minson and McLeod, 1970), which is characterized by chemical composition and digestibility. These factors are strongly affected by plant species, age at harvest and fertilization level. Grasses are the most common sources of energy for ruminants, for they contain a high percentage of cellulose, lignin and hemicellulosse (Arroyo-Aguilu and Oporta-Tellez, 1979).

Both the nutrient content and the extent to which the pasture is eaten, are influenced by the stage of maturity of the pasture. When grass matures, it goes through many different growth stages such as a vegetative stage, pre bloom, early bloom, full bloom, milk stage, dough stage, mature and overripe (Minson, 1990).

The nutrient value of forages depends primarily on the physiological and morphological development of grasses and legumes (Bratzler *et al.*, 1959). One of the first effects of maturity is the decline in total digestibility as the number of leaves decreases and the proportion of stem increases. In young plants the stem is more digestible than the leaf, but whereas the digestibility of the leaf fraction declines very slowly, that of the stem fraction declines rapidly (McDonald *et al.*, 1992).

As plants mature, the potential digestible components, such as total nonstructural carbohydrates (TNC) and proteins (cell contents), decline, while the proportion of lignin, cellulose and hemicelluloses (cell wall) increases (Whiteman, 1980). With advancing maturity the dry matter (DM) yield also increases. Figure 5 illustrates the influence of plant maturity on the chemical composition of plants (Holmes, 1980 as quoted by Van Niekerk, 1997).

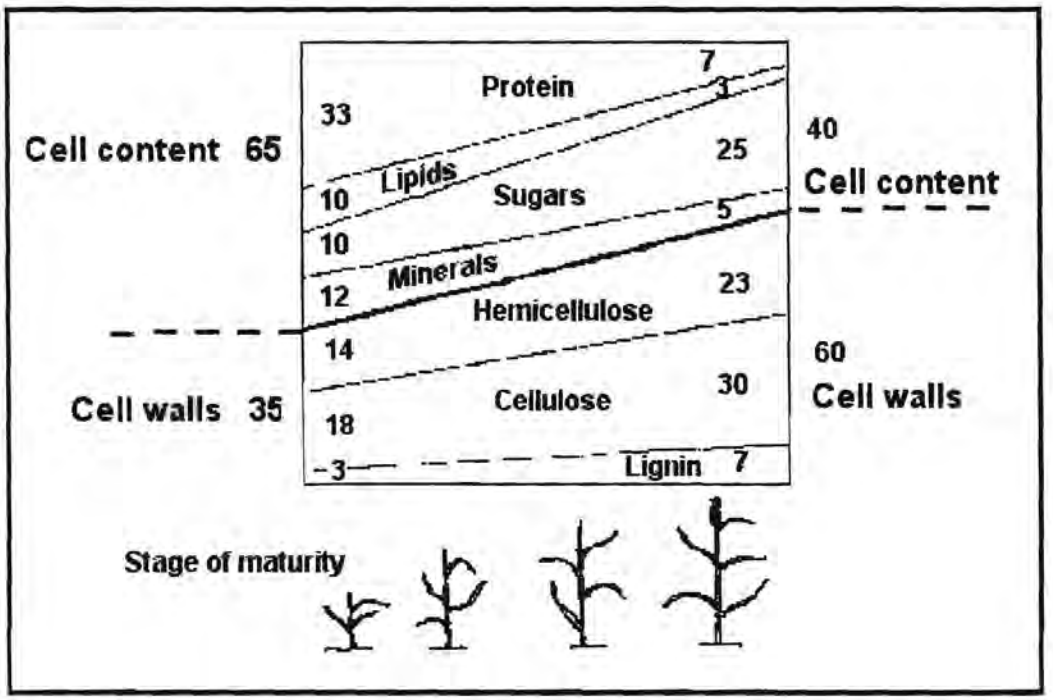


Figure 5 **A schematic scheme of the changes in chemical composition of plants as they go through different stages of maturity (Holmes, 1980 as quoted by Van Niekerk, 1997).**

In the following discussion the following changes, as plants mature, will be discussed:

- Nutrient value of grass at different stages of maturity
- Digestibility of grass as it matures
- Dry matter yield change as grass matures
- ADL change as grass mature
- Total non-structural carbohydrate changes with maturity
- Crude protein content and the changes with maturity
- Nitrate nitrogen content.

1.4.2 Digestibility of pastures as grass matures

Cellulose and hemicellulose represent the fraction in feeds responsible for variation in digestibility, since aside from the relatively indigestible lignin, they contain the bulk of truly indigestible substances (Van Soest, 1994). Published data generally show that there is a decline in digestibility as plants change from a leafy vegetative to a morphological stemmy growth as plants grow to maturity (Blaser, 1964).

Whiteman (1980) found that the percentage digestible cellulose decreased as the percentage lignin in the cellulose component increased. Reeves (1987) also reported a decline in digestibility as plants matured. Since the cell content is considered to be nearly 100% digestible (Van Soest, 1982), factors limiting ruminal digestion are mainly found in the cell wall fraction (Aman and Lindgren, 1983). The general effect is that as the plant matures, the proportion of fibre usually increases and the proportion of crude protein and non-structural carbohydrate content of the cell decreases (Minson, 1982).

There exists a linear decrease in digestibility as the concentration of lignin in cellulose increases (Whiteman, 1980). In a study by Hatfield *et al.* (1994) of the different methods to determine lignin concentration, these authors found that the general tendency is for leaves to have a higher concentration of crude protein and less NDF than the stems. It was also found that maturity is reflected in a general increase in NDF and a decrease in crude protein concentration as maturity increases. According to Minson (1971) the digestibility of tropical grasses decreased with 0.1 digestibility units per day as plants matured. Laredo and Minson (1973) found that the digestibility of leaf and stem fractions decreased by 0.34 and 0.25 digestibility units respectively as plants matured.

Cherney *et al.* (1992) found that cool season perennial grasses decreased in *in vitro* DM digestibility with increased maturity throughout the growing season. Increased lignin was postulated to be the major factor in reducing digestibility with increased maturity. *In vitro* digestibility is the best predictor of *in vivo* digestibility, although digestibility of forages is often predicted using ADF and ADL (Goering and Van Soest, 1970).

In a study of perennial grasses, Cherney *et al.* (1992) reported a decrease in digestibility from 79.7 to 44.2 % as plants matured. With this decline in digestibility they also found an increase in NDF from 40.0 to 62.7 % and an increase in lignin from 1.7 to 5.3 %. This is an increase of almost four times the original lignin content.

Minson (1971) found that there was a definite seasonal effect when one examined the decrease in digestibility of grasses. This author found that the digestibility of grass decreased with 0.07 digestibility units during autumn, while it decreased by 0.22 digestibility units during summer. In addition to this, Cherney *et al.* (1992) postulated that the magnitude and speed of quality decline with maturity, is more important than species differences in determination of digestion kinetics of perennial grasses. To ensure high quality forage, less emphasis should be placed on differences among species and much more emphasis should be placed on harvest management.

Calder and MacLeod (1968) found that *in vitro* digestibility is highly correlated with *in vivo* digestibility, so the need for animals in digestibility studies is eliminated or reduced as digestibility studies can be done in a laboratory.

1.4.3 Dry matter yield changes as grass matures

Over the years many scientists found that the DM yield of grasses increased linearly over time. A study, conducted by Oyenuga (1960) on *Panicum maximum* cv Jacq. reported that when the grass was cut at three weeks of age, the yield was about 20 t per acre, while it was about 28 tons per acre when cut at eight weeks of age. Blaser (1964) also found an increase in DM yield as plants mature. This author reported that alfalfa increased from less than 183.95 kg/ha during the vegetative stage to more than 919.76 kg/ha by the time the grass reached full bloom.

Although DM yield increases with advancing maturity, it must not be seen as an advantage since a decline in the nutrient value of the grass is correlated with advancing maturity and yield.

1.4.4 Acid detergent lignin changes with advancing stage of maturity

Lignin is the most difficult fraction of plant cell walls to define. Concepts tend to differ with this point of view. Thus, botanists regard it as a plastic, three-dimensional, substituted phenylpropane polymer. Wood chemists regard it as a plastic substance giving distinctive properties to wood. Nutritionists regard it as a structural substance protecting plant cell walls from microbial degradation (Van Soest, 1994). Whatever the case may be, lignin if of great interest to us, since it is associated with a decline in the digestibility of maturing plants. When lignin is removed, it has always produced a marked increase in the digestibility of plants (Harkin, 1973). Jung (1989) reported that the cell wall of plants had been characterized as cellulose microfibrils embedded in a ligno-hemicellulosic macro-molecule to which acetyl and phenolic acid groups are bound. The cellulose microfibrils are bound to the hemicellulose polymers by hydrogen bonding, but there is no evidence of covalent linkage of cellulose to other cell wall constituents. During plant cell development the primary cell wall is deposited initially and it contains cellulose, hemicellulose and pectins. Lignin becomes part of the cell wall during formation and thickening of the secondary cell wall. The phenolic constituents of forage that are linked to the cell wall can be divided into core and non-core lignincomponents (Jung, 1989). Core lignin is a highly condensed, high molecular polymer of cinnamyl alcohols. Klason type lignin preparations such as acid detergent lignin are considered core lignins. While core lignins generally have two covalent linkages between phenolic monomer units within the lignin molecule, non-core lignins are monomers which usually have only one covalent linkage of the phenolic compound, usually a cinnamic acid, to either core lignin or hemicellulose. Some non-core lignin units may posess a second linkage to the other cell wall components to act as a cross-linking agent of core lignin and cellulose (Jung, 1989).

With advancing maturity the lignin content of plants increases. Lignification increases rapidly as the plant nears the full bloom stage (Whiteman, 1955 as quoted by Cherney *et al.*, 1992). Lignin is a part of the cell wall and as the cell wall increases with age, the amount of lignin also increases with age. It can,

therefore, be understood how grasses with low lignification can have digestibilities as low as or lower than legumes with a higher lignin content. Cherney *et al.* (1992) reported that in perennial grasses lignin increased with stage of maturity and that it was highly correlated with fibre digestibility. Because of this correlation, lignin and other cell wall fractions can be used to develop a model to predict the *in vivo* digestibility of a grass from *in vitro* measurements (Andrighetto *et al.*, 1992). Reeves (1987) found that the composition of lignin varies greatly over the growing season and that the variations in composition are forage specific and appear to be influenced by frequency and date of harvest.

1.4.5 Total nonstructural carbohydrates changes as grass matures

The total nonstructural carbohydrates (TNC) are readily available sources of energy for ruminants. They are also rapidly and completely digested and represent readily available energy precursors. The main TNC's found in grasses and legumes are the sugars, consisting of glucose, fructose and sucrose, together with the polysaccharides, starch and fructosans (Jones and Wilson, 1987).

The form of stored structural carbohydrates in grass, differs according to the origin of grasses. Grasses of tropical origin have a C4 photosynthetic pathway, characterized by a specialized leaf anatomy, higher growth rates, a higher N use efficiency and accumulate starch as reserve polysaccharides (Jones and Wilson, 1987). Grass of temperate origin have a C3 photosynthetic pathway and characteristically accumulate fructosan as storage polysaccharide (Jones and Wilson, 1987).

The concentration of nonstructural carbohydrates that may occur in herbage may have several important advantages. The success of preservation of forage silage depends on the amount (at least 10 – 15 %) of readily fermentable carbohydrates present in the herbage. If the concentration of nonstructural carbohydrates is high, conditions are more favourable for the establishment and growth of strains of lactobacilli and the preservation is accomplished successfully with the fermentation of these carbohydrates to lactic acid. Total nonstructural

carbohydrates are also, as mentioned earlier, a source of readily available energy to the microbial population in the rumen.

The concentrations of nonstructural carbohydrates in different plant organs differ. In grasses of temperate origin the stem tissue usually contains a higher concentration of sugars and fructosans than the leaf tissue. Concentrations in leaf sheaths are also usually higher than in leaf blades, although sheaths are more similar to leaf blades than to the stem.

With advancing maturity the proportion of leaves decreases and that of the stem increases. Nonstructural carbohydrates, produced in excess of the needs of the plant, are translocated to and stored in the stem as fructosans. Since the stem increases with advancing stage of maturity, the nonstructural carbohydrates will also increase (Smith, 1973).

In a study conducted by Blaser (1964) it was found that one of the starch - like nonstructural carbohydrates, namely fructosan, is probably used very efficiently for energy by ruminants. With a study on ryegrass, it was found that fructosan increased with stage of maturity, but only till the fifth week of growth sampling. Thereafter it declined rapidly as it was apparently translocated or synthesized into structural material.

In a study of Troughton (1957), it was noted that the reserve carbohydrates in roots of several forage grasses generally decreased in carbohydrate concentration with early spring shoot growth and then gradually increased during late spring and summer. He also found that secondary herbage growth in late summer reduced reserve carbohydrate concentrations. Troughton (1957) associated maximum reducing sugars with rapid vegetative growth, maximum sucrose with differentiation and greatest quantity of "reserve polysaccharide" with the brief resting period prior to secondary growth.

1.4.6 Crude protein (nitrogen) content changes as grass matures

Chemically, the protein content of food is calculated from its N content (McDonald *et al.*, 1992). The term crude protein (CP) is used, since all nitrogen

does not come from proteins, but also comes from nitrites, nitrates and certain cyclic nitrogen (McDonald *et al.*, 1992). As plants mature, the CP content of grasses decreases.

Blaser (1964) found that nitrogen compounds made up progressively less of the dry matter and that there was a nett loss in protein after the mature stages of growth. This was because of the loss of leaves and the large decrease in leaf to stem ratios, as well as the accelerated rate of accumulating structural material. With increasing maturity the CP content of leaves also decreased slower than that of the stem (Stobbs and Minson, 1980).

Whiteman (1980) found that the CP content was highly soluble during the early period of rapid growth, but declined rapidly, as the grass matured and the proportion of cell content decreased. In a study with *Lolium perenne*, Van Vuuren *et al.* (1991) found a decrease in CP with increased grass maturity. It was also found that the fermentable fraction and rate of degradation of CP increased up to three weeks of age and then decreased between four and eight weeks of age.

Long *et al.* (1999) conducted a study on different grass species and found, for all species, that the N content decreased as the grass matured. This was despite the fact that the N content varied between the species. Table 3 represents the N content of different grass species to illustrate the decrease of N content over time.

Table 3 **Nitrogen content (%) of different grass species at different stages of maturity (on dry matter basis) (Long *et al.*, 1999)**

Species	Harvesting time month		
	August	September	October
E.nutans	1.91	1.17	0.61
R. kamoji	1.49	0.08	0.46
S. aliene	2.21	1.67	0.89
D. caespitosa	1.49	1.01	0.60
K. cristata	1.14	0.62	0.42
K. litwinowii	1.46	0.60	0.74
L. secalinum	1.80	1.54	0.83
Mean	1.64	1.06	0.65
SED	0.01	0.03	0.01

1.4.7 Nitrate nitrogen (NO_3-N) content in maturing grass

Most of the chemically combined N absorbed by plants is in the form of nitrate (Madison and Kenneth, 1963). Experiments involving periodic sampling of plants through a cycle of growth have shown that NO_3-N content first rises and then, after reaching a peak about the pre-bloom stage, declines as the plant matures (Madison and Kenneth, 1963). One reason for this decline in NO_3-N is that fruits and seeds usually contain very little NO_3-N and as they increase in DM the effect of high nitrate in other parts are diluted. Another reason is that the formation of seeds and fruits makes a very heavy demand on available N, and thereby decreasing the NO_3-N content (Whiteman, 1980).

1.5 Influence of nitrogen fertilization of grass pastures on DM yield and other chemical components

1.5.1 Introduction

While the availability of natural pastures for animal production is declining every year, the demand for animal products is increasing steadily. To ensure an adequate supply of animal products for the human population, the need to increase animal production per ha land must be addressed. Fertilization of pastures has, therefore, been employed to increase the forage quantity and quality and consequently, increase animal production.

Nitrogen fertilization is one of the most common fertilization practices, since this nutrient was found to be one of the most limiting factors influencing yield and chemical composition of grass pastures.

Numerous writers have reported that N fertilization increases DM yield markedly. Nitrogen fertilization will also increase the CP content as well as the NO_3-N content of a plant. Nitrogen fertilization normally has no effect on *in vitro* organic matter digestibility (IVOMD) of plants, but decreases the TNC content as well as NDF and cellulose content.

This chapter will describe in more detail the influence of N fertilization on DM yield as well as different chemical substances.

1.5.2 Yield

Increasing levels of N fertilization applied to pastures often result in a linear increase in yield. Reid (1970) found that yield response is linear at low N applications, reaching a maximum yield and subsequently declining at very high rates of application. The curve of increase in yield with increasing levels of applied N is generally sigmoid, the utilization of nitrogen often being less efficient at lower rates (Salette, 1970). The following graph shows this sigmoid trend of response to N fertilization.

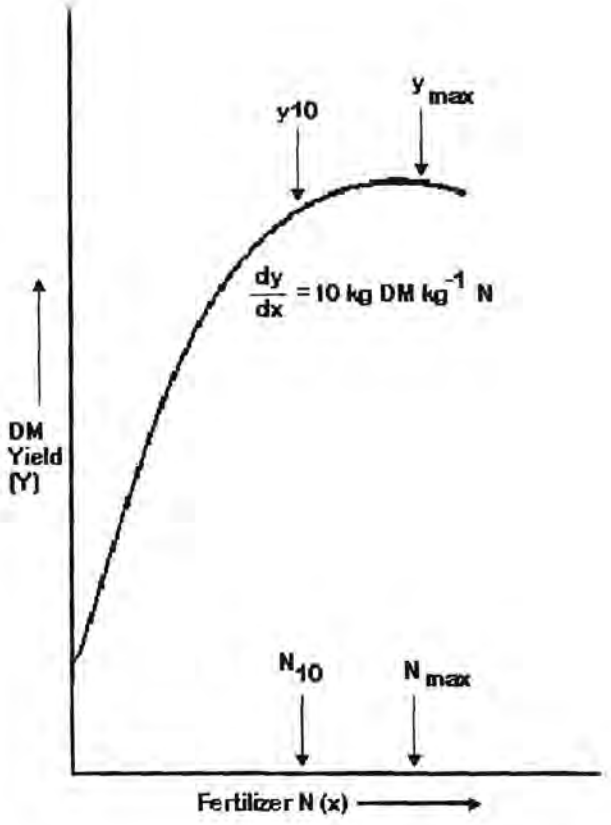


Figure 6: General form of the response of grassland to nitrogen fertilization (Morrison *et al.*, 1980)

Frederiksen and Kategile (1980) registered an increase in DM yield from 2.8 t/ha to 9.79 t/ha when grass was fertilized with 187.5 kg N/ha.

Wiedenfeld *et al.* (1985) found that Buffelsgrass (*Cenchrus ciliaris*) and 'Pretoria 90' bluestem (*Dichanthium annulatum*) fertilized with N, showed a quadratic response. As the N application rate decreased, the benefit from N decreased. Wiedenfeld *et al.* (1985) further found that established Buffelgrass showed a dramatic yield response when fertilized with N, while newly planted 'Pretoria 90' bluestem showed yield responses only after the residual nutrients had been depleted.

In an experiment reported by Omaliko (1984), the following results of N fertilization on the DM yield of *Anthephora ampulaceas* were found.

Tabel 4 Dry matter yield (t/ha) of *Anthephora ampulaceas* (Omaliko, 1984)

Nitrogen Rate kg/ha	Harvest 1	Harvest 2	Total
0	7.4	1.2	8.6
100	14.8	3.5	18.3
200	19.4	5.1	24.4
300	19.9	6.8	26.7
$LSD_{0.05}$	3.01	1.06	3.25

The yield response was accounted for by an increase in all yield components, especially tiller population and plant fractions (stubble, root, leaf and stem).

1.5.3 Nitrogen content

Nitrogen content of a grass is one parameter which is strongly influenced by N fertilization. Vincente-Chandler *et al.* (1959) found that total N content of herbage is consistently increased by the application of N fertilization. It was found that the N content of *Lolium multiflorum* increased from 4.7 %, when fertilized with 28 kg N/ha, to 5.7% when fertilized with 140 kg N/ha.

Saibro *et al.* (1978) reported that the total N concentration of a grass pasture increased linearly up to the maximum N rate, at all three the maturity stages. It was further found that the N concentration at each stage of maturity was much higher than the generally accepted 1.5 % N cut off for maintenance. Diets of ruminants containing over 3 % N were reported to be detrimental to animal production (Wilman, 1970). According to Eckard (1990), such levels could be expected at N fertilization levels of more than 350 to 375 kg N/ha per year.

Gomide *et al.* (1969), who studied the influence of N fertilization on tropical grasses, found that N fertilization increased the N content of grass and that most of the increase occurred soon after the application of N and declined as the plants matured.

In an experiment conducted by Gonzàlez Ronquillo *et al.* (1998) with *Cenchrus ciliaris*, it was found that while the N content increased with increasing levels of N fertilization, the NDF and ADF contents also tended to increase. The findings are tabulated in Table 5.

Table 5 Chemical composition (g/kg DM) of *Cenchrus ciliaris* as affected by level of N fertilization (Gonzàlez Ronquillo *et al.*, 1998)

Level of N	OM	N	NDF	ADF	ADL	ADIN
0 kg/ha	894	18.6	665	358	53	1.05
75 kg/ha	899	20.4	671	356	60	1.04
150 kg/ha	904	21.5	676	364	51	1.14

1.5.4 Total nonstructural carbohydrates

Total nonstructural carbohydrates are sources of readily available energy that enhance rumen microbial activity and forage utilization (Jung *et al.*, 1976).

When forage was fertilized with N, some authors, such as Nowakowski (1962), found that the concentration of TNC decreased considerably. The decrease was greater at higher rates of N application. Most of the reduction in TNC is found to be in the fructosans (Green and Beard, 1969). One of the main reasons for the decrease in fructosans is probably because the sugars are used in the vigorous growth of the leaves which resulted from N application (Nowakowski, 1962). The use of sugars evidently take place at the expense of carbohydrate reserves in the form of fructosans (Waite, 1970). Jones *et al.* (1965) also found a decrease in the TNC content of grasses and noted that this decrease was due mainly to a change in the proportion of fructosans in the plant, more than any other carbohydrate. Jones *et al.* (1962) found that micro-organisms use carbohydrates, especially glucose, sucrose and starch as a source of readily available energy. This source of energy must be available before the microorganisms can break down cellulose to carbohydrate molecules small enough to be used as a source

of energy. It was further reported that the soluble carbohydrate content of herbage is related to volatile fatty acid production in the rumen. The depression of carbohydrates by nitrogen fertilizers may, therefore, be of direct significance in the ability of the ruminant to make efficient use of food.

In a trial with defoliated Switchgrass (*P. virgatum*), George *et al.* (1989) found that N fertilization reduced TNC concentrations for all the defoliation treatments studied. Saibro *et al.* (1978) found that N fertilization substantially decreased the TNC concentration, regardless of growth stage at harvest.

From the above mentioned it is clear that N fertilization has a negative influence on the TNC content of plants.

1.5.5 Acid detergent lignin

Lignin is not a carbohydrate, but is very closely associated with this group of compounds (McDonald *et al.*, 1992). Lignin is found in the cell wall where it confers chemical and biological resistance to the cell wall and mechanical strength to the plant (McDonald *et al.*, 1992).

When grass is fertilized with N, it is often found that lignin content in plants increases. This increase can be explained as follows: Applied N stimulates biosynthesis of phenylalanine and tyrosine, which are precursors of phenylpropanoids from which lignin, is formed. Greater amounts of these substances are available when N supply is high. High N rates, however, also promote growth of new leaves and shoots low in lignin, which compensates for increases in lignin content of other tissues (Cherney *et al.*, 1992).

1.5.6 Nitrate Nitrogen

When nitrogen fertilization is applied to herbage, it is found that the nitrate nitrogen (NO_3-N) concentration in all plant fractions is increased with each increment of N fertilization (Friedrich *et al.*, 1977). Wilman (1965) found that this increase in NO_3-N content normally reaches a peak two weeks after the application of N fertilizer. When N above optimal rates is applied, the concentration of NO_3-N may exceed 0,5 %, which may be toxic to animals

(Deinum and Sibma, 1980). This toxic level of NO_3-N will not necessarily lead to the death of the animal, but can produce sub - clinical conditions which, may result in poor animal performance and general lack of condition (Walton 1983).

Nitrate poisoning is found when the animal takes in an excess of nitrate. The nitrate itself is not poisonous, but the ruminal microbes convert the nitrate to nitrite, which accumulates in the rumen (Jones *et al.*, 1965). The nitrite is then absorbed into the blood, where it converts hemoglobin to methemoglobin, a substance which is incapable of transporting oxygen (Walton, 1983). The rates of both nitrate and nitrite reduction in the rumen appear to be related to the overall metabolic rate of the microbial population. The ruminal contents of animals fed good quality rations can reduce nitrate and nitrite more rapidly than the ruminal content from animals fed rations containing less rapidly fermented material (Jones *et al.*, 1965). The TNC content of forage receiving high levels of N fertilization is reduced to a great extent. Animals receiving this herbage, therefore, have a much greater chance of nitrate poisoning. Marais (1980) also found that high nitrate levels reduced the digestion *in vitro* and that the rumen microbes did not seem to be able to adapt to these high nitrate levels.

According to Walton (1983), there are six common causes of high nitrogen content in forage tissues. They are:

- High applications of N fertilizer or high soil fertility,
- Drought conditions
- Damage to plant tissues (such as defoliation as a result of grazing or hail damage), which will stop or reduce photosynthetic activity.
- Low light intensity
- Plant species (some plants convert amino acids to proteins rather slowly)
- Management (if animals are made to graze closely, they will eat more of the lower stem tissue.

The correct level of N fertilization, as well as the correct management, is very important in preventing nitrate poisoning.

1.6 The influence of season on the nutritional value of grass

A major factor limiting the nutritional value of tropical pastures is the mature stage of growth at which most of the grass is grazed. This condition arises as a direct result of the seasonal nature and extreme variability of the rainfall, light intensity, and temperature which prevents pasture being stocked to the optimum during periods of active growth (Minson, 1971). Nothing can be done about the rainfall, but one can select the correct species and time to utilize a grass. The following sections will look at the influence of light intensity and temperature on the chemical composition of a grass.

1.6.1 Influence of light intensity on the chemical composition of grass

Light is the energy source for the plant as a living organism and the effect of light is exerted directly upon metabolism through photosynthesis. Several parameters are involved, including total light received, light intensity and day length. The end product of photosynthesis is glucose and added light promotes the accumulation of sugar and the general metabolism of N. Nitrate is reduced by increasing light through its reduction to ammonia and amino acid synthesis. Cell wall components decrease with increasing light, in all probability through dilution of the amounts of non-structural carbohydrates, amino acids and organic acids formed. It is almost universally agreed that the reduction in light intensity reduces the concentration of non-structural carbohydrates in the herbage of both grasses and legumes (Smith, 1973). Table 6 shows the water soluble carbohydrate concentration of perennial ryegrass after four weeks of growth at three different temperatures and light intensities.

Table 6 **Water soluble carbohydrate concentration in the herbage of perennial ryegrass after 4 weeks growth at 3 different temperatures and light intensities (Smith, 1973)**

Day/night temperature °C	Light intensity – cal/cm^2/day		
	490	350	90
25/20	21.2	18.8	8.9
20/15	26.7	21.2	7.9
15/10	33.2	28.4	9.0

1.6.2 The influence of temperature on the chemical composition of plants

Temperature is another environmental factor, which has a marked effect on the chemical composition of plants. Low digestibilities are associated with high temperatures and are the result of the combination of two main effects. Increased lignification of plant cell wall is apparently a peculiar effect of higher environmental temperatures. Increased temperature promotes more rapid metabolic activity, which decreases the pool size of metabolites in the cellular contents. Photosynthetic products are, therefore, more rapidly converted to structural components. This has the effect of decreasing nitrate, protein and soluble carbohydrates and increasing the structural cell wall components. Also, enzymatic activities associated with lignin biosynthesis are enhanced by increased temperature (Smith, 1973).

Smith (1973), found that the concentration of non-structural carbohydrates in leaves, stems or total herbage of grasses and legumes is generally higher in plants grown in cool rather than warm temperatures (Table 7).

Table 7 **Percentage of total water soluble carbohydrates in timothy plants at early anthesis following growth at 18/10 °C and 32/24 °C day/night temperatures with reversal of temperature regimes at inflorescence emergence (Smith, 1973)**

Plant part	Cool	Cool-warm	Warm	Warm-cool
Inflorescence	9.5	10.5	10.7	11.0
Leaf blades	16.4	7.0	8.1	10.1
Stems and sheaths	16.6	7.2	8.3	15.6
Stubble	25.5	17.6	19.0	19.0
Roots	8.4	3.2	6.5	8.2

The decrease in carbohydrate concentration appears to be more marked in the non-structural polysaccharide fraction (fructosans or starch) than in the sugar fraction (Smith, 1973). Temperature has its greatest overall effect on plant development in promoting the accumulation of structural matter. For example, plant species which remain vegetative, whether it is because of too low environmental temperatures during growth or because of a genetic characteristic, are almost always less lignified than those plants which develop to the flower stage under similar environmental conditions.

1.7 *Characteristics of Panicum maximum*

1.7.1 Habitat

P. maximum prefers damp conditions with fertile soils and is often found growing under trees or in shrubs and bushes (Pieterse *et al.*, 1997) or alongside rivers (Van Oudtshoorn, 1992). *P. maximum* is well adapted to a wide variety of soils with the exception of sandy and clay soils (Dickinson *et al.*, 1990).

The grass can withstand moderate frost conditions and needs a minimum of 500 mm of rain per year (Dickinson *et al.*, 1990). *P. maximum* will grow in acid or

alkaline soils, but the best results are obtained on neutral to slightly alkaline soils (Du Pisani *et al.*, 1989 as quoted by Relling, 1998).

Studies by Du Pisani *et al.* (1989) as quoted by Relling (1998), showed that soil acidity adversely affects dry matter yield as well as CP, P, Ca and Zn content of the grass.

1.7.2 Description of *P. maximum*

Panicum maximum is indigenous to South Africa. It is a perennial tufted grass and can reach heights of between 1 and 2 meters. The grass is described as follows by Van Oudtshoorn (1992): " A tufted perennial, sometimes with a short rhizome, culms of up to 2.5 m tall, occasionally rooting at the lower nodes. Inflorescence is an open panicle up to 400 mm long, with particularly the lower branches arranged in a whorl. Flowers from November to July. Spiculets up to 4 mm long, glabrous and hairy, often tinged with purple or entirely purple. Leaf blade up to 30 mm wide, flattened, glabrous or hairy, especially at ligule. Leaf sheath often densely hairy. Ligule an inconspicuous, short membrane".

1.7.3 General

P. maximum is considered a palatable grass and is very valuable as a pasture grass. Selected cultivars such as 'Green Panic' produces hay and standing hay of high quality. This grass is one of the best planted pastures and responds well to N fertilization (Van Oudtshoorn, 1992). A deficiency of N is characterized by poor leaf growth and a yellowish appearance. A soil P status of 15 to 20 mg/kg and a pH of between 4.5 and 6.5 is recommended for *P. maximum* (Dickinson *et al.*, 1990). With sufficient fertilization, a yield of 1 to 1.5 t dry matter /ha / 100 mm of summer rain, can be expected from this grass.

One of the disadvantages of *P. maximum*, is that is loses its vigour when over-grazed in the summer months.

1.7.4 *Panicum maximum* cultivars

According to different growth habits, *P. maximum* can be divided into two main groups, namely the medium to tall growing type such as Hamil, Vencidor and Coloniao and the low growing type such as Green Panic, Mutale, Gatton and Sabi (Steenekamp, 1995). The following is a brief description of each.

1.7.4.1 Green Panic

This cultivar has relatively fine leaves and stems and seldom grows taller than 1 m. Green Panic is much finer than Hamil and grows much more erect than Sabi, while the leaves are less blue-green. Although Green Panic is relatively drought resistant, it can also be planted in areas with a rainfall of up to 1700 mm per year. This cultivar is, however, not resistant to water logging. Green Panic will form seed through-out the whole summer (Dickinson *et al.*, 1990).

1.7.4.2 Gatton

This cultivar is very similar to Green Panic, except that it forms much larger tufts. More information on the ability of Gatton to withstand winters is needed to be able to make recommendations about its adaptability to different conditions (Dickinson *et al.*, 1990).

1.7.4.3 Hamil

This cultivar was imported to South Africa in 1986 as an alternative for Green Panic. It, however, developed a much coarser stem and broader leaves than was expected. Although Hamil is easily damaged by frost, it is still very palatable in winter. It seems as if this cultivar is very good for planting in warm, high rainfall regions where it has a high production potential (Dickinson *et al.*, 1990).

1.7.4.4 Sabi

This cultivar is indigenous to the Sabi River Valley in Zimbabwe and is the only one used commercially in that region. Sabi is resistant to Rootknot eelworm and can be used in rotation systems with tobacco. Sabi *Panicum* is very good for

making silage and standing hay and can grow on a great variety of soils (Dickinson *et al.*, 1990).

1.7.4.5 Mutale

It seems as if this cultivar has a wide adaptation, but too little information is available to make long term predictions. Mutale is very leafy and remains in the vegetative stage till late in the growing season. Because seed is only formed late in the season, Mutale is very good as standing hay (Dickinson *et al.*, 1990).

1.8 Voluntary intake of a grass and the different parameters that influence voluntary intake.

Voluntary intake (VI) of an animal can be defined as the amount of feed that will be eaten by an animal or group of animals in a specific time (Forbes, 1995). Different factors can influence the VI of animals. These factors include:

- Species difference
- Cultivar and selections
- Plant parts
- Stage of growth
- Soil fertility
- Climate
- Processing

When one examines the intake of different plant parts with different digestibilities, large differences are noted. Jarrige *et al.* (1974) found in a study with 75 dairy cows, that 75 % of the drop in intake was due to a decrease in digestibility *per se*. This decrease in intake, due to a decrease in digestibility was also observed by Minson (1984) in a study of the digestibility of five *Digitaria* species for sheep. The drop in intake can also be caused by the following three factors (Minson 1990):

- an increase in the proportion of stem
- a fall in the VI of both leaf and stem fraction and
- a nutrient deficiency in mature forages.

Stem is eaten in smaller quantities than leaf (Minson, 1990). Minson (1973) proved this with an experiment where leaf and stem fractions were fed separately to eight sheep. It was found that the mean VI of leaf was 46 % higher than that of stem. The higher intake of leaf can be associated with a shorter retention time of DM in the reticulo-rumen (mean 23.8 *v.* 31.8) which appeared to be caused by the large surface of the leaf fraction initially available to bacterial degradation (mean 128 v. 41 sq cm/g) (Minson, 1973). McLeod *et al.* (1990) also found the intake of leaf fractions to be higher than that of stem. These authors found that the intake of Western Wheatgrass leaves to be 8.23 kg / day, while that of stem was only 3.67 kg /day by cattle. Poppi *et al.* (1980) found that sheep ate 21 % more leaf than stem of Pangola grass (*Digitaria decumbens*) and Rhodes grass (*Chloris gayana*). This larger consumption of leaves was associated with a shorter retention time in the rumen rather than differences between the digestibility of stem and leaves (See Table 8).

Tabel 8 **Voluntary intake of leaf and stem fractions of tropical grasses, proportional breakdown of large particles during eating and time forage is retained in the rumen (Poppi et al., 1980)**

Measurement	Animal	Leaf	Stem	Difference
Large particle breakdown during eating (%)	Sheep	34	19	15
	Cattle	32	12	20
Time dry matter retained in rumen (hr)	Sheep	27	33	6
	Cattle	35	45	10
Voluntary intake (g/kg $W^{0.75}$)	Sheep	50	41	9
	Cattle	72	53	19

Except for the above mentioned factors, chemical composition of plants can also contribute to a change in intake. Van Soest (1965) stated that the chemical composition determines the nutritive value of forages. A chemical substance such as lignin is negatively associated with digestibility and influences the VI of plants negatively. As plants mature and go through different stages of maturity, such as pre-bloom, full bloom, dough stage and overripe, lignin and other structural carbohydrates such as NDF and ADF increase and the digestibility decreases. Laredo and Minson (1973) found that when plants mature the VI of both leaf and stem decrease. While the VI of leaf fractions declined from 69 to 52 g/kg $W^{0.75}$/day over 37 days, the corresponding decrease in VI of the stem fraction was from 49 to 35 g/kg $W^{0.75}$/day. This fall in VI was associated with an increase in lignin and other cell wall contents, as described by Van Soest (1965). Figure 7 shows the fall in VI with increasing maturity (Troelsen and Campbell, 1969).

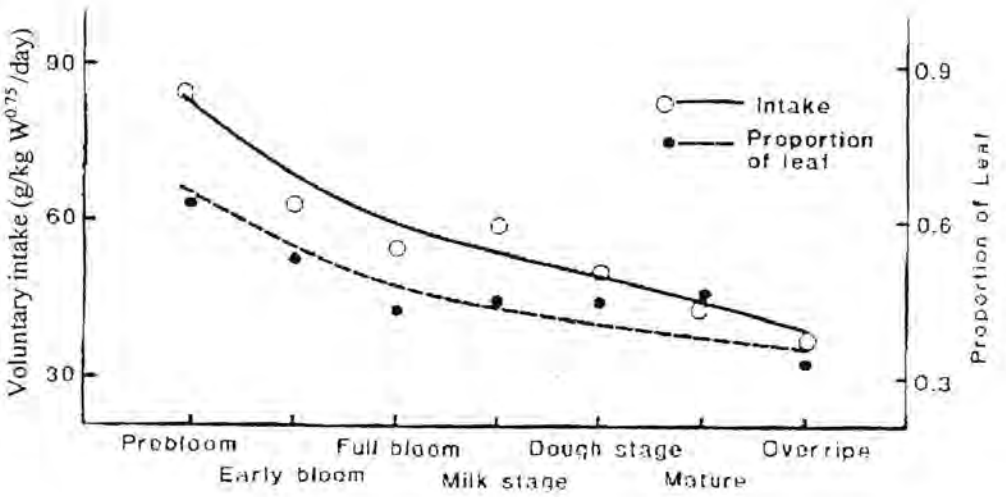


Figure 7 **Mean voluntary intake by sheep and proportion of leaf in four grasses cut at different stages of growth. (Data from Troelsen and Campbell (1969)**

A nutrient deficiency in mature forages can also cause a decrease in VI. Minson (1973) suggested that when nitrogen fertilization changes DM digestibility of tropical grasses, VI changes in the same direction. This tendency was also found by White (1985), who reported that N fertilization increased the average *in vitro* DM digestibility of forages by 0.1 percentage units. Another example of the influence of nutrient deficiency was reported by Minson and Milford (1967), as quoted by Minson (1973), who found that stem fractions from an 87-day regrowth of grass contained less than 1% nitrogen. With such feeds VI was likely to be depressed by a nitrogen deficiency. Minson (1973), reported in an experiment with *Chloris gayana*, *Digitaria decumbens* and *Pennisetum clandestinum*, that the intake of the tropical grasses was increased by 10 to 78 percent when the grass was fertilized with N. The control diet used in this trial contained less than 1 % N, a level considered necessary if voluntary intake is not to be limited by a protein deficiency. In the absence of a protein deficiency, it is possible that N applications to tropical grass pastures could change intake by affecting leafiness, flowering and dilution of other components of the diet.

1.9 Partial digestibility

1.9.1 Rate of passage of digesta through the rumen.

Ingested food and water can disappear from a compartment in two different ways: 1) through digestion and absorption and 2) through passage. Only escaping undigested matter passes down the tract to the next compartment. According to Van Soest (1994), the rate of passage of digesta refers to the passage of undigested matter through the rumen. Outflow from the rumen includes bacteria and some potentially digestible feed residues in addition to lignified fibre. At subsequent stages more digestion of bacteria and feed matter occurs. The final fecal residue is comprised mainly of bacteria and plant cell walls and some endogenous matter. The microbial and endogenous components arise during the course of digestion and passage and, to a limited extend, counterbalance the disappearance of matter through digestion (Van Soest, 1994).

The rate of passage of feed particles out of the rumen is believed to depend on level of feed intake and diet type as well as the shape and physical characteristics of the feed particles (Tamminga *et al.*, 1989). Grabber *et al.* (1992) reported that the digestion of fibre and reduction of its volume in the rumen depend, in part, on the digestion characteristics of its component cell types, such as parenchyma and sclerenchyma. The digestion characteristics of cell types depend on both extramural and chemical factors. Parenchyma cells are usually rapidly and extensively digested, due in part to their small size, thin cell walls, anatomical arrangement and large surface area exposed to rumen microorganisms after mastication. In contrast with this, sclerenchyma cells are slowly and incompletely digested, due in part to their large size, thick cell walls, association with poorly digested tissues and low surface area exposed to digestion after mastication (Akin, 1989). Chesson *et al.* (1986) found that the digestibility of leaf parenchyma (mesophyll) was greater than that of leaf sclerenchyma, even when extramural factors were eliminated by milling the isolated cells prior to digestion. Akin (1989) as well as Chesson *et al.* (1986) reported that lignification was associated with reduced digestibility of plant cells.

Grabber *et al.* (1992) found a reduced digestibility of grass stems as plants mature and indicated that this reduced digestibility was associated with a reduced digestibility of parenchyma.

It was found that by increasing the rate of feed intake, the passage of feed from the rumen could also be increased (Tamminga *et al.*, 1989). Owens and Goetsch (1986) as quoted by Tamminga et al. (1989), reported that by increasing the proportion of long roughage in the diet, the rate of passage of small particles also increased, although the increase was not linear.

1.10 Rumen parameters as influenced by stage of maturity and level of N fertilization

1.10.1 Volatile fatty acids

Volatile fatty acids (VFA) are the end products in the metabolism of carbohydrates (cellulose, glucose, sucrose, fructans etc.) by the rumen micro-organisms (McDonald *et al.*, 1992). The three most important VFA are propionic, acetic and butyric acids. Rumen VFA have been estimated to provide up to 70% of the metabolisable energy (ME) absorbed by sheep (Annison and Armstrong, 1970).

The molar proportions of VFA found in the rumen are generally assumed to represent the proportion in which the different VFA are produced (MacLeod *et al.*, 1984). The VFA concentrations in the rumen are influenced by the composition of the diet entering the rumen. Terry and Tilly (1961) reported an inverse relationship between the soluble carbohydrate level and the molar proportion of acetic acid in the rumen liquor of sheep fed on different ryegrass swards, although this association was lost when other grass species were included. The molar ratio of acetic acid in the rumen liquor has been found to be positively related to the fibre content of many diets. Michell (1974) found that the proportion of acetate in the rumen was the lowest in the spring and increased as the plants matured. This author claimed that the total concentration of VFA and therefore the apparent rate of VFA production, was most closely related to dry

matter digestibility and this indicates that pasture of high digestibility has high rates of VFA production.

Rumen pH also has a marked influence on VFA ratios and absorption. Michell (1974) found that the proportion of propionate was most closely related to total VFA concentration and to water soluble carbohydrate content. A number of authors have shown that feeds producing a low rumen pH, tend to produce a high proportion of propionate and also showed in an *in vitro* system that the artificial lowering of rumen pH caused a low acetic : propionic acid ratio. It is possible that pasture having a rapid VFA production rate can cause a low rumen pH and so a high proportion of rumen propionate (Michell, 1974).

Michell (1974) further reported a positive relation between propionate and water soluble carbohydrate concentration, which is probably due to the metabolic paths involved. This author also found that the correlation between the proportion of propionate and digestibility and intake showed that feed which produced high rumen propionic acid levels, are not necessarily feeds that have high digestible energy intakes.

1.10.2 Nitrate-ammonia concentrations in the rumen

The overall use of food N in ruminants can be affected by the efficiency of N use in the rumen for microbial synthesis as well as by the quality of food protein which reaches the intestine undegraded, compared with that of microbial protein (Oldham *et al.*, 1977). In the rumen NH_3-N is the major precursor of microbial protein. The need of rumen microbes for ammonia is satisfied at a concentration of 5mMol ammonia per 100 ml rumen fluid. Ammonia in excess of this will be absorbed from the rumen and lost as urinary urea (Oldham *et al.*, 1977).

1.10.3 Rumen pH as affected by stage of maturity and N fertilization

The normal pH in the rumen is kept between 5.5 and 6.5 (McDonald *et al.*, 1992). Krysl *et al.* (1987) reported that rumen pH increased with increasing levels of N fertilization.

Although Krysl *et al.* (1987) did not find an increase in rumen pH when plants matured, these authors suggested that there should be an increase in pH, since a smaller quantity of VFA was buffered by more saliva associated with increasing chewing time and rumination of dormant forages.

CHAPTER 2

THE INFLUENCE OF NITROGEN FERTILIZATION AND STAGE OF MATURITY ON THE DRY MATTER YIELD AND QUALITY OF *PANICUM MAXIMUM* CV GATTON DURING AUTUMN

2.1 Abstract

The effect of level of nitrogen fertilization and stage of maturity on the dry matter (DM) yield and chemical composition of *Panicum maximum* cv Gatton, during autumn, was studied. Seven nitrogen (N) treatments (0, 25, 50, 75, 100, 125 and 150 kg N/ha) were applied and the different parameters were measured at three stages of maturity, namely vegetative stage, early bloom and full bloom.
Nitrogen was applied in late summer. Samples were harvested at each stage and dry matter (DM) content and yield were determined. The samples were analyzed for N concentration, total nonstructural carbohydrates (TNC), nitrate-nitrogen (NO_3-N), neutral detergent fiber (NDF), acid detergent lignin (ADL) and *in vitro* organic matter digestibility (IVOMD).
Dry matter yields increased with increasing levels of N fertilization. Nitrogen, NO_3-N and ADL also increased significantly ($p \leq 0.05$), while TNC and NDF decreased significantly ($p \leq 0.05$) with increasing levels of N. There was no significant change in IVOMD.
With advancing maturity, N, NO_3-N and IVDOM decreased, while DM yield, TNC, NDF and ADL concentrations increased.

2.2 Introduction

With the increase in human population in South Africa, less land is available for animal production. On these limited areas, there is a growing interest in intensive grassland production (Salette, 1970). According to Wiedenfeld *et al.* (1985), numerous management techniques can be employed to enhance the

yield and quality of grass pastures. Nitrogen fertilization is one such strategy. It can be used to stimulate early spring or late autumn grass growth (Cook and Mulder, 1984), increase total pasture production and grass DM yields (Quinlan *et al.*, 1981) and alter the quality of a grass.

It is known that N fertilization will increase the N concentration of a grass, decrease soluble carbohydrate concentration (Minson, 1990), and increase NO_3-N concentration (Van Soest, 1994). These changes might be expected to have a major effect on dry matter digestibility, but most studies have shown only small differences.

Panicum maximum is a perennial tufted grass and can reach heights of one to two meters. In experiments, reported by Rodel and Boultwood (1971), it was found that *P. maximum* was one of the highest yielding tufted grasses.

Little work has, however, been reported on the *P. maximum* cultivar, Gatton. This paper describes the influence of a range of N fertilization levels and stage of maturity on dry matter (DM) yield, N concentration, total nonstructural carbohydrate (TNC), NO_3-N concentration, neutral detergent fiber (NDF), and acid detergent lignin (ADL) and *in vitro* organic matter digestibility (IVOMD) of *P. maximum* cv Gatton, during autumn, under dry land conditions.

2.3 Materials and methods

A small plot experiment was conducted during autumn, on a three year old stand of *P. maximum* cv Gatton, that was established on a deep red Hutton soil on the Hatfield Experimental Farm of the University of Pretoria at an altitude of 1372 m. Maximum temperatures varied between 18 and 32 ^{o}C with an annual rainfall of ± 700-mm, occurring mainly in the summer.

The trial was conducted as a simple factorial with seven levels of N, three stages of maturity and three replications laid out in a randomized block design. The N levels were 0, 25, 50, 75, 100, 125 and 150 kg N /ha and each N level was harvested at three stages of maturity, namely:

Vegetative stage ..... 20 - 30 cm high
Early bloom..... 30 - 40 cm high
Full bloom..... ..... 60 - 90 cm high

Each plot was 2m x 5m or 0.001 ha.

Nitrogen, in the form of limestone ammonium nitrate (LAN) (28 % N), was applied in mid-February. All the plots were also fertilized with 300 kg KCl to prevent any potassium deficiencies.

DM yield was determined by harvesting an area of 1 x 5m in the middle of each plot and determining the oven dry DM content.

Hand clipped samples were taken at each maturity stage. The grass samples were clipped weekly at 08h00 and frozen immediately. At the end of the sampling period of 10 weeks, the weekly samples were pooled and the samples for each maturity stage were weighed, freeze-dried, ground and stored in glass bottles for further analysis.

The following analyses were conducted. Dry matter concentration was determined according to AOAC (1980). All results were calculated on a dry matter basis.

Total N was determined by Kjeldahl procedures and included both organic and inorganic N. The TNC was determined using the technique described by Marais (1979). Total nonstructural carbohydrates (TNC) were analyzed as reducing sugars after quantitative hydrolysis to monosaccharides by means of carefully controlled acid hydrolysis procedures (Marais, 1979). The reducing sugars formed during hydrolysis were determined quantitatively by the modified Nelson-Somogyi method (Morrison and Boyd, 1966). This procedure is not suitable for samples containing starch, but can be used for analyzing temperate (C_3) grasses, storing fructose and sub-tropical grasses (C4), storing glucose.

For the determination of NO_3-N, the analysis was based on the procedures described by Cataldo *et al.* (1975). These are based on the nitration of salicylic acid under highly acidic conditions and the calorimetric determination of the

resulting coloured complex which absorbs maximally at 410 nm in basic (pH > 12) solutions.

Acid detergent lignin was determined using the procedures described by Goering and Van Soest (1970), while NDF was determined using the procedures described by Robertson and Van Soest (1981).

In vitro organic matter digestibility of the samples was determined by making use of the technique of Tilley and Terry (1963) as modified by Engels and Van der Merwe (1967).

2.4 Statistical analysis

For this trial, the GLM (General Linear Modules) procedure by Statistical Analysis Systems (SAS, 1995), was used to analyze the data.

All main effects (treatment, replication and period) as well as all possible first-order interactions were included in the initial model. Interactions that did not make a significant ($p > 0.05$) contribution to the variance, were omitted in subsequent analyses.

Models were tested for the dependent parameters namely DM yield, N, NDF, NO_3-N, TNC, ADL and IVDOM.

Significance of difference between least square means was determined by Bonferoni's test (Van Ark, 1981).

2.5 Results and discussion

2.5.1 Dry matter yield

DM yields, as affected by N fertilization levels, are presented in Figure 8.

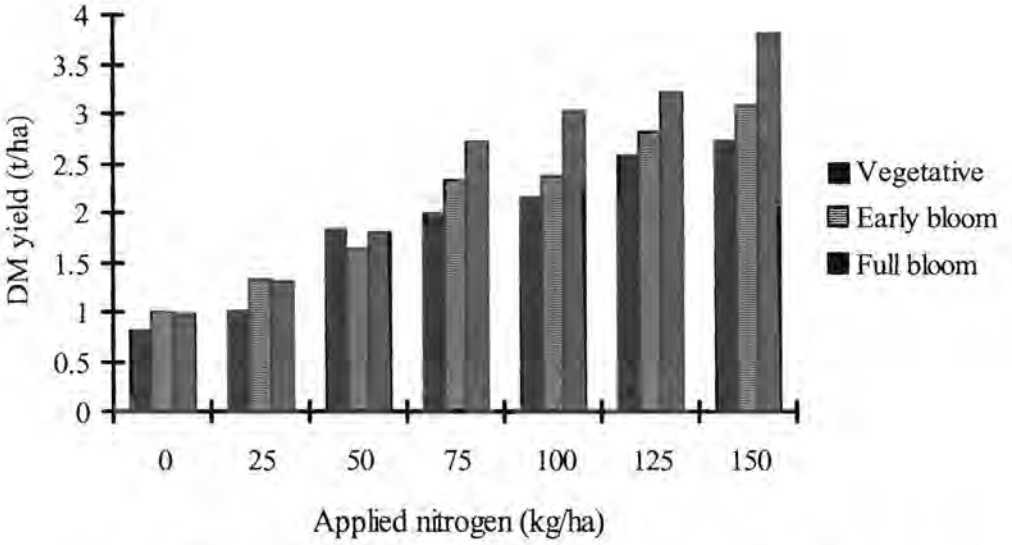


Figure 8 DM yield (t/ha) of *P. maximum* cv Gatton, as affected by level of N fertilization and stage of maturity during autumn

Dry matter yield increased linearly (Fig. 9) with increasing levels of fertilization. This response did not, however, exhibit the characteristic response curve of grasses to fertilizer N, probably because of the relative small range of N levels used in this experiment or the limited growth period (production was only assessed in the latter part of the growing season). Morrison and Russell (1980) summarized the response curve as follows: 'Response is linear at low N applications, reaching a maximum yield and subsequently declining at high rates of application'. In this experiment only the linear response was observed. Nitrogen fertilization levels were, therefore, not high enough for DM yield to reach a maximum and to start declining. Reid (1967) found that with a pure grass sward DM yield started to decline at N levels of about 150 kg/ha.

DM yield also increased with advancing maturity (Fig. 8). Forwood *et al.* (1988) also found that Caucasian Bluestem exhibited a linear increase in DM yield as the grass matured.

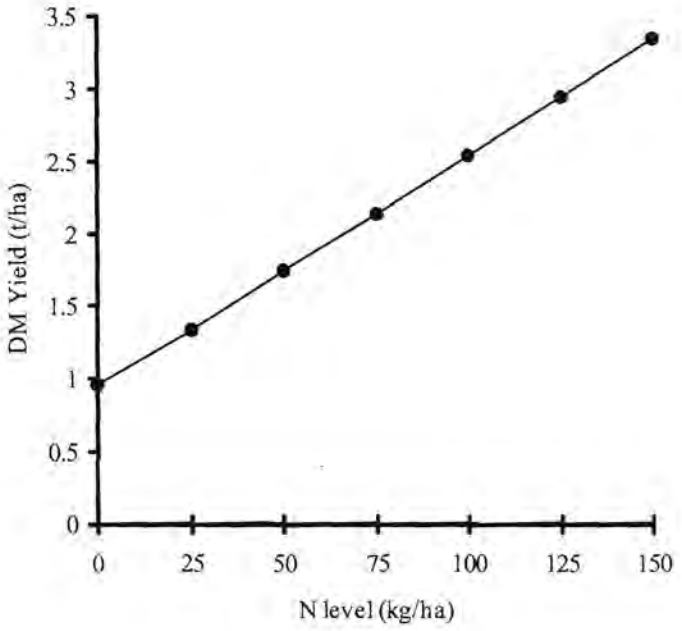


Figure 9 **The relationship between DM yield and N level of *P. maximum* cv Gatton ($Y = 0.016x + 0.94$, $r^2 = 0.71$)**

2.5.2 Nitrogen concentration

Nitrogen fertilization had a marked influence on the N concentration of plants. Figure 10 illustrates the influence of seven levels of N fertilization on the N concentration of plants during three stages of maturity.

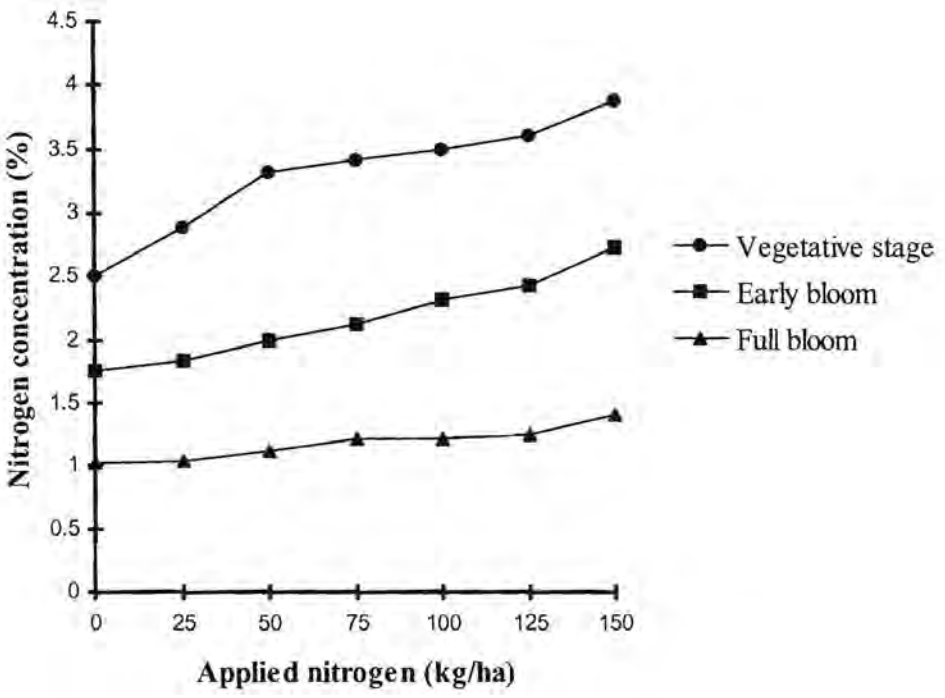


Figure 10 **Nitrogen concentration of *P. maximum* (cv. Gatton) during three stages of maturity as influenced by level of N fertilization**

The N concentration of plants increased significantly ($p \leq 0.05$) with increasing levels of N fertilization, but decreased with advancing maturity (Fig. 10). These findings correspond well with work done by Blaser (1964), Gomide *et al.* (1969) and Van Niekerk *et al.* (1993). The highest N concentration in plants was observed shortly after the application of N in the vegetative stage at 150-kg N/ha and declined significantly with maturity. According to Jones and Wilson (1987) N concentration is strongly influenced by the stage of growth. Young vegetative growth is high in N, but the concentration falls rapidly as the proportion of leaf decreases (Jones and Wilson, 1987) and that of flowers and stems increases (Fleischer *et al.*, 1983). Stems and flowers are generally lower in N than green leaves, which probably explains the lower N concentration with advancing maturity (Fleischer *et al.*, 1983).

2.5.3 Total nonstructural carbohydrates (TNC)

Figure 11 illustrates the influence of N fertilization, during three stages of maturity, on the TNC concentration of grass.

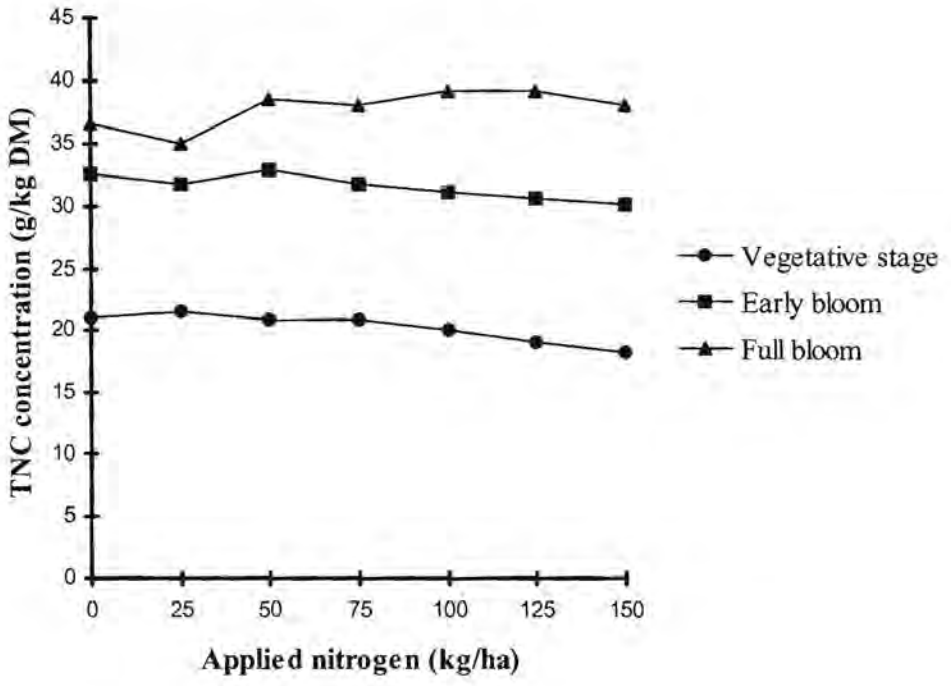


Figure 11 TNC concentration of *P. maximum* cv Gatton during three stages of maturity as influenced by level of N fertilization

Increased levels of N fertilization had only a slight, non-significant ($p \geq 0.05$) depressing effect on the TNC concentration of plants. This decrease was probably due to the accelerated growth of herbage which was promoted by N fertilization (Jones *et al.*, 1965; Waite, 1970; Jones and Wilson, 1987) and appears to be the result of a change in the proportion of fructosans relative to that of the soluble sugars, namely glucose, fructose and sucrose (Nowakowski, 1962). Accelerated growth reduces fructosans, since they are used for the vigorous growth of leaves that results from the application of N fertilization. The use of sugars evidently take place at the expense of carbohydrate reserves in the form of fructosans (Nowakowski, 1962).

As plants became older the TNC concentration increased significantly ($p \leq 0.05$) (Fig 11). This may be explained by a slower growth rate of the plants, but also by the ratio of stem to leaf tissue. With advancing maturity more stems developed, which are the storage areas for fructosans. With an increase in stems the TNC concentration will, therefore, increase (Smith, 1973).

2.5.4 Nitrate-nitrogen concentration

In Figure 12 the influence of N fertilization and stage of maturity on the NO_3-N concentration of plants, is illustrated.

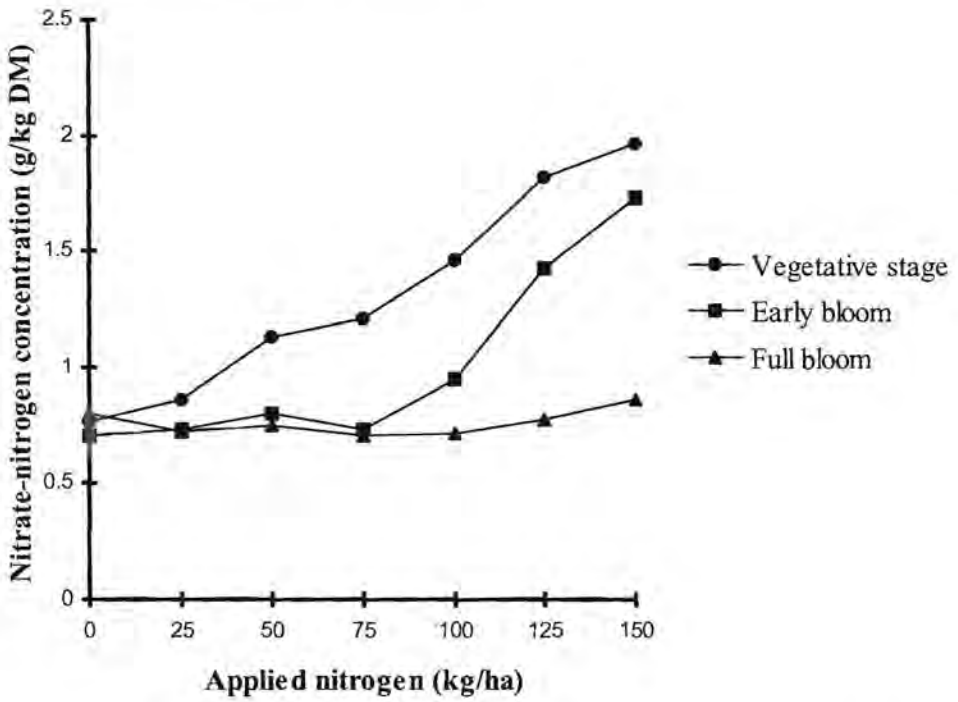


Figure 12 The NO_3-N concentration of *P. maximum* cv Gatton at three stages of maturity as influenced by level of N fertilization

Nitrogen fertilization had a strong significant ($p \leq 0.05$) influence on the NO_3-N concentration of a plant. During the vegetative stage, shortly after N application, the NO_3-N concentration of plants receiving 150 kg N/ha, was more than double that of the control plants which received no N (Fig. 12).

As plants matured the NO_3-N levels were significantly ($p \leq 0.05$) lower, especially at higher levels of N fertilization. By the time plants reached full bloom, there was little difference in NO_3-N concentration of plants receiving 0 kg N/ha and those receiving 150 kg N/ha.

These results confirm the findings of Madison and Kenneth (1963) who reported that most of the chemically combined N absorbed by plants is in the form of NO_3-N. The accumulation of NO_3-N, therefore, implies that the rate of assimilation has not kept pace with the rate of uptake. Such concentrations are often only temporary, diminishing as the plant ages, until at maturity little or no NO_3-N can be detected (Madison and Kenneth, 1963). The rate of N uptake is related to the specific species involved. Age also plays a role in the uptake of N. As plants mature, less and less NO_3-N is taken up. The importance of age itself is unknown, but alternative explanations for the decline in NO_3-N uptake, are available. One of these is the changing proportion of stem, leaf and fruit with maturation. Fruits and seed usually contain very little NO_3-N, and as they increase in DM the effect of high NO_3-N concentration in other parts tends to be diluted. Secondly, the formation of fruit or seed makes a heavy demand on NO_3-N (Madison and Kenneth, 1963) which will further lower the NO_3-N concentration of mature plants.

When grass is fertilized at different levels of N, different effects on NO_3-N and TNC concentration of plants are observed. When high levels of N are applied, NO_3-N concentrations are high while the TNC concentration is low, but when N fertilization is low it does not have as large an influence on NO_3-N as it has on lowering the TNC concentration. There seemed, therefore, to be a negative relationship between TNC concentration and NO_3-N accumulation. This negative relationship was confirmed by Jones *et al.* (1962) in work on perennial ryegrass. The presence of a large supply of nutrient N stimulates the plant to draw upon it's reserves of available carbohydrates for reductive energy and carbon skeletons, and eventually the carbohydrate reserves may be insufficient to keep pace with NO_3-N uptake. In experiments with corn seedlings grown in nutrient solutions,

Burt (1963), as quoted by Madison and Kenneth (1963), found that as the total carbohydrate concentration of the DM decreased from 22 % to 14 %, the NO_3-N concentration rose from 0,02 to 0,80 %. It is, therefore, evident that a plant high in NO_3-N is likely to be low in readily available carbohydrates.

2.5.5 *In vitro* organic matter digestibility (IVOMD)

The influence of N fertilization and stage of maturity on the IVOMD of *P. maximum* cv Gatton is illustrated in Table 9.

Table 9 ***In vitro* organic matter digestibility (%) of *P. maximum* cv Gatton as influenced by level of N fertilization and stage of maturity, during autumn**

kg N/ha	Vegetative stage	Early bloom	Full bloom	Mean
0	$73.9^{a}{}_{1}$	$70.9^{a}{}_{1}$	$65.0^{ab}{}_{2}$	**69.9**
25	$74.8^{a}{}_{1}$	$71.9^{a}{}_{1}$	$64.8^{ab}{}_{2}$	**70.5**
50	$75.4^{a}{}_{1}$	$71.2^{a}{}_{2}$	$64.3^{ab}{}_{3}$	**70.3**
75	$74.8^{a}{}_{1}$	$71.6^{a}{}_{1}$	$65.1^{ab}{}_{2}$	**70.5**
100	$75.6^{a}{}_{1}$	$72.3^{a}{}_{1}$	$64.9^{ab}{}_{2}$	**70.9**
125	$74.8^{a}{}_{1}$	$71.3^{a}{}_{1}$	$63.6^{b}{}_{2}$	**69.9**
150	$74.7^{a}{}_{1}$	$71.7^{a}{}_{1}$	$67.1^{a}{}_{2}$	**71.2**
Mean	**74.86**	**71.56**	**64.97**	

Values in the same column and in the same row with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$)

The IVOMD of the forage was generally not affected by N fertilization. Though the values seemed to increase slightly with higher N levels, the increase was not statistically meaningful ($p > 0.05$). According to Spedding and Diekmahns (1972) there is no evidence that the application of N fertilization significantly affects digestibility. Prins and Van Burg (1979), however, pointed out that N fertilization allows more frequent harvesting and hence indirectly increases herbage

digestibility. Van Niekerk *et al.* (1993) found no significant increase in IVOMD of *P. maximum* cv Gatton as N levels increased. Saibro *et al.* (1978) also reported that N fertilization did not affect the IVOMD of plants during the vegetative stage, but it did tend to depress the IVOMD slightly at the seed stages, although this decrease was not significant. This trial confirms reports that N fertilization does not alter IVOMD of herbage significantly.

As plants matured, however, IVOMD declined. Such a decline was also reported by Forwood *et al.* (1988) in work done on Caucasian Bluestem. In very young plants the stem is often more digestible than the leaves. As plants mature, however, the digestibility of the leaf fraction decreases slowly, while that of the stem fraction declines rapidly. In mature plants, stem comprises a much larger proportion of the whole plant than leaves. *In vitro* organic matter digestibilities in mature plants will, therefore, decrease because of the large amount of less digestible stems (McDonald *et al.*, 1992).

2.5.6 Acid detergent lignin (ADL)

The ADL concentration of *P. maximum* cv Gatton as influenced by N fertilization and stage of maturity, is represented in Table 10.

Table 10 Acid detergent lignin (%) of *P. maximum* cv Gatton as affected by level of fertilization and stage of maturity during autumn

kg N/ha	Vegetative stage	Early bloom	Full bloom	Mean
0	$4.0^{a}{}_{1}$	$4.3^{a}{}_{1}$	$4.2^{b}{}_{1}$	**4.2**
25	$3.9^{ab}{}_{2}$	$3.9^{a}{}_{2}$	$4.4^{ab}{}_{1}$	**4.1**
50	$4.0^{a}{}_{2}$	$4.2^{a}{}_{2}$	$4.5^{ab}{}_{1}$	**4.2**
75	$3.8^{ab}{}_{2}$	$3.9^{a}{}_{1,2}$	$4.2^{b}{}_{1}$	**4.0**
100	$3.8^{ab}{}_{1}$	$3.9^{a}{}_{1}$	$4.6^{a}{}_{2}$	**4.1**
125	$3.6^{b}{}_{1}$	$4.0^{a}{}_{2}$	$4.4^{ab}{}_{3}$	**4.0**
150	$4.1^{a}{}_{1}$	$4.0^{a}{}_{1}$	$4.2^{b}{}_{1}$	**4.1**
Mean	**3.9**	**4.0**	**4.4**	

Values in the same column and in the same row with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).

Acid detergent lignin concentration of herbage increased significantly ($p \leq 0.05$) with advancing maturity. This is probably because of an increase in cell wall concentration with maturity (Biblack and Buxton, 1992).

Vicente-Chandler *et al.* (1959) found that when N fertilization was applied to herbage, the lignin concentration of plants was increased. This is in contrast with what was found in this experiment, since no significant change in lignin concentration could be found in this experiment. Kaltofen (1988) gave the following explanation for this: "High N rates promote growth of new leaves and shoots low in lignin which compensates for increase in the lignin concentration of other tissues".

The interest in lignin lies in the influence it has on the decline in IVOMD of grass as it matures. Lignin is limited to the cell wall (Van Soest, 1975) and since cell wall components increase with maturity, lignin also increases. Lignin can inhibit digestion of grass in the rumen by preventing the physical attachment of bacteria to the cell walls (Richards, 1976, as quoted by Jones and Wilson, 1987) or by inhibiting enzyme attack through linkage to cell wall polysaccharides.

2.5.7 Neutral detergent fibre (NDF)

Table 11 illustrates the influence of N fertilization and stage of maturity on the NDF concentration of *P. maximum* cv Gatton, during autumn.

Table 11 NDF (%) concentration of *P. maximum* cv Gatton as influenced by level of N fertilization and stage of maturity during autumn

kg N/ha	Vegetative stage	Early bloom	Full bloom	Mean
0	$59.3^{a}{}_{3}$	$62.7^{a}{}_{2}$	$65.9^{a}{}_{1}$	**62.6**
25	$58.3^{ab}{}_{3}$	$62.5^{a}{}_{2}$	$65.5^{a}{}_{1}$	**62.1**
50	$56.8^{bc}{}_{3}$	$63.5^{a}{}_{2}$	$65.8^{a}{}_{1}$	**62.0**
75	$56.8^{c}{}_{3}$	$62.7^{a}{}_{2}$	$65.9^{a}{}_{1}$	**61.8**
100	$56.7^{c}{}_{3}$	$63.0^{a}{}_{2}$	$65.4^{a}{}_{1}$	**61.7**
125	$56.6^{c}{}_{3}$	$63.3^{a}{}_{2}$	$65.8^{a}{}_{1}$	**61.9**
150	$56.3^{c}{}_{3}$	$60.3^{b}{}_{2}$	$65.3^{a}{}_{1}$	**60.6**
Mean	**57.26**	**62.57**	**65.66**	

Values in the same column and in the same row with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).

As N fertilization levels were increased, there was a significant ($p \leq 0.05$) decrease in NDF concentration of plants. This decrease was, however, only evident during the vegetative stage and with the highest N level of the early bloom stage. Van Niekerk *et al.* (1993) also found that in *P. maximum* cv Gatton, NDF concentration decreased with increasing levels of N fertilization. With advancing maturity there was a significant ($p \leq 0.05$) increase in NDF concentration of plants. These findings correspond well with those reported by Rouquette *et al.* (1972). NDF consists of cellulose, hemicellulose and lignin. As plants mature, these structural carbohydrates increase to serve as a support system for the plants.

2.6 Conclusion

Nitrogen fertilization had many different effects on the yield and quality of *P. maximum* cv Gatton. During the autumn, N fertilization increased the DM yield from 0.94 t/ha, when no N was applied, to 3.21 t/ha when 150 kg/ha N was applied. Nitrogen fertilization also increased the N concentration of plants, but tended to decrease the TNC and NDF concentration. One further disadvantage of high levels of N is that it increased the NO_3-N concentration of plants from a mean of 0.88 g/kg DM, when no N was applied, to a mean of 1.62 g/kg DM, when 150 kg/ha N was applied. This increase is especially evident shortly after N application in the vegetative stage.

Stage of maturity is another factor affecting the quality and quantity of *P. maximum* cv Gatton. With advancing maturity DM yield was increased from a mean of 1.88 t/ha to 2.41 t/ha.

As plants grew older the quality declined. Acid detergent lignin and NDF increased from 3.9 to 4.4 % and from 57.26 to 65.66 % respectively, while the IVOMD decreased with 10 percentage units from 74.86 % to 64.97 %.

With advancing maturity the very high NO_3-N concentration that was seen during the vegetative stage, declined from 1.31 g/kg DM to a more acceptable 0.76 g/kg DM, while TNC concentration increased from 20.24 g/kg DM to 37.79 g/kg DM. Nitrogen concentration also increased as the grass matured.

From this trial it is evident that there is a very fine compromise between the amount of nitrogen fertilization to be used and the stage of maturity at which the grass must be utilized.

CHAPTER 3

THE INFLUENCE OF NITROGEN FERTILIZATION AND STAGE OF MATURITY ON INTAKE, RUMEN CONDITIONS AND ORGANIC MATTER DISAPPEARANCE IN SHEEP GRAZING *PANICUM MAXIMUM* CV GATTON DURING AUTUMN.

3.1 Abstract

A study was conducted during autumn to determine the influence of nitrogen (N) fertilization and stage of maturity on the quality and voluntary intake of *P. maximum* cv Gatton as well as the disappearance of digesta in the digestive tract of sheep. Pastures were fertilized with 0, 75 and 150 kg N /ha. Sheep fitted with rumen, abomasum and ileal fistulas were used to obtain samples of digesta flow. Three sheep fitted with oesophageal cannulas, were used to obtain samples of pasture selected by the grazing sheep.

Nitrogen fertilization increased the N concentration of plants, while it had no effect on neutral detergent fibre (NDF), acid detergent lignin (ADL) or *in vitro* digestibility (IVDOM) of plants. With advancing stage of maturity nitrogen concentration and digestibility of plants decreased, while NDF and ADL concentration increased.

The disappearance of organic matter (OM) from the rumen was increased when animals grazed pasture fertilized with 0 or 150 kg N/ha, while it was decreased when animals grazed pasture fertilized with 75 kg N/ha. More OM therefore, seemed to be absorbed in the small intestine as amino acids in the latter treatment.

3.2 Introduction

It is known that N fertilization will increase the dry matter (DM) yield (Quinlan *et al.*, 1981), N concentration and nitrate-N concentration of a grass, but depress the carbohydrate concentration, while stage of maturity will increase DM yield, ADL and carbohydrate concentration and depress N and nitrate concentration.

This is also true for *P. maximum* cv Gatton. The increase in DM yield with increasing levels of N fertilization is especially important, since areas for increased animal production in South Africa are limited (Salette, 1970).

All the above parameters reflect the influence of N fertilization and stage of maturity on the grass itself. How will this fertilization, at different stages of maturity, influence the grass utilization by the grazing animal?

This report describes the influence of N fertilization and stage of maturity on voluntary intake, different rumen conditions and the passage of digesta through the digestive tract of the animal.

3.3 Materials and Methods

For this experiment nine paddocks of three different sizes (0.04 ha, 0.08 ha and 0.16 ha) were used. The camps were situated on the Hatfield Experimental Farm of the University of Pretoria, South Africa. The site description on Hatfield Experimental Farm, is as follows:

Locality:	28° 16' E, 25° 45'S
Altitude:	1372 m
Average annual rainfall:	709 mm
Average annual rainfall for experimental period:	Average rainfall was simulated to achieve the long term average of 709 mm per annum.
Average annual temperature:	30°C (Jan), 2°C (June)
Average temperature for experimental season:	29.7oC (Jan), 5.5°C (June)

The pastures used were established in the summer of 1988 on a deep red Hutton soil. For this trial, the camps were mown at the beginning of February. After mowing, 0, 75 and 150 kg of N per ha were applied to the different paddocks. This was done as follows: The 0.04 ha camps received 150 kg N / ha, the 0.08 ha camps received 75 kg N / ha and those of 0.16 ha received no N. All the camps received 300 kg of KCl /ha, on the basis of soil analysis, to prevent any K

deficiencies during the investigation. No P fertilizer was applied because the phosphorous status of the soil was relatively high (approximately 30 ppm).

Each N fertilization level was evaluated at three stages of maturity; namely vegetative stage, early bloom and full bloom.

For the partial digestibility experiment, five Döhne merino wethers, were used. Each animal was fitted with a rumen cannula and T-shaped cannula in the abomasum as well as in the terminal ileum. Each animal was also fitted with a harness and faeces bag. Because the experiment was conducted in the field and not in metabolism cages, each animal was also fitted with a peristaltic pump and two marker bags on it's back (Corbett *et al.*, 1976). The animals were allowed free access to water during the trial. Before the start of the experiment, animals were adapted to the specific pasture for seven days. After the adaptation period, Cr-EDTA was prepared according to the procedure of Binnerts *et al.* (1968), and continuously infused (240 mg Cr/day) by a peristaltic pump fitted to the back of each animal, into the rumen of the sheep. Yb acetate, dissolved in distilled water, was also infused (100 mg Yb/day) into the rumen.

Cr-EDTA and Yb acetate were infused into the rumen via different infusion lines from two separate marker bags. The reason for this was that if Cr-EDTA and Yb acetate were mixed, a precipitate might be formed on standing (Siddons *et al.*, 1985). The technique of Faichney (1980) was used to estimate, via the two markers, digesta flow in the digestive tract.

The infusion of markers was done for four days, starting with a prime dose, before sampling started on day five (Faichney, 1980). Samples were taken from the rumen, abomasum and ileum. Samples were collected every 12 hours as follows:

Day 5	06:00	and	18:00
Day 6	09:00	and	21:00
Day 7	12:00	and	00:00
Day 8	15:00	and	03:00

A complete collection of faeces was also done during the four days of sampling.

3.3.1 Treatment of collected samples

Each rumen sample was filtered and the pH was recorded after filtration. Twenty ml of each rumen sample was acidified with 4 ml 0.5 M H_2SO_4 for ammonia analysis, while another 20 ml was preserved with 2 ml of a 10 % NaOH solution for volatile fatty acid analysis. After these treatments, the samples were frozen and kept for further analyses. Fifty ml of each abomasum and ileum sample was stored frozen without any added preservatives. Faeces were collected twice in every 24-h period. The two collections were mixed together, weighed and a 10 % sample was stored frozen. Faeces were collected for four days. Each sheep's samples, collected over the four day sampling period, were stored together and analyzed as one sample for each sheep.

Five sheep, fitted with oesophageal fistulas, were used to obtain a sample of the grass selected by the grazing animals. Ten samples were taken from each camp, five at the beginning and five at the end of a sampling period. The first and second samples were analyzed separately and a mean value was then calculated from the values obtained.

Oesophageal collected samples were obtained by initially fasting animals for three hours. The animals were then put to the pasture for 45 minutes while samples were collected in oesophageal bags fastened around their necks. Most of the saliva was squeezed out of the samples using a double layer of cheesecloth, before they were frozen.

3.3.2 Preparation of samples

All the samples were thawed overnight. Separation of the soluble and particulate matter of the thawed samples of the rumen, abomasum and ileal digesta was achieved by high-speed centrifugation at 5000 rpm for 20 minutes. The clear centrifuge of all the samples were stored in glass bottles in the refrigerator.

Abomasum samples were dried at 55 ^{0}C for 48 hours, while ileum samples were freeze - dried. The dried abomasum and ileum samples were milled and stored in bottles.

Samples of the dried faeces samples were also milled and stored in bottles.

3.3.3 Analytical methods

Dry matter (DM) of abomasum and ileum samples were determined by drying 5 ml of the samples for 24 hours at 100 ^{0}C. Dry matter of faeces was also obtained by drying a small sample for 24 hours at 100 ^{0}C.

Organic matter of all the samples was determined by ashing the dried samples at 550 ^{0}C for 3 hours.

Total N in abomasum, ileum and faeces samples were determined by the Kjeldahl procedure and ammonia-N in the rumen by the use of an Auto Analyzer II. The concentration of N in the rumen samples was calculated as follows:

$$\text{mmol N / litre in the test sample} = \text{mmol N / litre in the standard} \times \frac{\text{peak height for sample}}{\text{peak height for standard}}$$

Corrections must be made for dilution of the samples e.g. the addition of the sulphuric acid solution as preservative.

$$\text{mmol N / litre} \times 14 = \text{mg N / litre rumen digesta.}$$

Volatile fatty acids were determined by means of injecting the "supernatant" into a gas chromatograph. The Carlo Erba 4200 gas chromatograph with flame ionisation detector was used, fitted with a 20 m glass column (3mm internal diameter) packed with 60/80 Carbopack c/0.3 % carbowax 20 M /0.1 % H_3PO_4. The column was conditioned overnight at 153 ^{0}C and a flow of ±15 ml N per minute.

ADL was determined using methods described by Goering and Van Soest (1970), while NDF was determined by the methods of Robertson and Van Soest (1981). *In vitro* digestibility of the oesophageal samples was determined by making use of the technique described by Tilley and Terry (1963) and modified by Engels and Van der Merwe (1967).

3.4 Statistical analysis

The GLM (General Linear Modules) procedure by Statistical Analysis Systems (SAS, 1995), was used to analyze the data.

All main effects (treatment, replication and period) as well as all possible first-order interactions were included in the initial model. Interactions that did not make a significant ($p > 0.05$) contribution to the variance, were omitted in subsequent analysis.

Models were tested for the dependent parameters namely DM yield, N, NDF, TNC, NO_3-N, ADL, IVDOM, intake, rumen pH, volatile fatty acids, rumen ammonia (NH_3-N) and organic matter (OM) disappearance in the rumen and small intestine.

Significance of difference between least square means was determined by Bonferoni's test (Van Ark, 1981).

3.5 Results and discussion

3.5.1 Intake study

The quality parameters of grass samples collected from oesophageal fistulated animals are reported in Table 12.

Table 12 Quality parameters of oesophageal samples of *P. maximum* cv Gatton during autumn

Parameters	kg N/ha	Vegetative	Early Bloom	Full Bloom
N (g/kg DM)	0	$2.38^{a}{}_{3}$ (±0.2)	$1.43^{b}{}_{3}$ (±0.1)	$1.56^{b}{}_{2}$ (±0)
	75	$2.62^{a}{}_{2}$ (±0.2)	$1.85^{b}{}_{2}$ (±0.1)	$1.63^{b}{}_{2}$ (±1.0)
	150	$2.98^{a}{}_{1}$ (±0.2)	$3.08^{a}{}_{1}$ (±0.1)	$2.35^{b}{}_{1}$ (±0.1
NDF (%)	0	$63.66^{b}{}_{1}$ (±2.2)	$68.45^{a}{}_{1}$ (±2.2)	$61.15^{b}{}_{2}$ (±1.1)
	75	$63.92^{b}{}_{1}$ (±3.2)	$68.20^{a}{}_{1}$ (±1.3)	$66.05^{ab}{}_{1}$ (±3.2)
	150	$63.50^{b}{}_{1}$ (±1.8)	$62.32^{b}{}_{2}$ (±2.6)	$67.67^{a}{}_{1}$ (±2.3)
ADL (%)	0	$4.10^{ab}{}_{1}$ (±0.6)	$3.58^{b}{}_{1}$ (±0.5)	$4.65^{a}{}_{1}$ (±0.6)
	75	$4.28^{a}{}_{1}$ (±0.7)	$4.15^{a}{}_{1}$ (±0.4)	$5.00^{a}{}_{1}$ (±0.8)
	150	$3.5^{b}{}_{1}$ (±0.3)	$3.5^{b}{}_{1}$ (±0.3)	$4.58^{a}{}_{1}$ (±0.5)
IVDOM (%)	0	$72.^{a}{}_{1}$ (±0)	$70.1^{ab}{}_{1}$ (±0)	$65.6^{b}{}_{1}$ (±0)
	75	$75.2^{a}{}_{1}$ (±0)	$66.5^{b}{}_{1}$ (±0)	$61.8^{b}{}_{1}$ (±0)
	150	$73.8^{a}{}_{1}$ (±0)	$66.3^{b}{}_{1}$ (±0)	$60.2^{b}{}_{1}$ (±0)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).
The values in brackets indicate standard deviation.

Samples collected from oesophageal fistulated animals had a high N concentration for all the N treatments. Nitrogen fertilization increased the N concentration of the selected diet significantly ($p \leq 0.05$), while there was a significant ($p \leq 0.05$) decrease in N in the selected diet as plants matured. This could be seen for all the treatments. These findings are supported by workers, such as Wiedenfeld *et al.* (1985), who found a linear increase in N concentration in their study of Buffelsgrass and 'Pretoria 90' Bluestem's response to N fertilization. Saibro *et al.* (1978) also found an increase in N concentration when grass was fertilized with increasing levels of N. According to Nowakowski (1962), pastures fertilized with high levels of N may supply animals with a

unbalanced ration of N which may exceed requirements. This may result in nutritional disorders, particularly in dairy cattle.

Neutral detergent fibre concentration in selected diets decreased with advancing maturity when no N fertilization was applied, but increased with advancing maturity when grass was fertilized with either 75 or 150 kg N/ha. During the vegetative stage NDF concentration tended to decrease with increasing levels of fertilization, although this decrease was not significant ($p \leq 0.05$). During the early bloom stage NDF decreased with 6 percentage units with increasing N levels, while NDF increased with 6 percentage units during full bloom. Rouquette *et al.* (1972), found that, on a whole plant basis, the percentage NDF was reduced by about 5 percentage units by the addition of N, while the percentage NDF increased with age.

Although not always statistically significant, ADL concentration showed a tendency to increase with advancing maturity, while there was no significant influence on lignin concentration with increasing levels of N fertilization. This is in contrast with results of Vicente-Chandler *et al.* (1959) and Rusoff *et al.* (1961) as quoted by Harkin (1973), who found that high levels of N resulted in an increase in lignin concentration of herbage.

Although there appeard to be a slight decrease in *in vitro* digestible organic matter (IVDOM) with increasing N fertilization levels, these trends were not significant. This confirms other reports that N fertilization did not affect the digestibility of pasture grasses appreciably (Saibro *et al.*, 1978; Grunow *et al.*, 1985). As expected IVDOM decreased significantly ($p \leq 0.05$), with advancing maturity. As plants mature the amount of cell wall increases. Lignin is a component with an inert nature (Biblack and Buxton, 1992) and is limited to the cell wall (Van Soest, 1975). Lignin is thought to be the major chemical constituent contributing to lowered digestibility as forage mature. The finding, that IVDOM decreases with advancing maturity, therefore, corresponds with the above mentioned results.

3.5.2 Intake

The DM and OM intake on a g/day basis as well as on a metabolic mass basis is presented in Table 13.

Table 13 Dry matter and OM intake of sheep grazing *P. maximum* cv Gatton at three stages of growth and at different levels of N fertilization during autumn

Parameters	kg N/kg	Vegetative	Early Bloom	Full Bloom
DM intake (g/day)	0	$1788.4^{a}{}_{1}$ (±130)	$1685.3^{a}{}_{1}$ (±137)	$1665.8^{a}{}_{1}$ (±51.9)
	75	$1328.2^{a}{}_{2}$ (±1328)	$1159.0^{a}{}_{2}$ (±297)	$1180.7^{a}{}_{2}$ (±313)
	150	$1416.0^{a}{}_{2}$ (±131)	$1334.7^{a}{}_{2}$ (±243)	$1164.0^{a}{}_{2}$ (±212)
DM intake (g/kg $W^{0.75}$/day)	0	$46.0^{a}{}_{1}$ (±3.0)	$40.4^{ab}{}_{1}$ (±4.0)	$37.7^{b}{}_{1}$ (±3.5)
	75	$37.5^{a}{}_{2}$ (±3.4)	$29.2^{b}{}_{2}$ (±5.2)	$26.6^{b}{}_{2}$ (±4.6)
	150	$38.9^{a}{}_{2}$ (±5.5)	$36.2^{a}{}_{1}$ (±3.6)	$25.5^{b}{}_{2}$ (±3.4)
OM intake (g/day)	0	$1469.8^{a}{}_{1}$ (±123)	$1440.4^{a}{}_{1}$ (±112)	$1362.6^{a}{}_{1}$ (±42.7)
	75	$1104.6^{a}{}_{2}$ (±185)	$1030.2^{a}{}_{2}$ (±263)	$1041.2^{a}{}_{2}$ (±276)
	150	$1191.6^{a}{}_{2}$ (±99)	$1179.6^{a}{}_{2}$ (±218)	$1041.2^{a}{}_{2}$ (±193)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).
The values in brackets indicate standard deviation.

Voluntary intake (VI) of an animal can be defined as the amount of feed that will be eaten by an animal or group of animals in a specific time (Forbes, 1995). Different factors can influence the VI of animals. These factors include:

- Species difference
- Cultivar and selections
- Plant parts
- Stage of growth
- Soil fertility
- Climate
- Processing

In this experiment the stage of maturity and the influence of N fertilization on intake was studied. Stage of maturity can be divided into different stages; vegetative, pre bloom, early bloom, full bloom, milk stage, dough stage, mature and overripe (Minson, 1990). Although the differences were not statistically meaningful, it can be seen from Table 13 that intake showed a slight decrease as plants matured. When intake was quantified as g/kg $W^{0.75}$, it was clear that intake decreased with advancing maturity.

An examination of the intake of different plant parts and different digestibilities reveals large differences in intake. Jarrige *et al.* (1974) reported that 75 % of the decline in intake was due to a fall in digestibility *per se*. This decrease in intake due to a decrease in digestibility was also observed by Minson (1984) in his study of the digestibility of five *Digitaria* species for sheep. The decrease in intake can also be ascribed to the following three factors (Minson, 1990):

- an increase in the proportion of stem;
- a decrease in the VI of both leaf and stem fractions and
- a nutrient deficiency in mature forages.

Stem is consumed in smaller quantities than leaf (Minson, 1990). Minson (1973) proved this with an experiment where leaf and stem fractions were fed separately to eight sheep. The mean VI of leaf was 46 % higher than that of stem. The higher intake of leaf was associated with a shorter retention time of DM in the reticulo-rumen (mean 23.8 *v.* 31.8), which appeared to be caused by the large surface of the leaf fraction initially available to bacterial degradation (mean 128 v. 41 sq cm/g) (Minson, 1973). McLeod *et al.* (1990) also reported the intake of leaf fractions to be higher than that of stem. The intake of Western Wheatgrass leaves by cattle was 8.23 kg / head / day, while that of stem was only 3.67 kg /day.

A nutrient deficiency in mature forages can also lead to a decrease in VI. From Table 13 it is evident that there is a statistically meaningful decrease in VI when VI is considered on a $W^{0.75}$ basis. Minson (1973) found that when nitrogen fertilization changed DM digestibility of tropical grasses, VI changes in the same direction. This tendency was also found by White (1985), who reported that

nitrogen fertilization increased the average forage *in vitro* DM digestibility by 0.1 percentage units. Another example of the influence of nutrient deficiency was found by Milford and Minson (1964), as quoted by Minson (1973). These authors found that stem fractions from an 87-day regrowth of grass contained less than 1 % nitrogen, and that with such feeds VI was likely to be depressed by a nitrogen deficiency.

3.6 Partial digestibility study

3.6.1 Rumen conditions

3.6.1.1 pH

Table 14 represents rumen pH as it was measured during this trial.

Table 14 Rumen pH of animals which grazed fertilized *P. maximum* cv Gatton at different stages of maturity during autumn

kg N/ha	Vegetative	Early Bloom	Full Bloom
0	$6.11^{b}{}_{1}$ (±0.1)	$6.32^{a}{}_{1}$ (±0.1)	$6.40^{a}{}_{1,2}$ (±0.1)
75	$6.05^{b}{}_{1}$ (±0.2)	$6.24^{a}{}_{1}$ (±0.2)	$6.29^{a}{}_{2}$ (±0.1)
150	$6.11^{b}{}_{1}$ (±0.1)	$6.23^{b}{}_{1}$ (±0.1)	$6.56^{a}{}_{1}$ (±0.1)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).
The values in brackets indicate standard deviation.

Rumen pH never reached a value of less than 6. Between N treatments there did not seem to be any difference in rumen pH ($p > 0.05$). These findings are in contrast with those found by Krysl (1986), who found that an increase in N fertilization increased rumen pH. This increase in pH on fertilized pasture was also found by Krysl *et al.* (1987).

In this study it was found that pH was higher with advancing maturity of plants for all three treatments. pH increased from a mean of 6.09 during the vegetative stage to a mean of 6.42 during full bloom. Although Krysl *et al.* (1987) did not report such an increase in pH in steers grazing fertilized Blue Grama rangeland,

it was stipulated that there should be an increase in pH since a smaller quantity of volatile fatty acids (VFA) were buffered by more saliva associated with increased chewing time and rumination of dormant forages.

3.6.1.2 Rumen ammonia

The rumen ammonia levels, as influenced by N fertilization and stage of maturity, are represented in Table 15.

Table 15 Rumen ammonia-N (mg/100 ml rumen fluid) levels of animals grazing *P. maximum* cv. Gatton fertilized with different levels of N during autumn

kg N/ha	Vegetative	Early Bloom	Full Bloom
0	$15.06^{a}{}_{3}$ (±4.7)	$8.06^{b}{}_{2}$ (±2.5)	$8.94^{b}{}_{1}$ (±1.4)
75	$26.26^{a}{}_{2}$ (±3.3)	$14.16^{b}{}_{1}$ (±3.1)	$13.48^{b}{}_{1}$ (±3.8)
150	$38.14^{a}{}_{1}$ (±6.2)	$20.14^{b}{}_{1}$ (±4.7)	$14.30^{b}{}_{1}$ (±2.1)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).
The values in brackets indicate standard deviation.

During the vegetative stage, just after N fertilization, the ammonia-N concentration in the rumen was extremely high but declined significantly as the plants matured. The rumen ammonia-N concentration of animals grazing pastures fertilized with no N, was 15.06 mg/100 ml rumen fluid, while that of animals on pasture fertilized with 150 kg N /ha, reached a high of 38.14 mg/100 ml rumen fluid.

Ammonia in the rumen originates from the degradation of protein and non-protein N and is used by rumen microbes to form microbial protein (McDonald *et al.*, 1992). The needs of rumen microbes are satisfied at a concentration of 5 mg ammonia per 100-ml rumen fluid (Oldham *et al.*, 1977; Morrison *et al.*, 1988). Ammonia in excess to this may have a negative influence on the animal since the excess ammonia will be absorbed from the rumen and lost as urinary urea (Lewis

et al., 1957). This is an energy consuming process and can be a problem especially when energy intake by the animal is low.

The high ammonia levels for the first stage of maturity corresponds well with work done by McIntyre (1970), who reported that rumen ammonia levels of sheep were between 20 - 30 mg/100ml when sheep received roughage providing them with 37.3 g N / day.

3.6.1.3 Volatile fatty acids

The following table shows the different VFA as produced in the rumen of sheep grazing grass fertilized with N, at different stages of maturity.

Table 16 **Volatile fatty acid (mmol/100 ml rumen fluid) production in the rumen of sheep grazing *P. maximum* cv Gatton at three stages of maturity and different levels of N fertilization**

Parameters	kg N /ha	Vegetative	Early bloom	Full bloom
Total volatile fatty acids	0	$13.3^{a}{}_{2}$ (±0.9)	$13.5^{a}{}_{1}$ (±1.3)	$14.4^{a}{}_{1}$ (±0.6)
	75	$16.3^{a}{}_{1}$ (±0.7)	$13.8^{b}{}_{1}$ (±1.8)	$12.5^{b}{}_{2}$ (±1.1)
	150	$16.7^{a}{}_{1}$ (±0.7)	$14.1^{b}{}_{1}$ (±1.2)	$12.3^{c}{}_{2}$ (±1.3)
Acetic acid	0	$9.7^{a}{}_{2}$ (±0.8)	$9.9^{a}{}_{1}$ (±0.9)	$10.7^{a}{}_{1}$ (±0.3)
	75	$11.3^{a}{}_{1}$ (±0.5)	$9.8^{b}{}_{1}$ (±1.2)	$9.3^{b}{}_{2}$ (±0.8)
	150	$11.5^{a}{}_{1}$ (±0.3)	$10.1^{b}{}_{1}$ (±0.8)	$8.7^{c}{}_{2}$ (±0.8)
Propionic acid	0	$2.4^{a}{}_{2}$ (±0.3)	$2.3^{a}{}_{1}$ (±0.3)	$2.3^{a}{}_{1}$ (±0.2)
	75	$3.1^{a}{}_{1}$ (±0.3)	$2.5^{b}{}_{1}$ (±0.2)	$2.0^{b}{}_{1}$ (±0.3)
	150	$3.2^{a}{}_{1}$ (±0.3)	$2.6^{b}{}_{1}$ (±0.3)	$2.2^{b}{}_{1}$ (±0.3)
Butyric acid	0	$1.1^{a}{}_{2}$ (±0.1)	$1.1^{a}{}_{1}$ (±0.2)	$1.2^{a}{}_{1}$ (±0.1)
	75	$1.5^{a}{}_{1}$ (±0.2)	$1.3^{ab}{}_{1}$ (±0.3)	$1.1^{b}{}_{1}$ (±0.1)
	150	$1.6^{a}{}_{1}$ (±0.1)	$1.2^{b}{}_{1}$ (±0.2)	$1.2^{b}{}_{1}$ (±0.1)
A : P*	0	$4.1^{a}{}_{1}$ (±0.8)	$4.4^{a}{}_{1}$ (±0.4)	$4.6^{a}{}_{1}$ (±0.4)
	75	$3.6^{b}{}_{1}$ (±0.3)	$3.9^{b}{}_{1}$ (±0.2)	$4.7^{a}{}_{1}$ (±0.6)
	150	$3.6^{a}{}_{1}$ (±0.3)	$3.9^{a}{}_{1}$ (±0.4)	$4.0^{a}{}_{2}$ (±0.4)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).
*A:P – Acetic to propionic acid ratio

The volatile fatty acids (VFA), acetic, propionic and butyric acid are the results of anaerobic fermentation of pastures in the rumen of an animal and represent the form in which most of the energy in pastures will be absorbed (Van Niekerk, 1997).

On a fibre based diet, as in this experiment, acetate will tend to dominate, while it will decline when a animal is fed a grain based diet (Chesworth, 1992).

In this experiment fertilization had a significant ($p \leq 0.05$) influence on total VFA production. Animals grazing pastures fertilized with 150 kg N/ha had the highest total VFA production in the rumen. When the separate VFA were taken into account, it was found that all of the VFA's increased with increasing levels of N fertilization. This was especially marked during the first stage after fertilization. After the first stage of maturity this effect was not as marked. With advancing maturity total VFA increased when pasture was not fertilized with N, but decreased when pasture was fertilized with N. An increase in acetic acid was found when no N was applied, but when pastures were fertilized acetic acid decreased with advancing maturity.

Both propionic and butyric acid decreased with advancing maturity. Acetic acid production is associated with cell wall digestion. The lower the digestibility, the more cell wall there is and the more acetic acid is produced (Parks *et al.*, 1962). When acetic acid increase, propionic and butyric acids decrease.

From this study the tendency for acetic acid to increase with advancing maturity can only be seen in animals grazing pasture that was not fertilized with N. The moment the pasture is fertilized, acetic acid decreased significantly with advancing maturity. Parks *et al.* (1962) found that acetate increased if soluble sugar decreased. This can explain why acetic acid increases with increasing levels of N fertilization, when soluble carbohydrates decreased.

The acetic acid to propionic acid ratio did not decrease significantly ($p > 0.05$) with increasing levels of N fertilization, while it tended to increase with advancing maturity.

3.6.1.4 Organic matter (OM) disappearance

Organic matter disappearance in the stomach

Table 17 represents the disappearance of organic matter in the rumen of sheep grazed on fertilized pasture.

Table 17 OM disappearance in the stomach of sheep grazing *P. maximum* cv Gatton at three stages of maturity and three different levels of N fertilization

Parameters	kg N/ha	Vegetative	Early Bloom	Full Bloom
OM disapp. in the stomach (g/d)	0	$782.5^{a}{}_{1}$ (±128)	$735.6^{a}{}_{1}$ (±163)	$685.7^{a}{}_{1}$ (±68.7)
	75	$538.1^{a}{}_{1}$ (±178)	$418.9^{a}{}_{2}$ (±301)	$456.5^{a}{}_{1}$ (±237)
	150	$751.6^{a}{}_{1}$ (±89)	$673.6^{ab}{}_{12}$ (±18)	$442.8^{b}{}_{1}$ (±124)
OM disapp. as % of total OMI	0	$53.0^{a}{}_{1}$ (±5.1)	$50.6^{a}{}_{1}$ (±7.8)	$50.3^{a}{}_{1}$ (±5.3)
	75	$48.8^{a}{}_{1}$ (±13.2)	$37.6^{a}{}_{1}$ (±20.1)	$43.5^{a}{}_{1}$ (±17.5)
	150	$63.1^{a}{}_{1}$ (±5.0)	$56.6^{ab}{}_{1}$ (±9.7)	$42.1^{b}{}_{1}$ (±6.2)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).
A:P – Acetic to propionic acid ratio

Both feed and animal factors affect the rate of passage of feed from the rumen. The predominant ones being feed intake, the chemical (Mir *et al.*, 1991) and physiological nature of the diet, the physiological state of the animal and the climatic conditions under which the animal "lives" (Faichney and Black, 1984).

The OM disappearance in the stomach, taken as g/day, gives a somewhat confusing picture. When OM disappearance is taken as a percentage of OM intake, a clearer picture is obtained. Although it appears as if OM disappearance in the stomach decreases with advancing maturity, the decrease is not significant. For all three stages of maturity, treatment of pasture with 75 kg N/ha tended to decrease the amount of OM that disappeared in the rumen, while treatment with 150 kg N/ha tended to increase the amount of OM disappearing in the rumen. This trend is, however, only true for the first two stages of maturity, because a further decrease of OM absorption occurred during full bloom. The above mentioned increases are not significant, because of the large differences between individually observed values.

Rate of digestion of food refers to the quantity of food that can be digested per unit of time. It is essentially a function of time. The speed of digestion is determined by the composition of the diet, its quality, deficiencies, excesses and availability of nutrients. Generally soluble components such as sugars, are fermented very rapidly, while less soluble substrates are digested more slowly (Van Soest, 1982). The more slowly components are therefore digested, the longer the retention time will be. Meissner and Du Plessis (1992) found that more OM disappeared in the stomach when the retention time was longer. It would appear that the retention time of digesta in the rumen increased on the highly fertilized pasture. This is undesirable since OM is absorbed in the rumen as VFA, while if it was digested in the small intestine, it would be absorbed as amino acids, which is a more desirable trend.

High levels of N fertilization caused the crude protein (CP) concentration as well as sometimes the lignin concentration of pastures to increase, while soluble carbohydrates decreased because of the rapid growth of plants. Such characteristics can cause a disturbance in the rumen of the animal (Nowakovski, 1962).

The main N product when protein breaks down in the rumen, is ammonia (Nowakovski, 1962). This process, however, needs energy which microbes obtain by fermenting soluble components, such as soluble carbohydrates. When there is a energy shortage microbial growth can be limited (Steinhour and Clark, 1980). With fewer microbes in the rumen, less of the de-aminated protein will be built into microbial protein. This process will also take longer, and more OM can, therefore, be absorbed in the stomach parts.

3.6.1.5 Disappearance of organic matter in the small intestine.

The disappearance of OM in the small intestine is presented in Table 18.

Table 18 OM disappearance in the small intestine of sheep grazing *P. maximum* cv Gatton fertilized with N at different stages of maturity

Parameters	kg N/ha	Vegetative	Early Bloom	Full Bloom
OM disapp. in the small Intestine (g/d)	0	$128.4^{a}{}_{1}$ (±84.9)	$189.6^{a}{}_{1}$ (±76.3)	$191.7^{a}{}_{1}$ (±84.1)
	75	$254.2^{a}{}_{1}$ (±118)	$190.6^{a}{}_{1}$ (±226)	$204.5^{a}{}_{1}$ (±221)
	150	$102.3^{a}{}_{1}$ (±44.1)	$166.9^{a}{}_{1}$ (±74.1)	$183.3^{a}{}_{1}$ (±109)
OM disapp. as % of total OMI	0	$8.7^{a}{}_{1}$ (±5.9)	$13.2^{a}{}_{1}$ (±5.7)	$14.0^{a}{}_{1}$ (±6.0)
	75	$22.8^{a}{}_{1}$ (±9.3)	$20.4^{a}{}_{1}$ (±22.2)	$20.1^{a}{}_{1}$ (±21.7)
	150	$8.7^{a}{}_{1}$ (±3.8)	$14.4^{a}{}_{1}$ (±7.8)	$17.2^{a}{}_{1}$ (±0.5)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).
The values in brackets indicate standard deviation.

Although the individual values differed a lot, there is no significant difference between either the disappearance of OM during the different stages of maturity, or between the different N treatments.

Although no significant differences were obtained, the percentage disappearance of OM in the small intestine was higher, when sheep grazed pasture fertilized with 75 kg N/ha. This increased disappearance rate in the small intestine indicates that at least part of the OM, not absorbed in the stomach, had reached the small intestine and was digested and absorbed there (Van Niekerk, 1997).

This corresponds well with values obtained in Table 17, where it was found that less OM disappeared in the rumen of sheep grazing pasture that was fertilized with 75 kg N/ha, while much less OM disappeared in the small intestine, when large amounts of OM were absorbed in the rumen.

3.7 Conclusion

Nitrogen fertilization increased the N concentration of grass, but didn't have a large influence on NDF, ADL or IVDOM. The N concentration decreased with

stage of maturity while the NDF and ADF concentration increased. Stage of maturity had no effect on the IVDOM of *P. maximum* cv Gatton.

When fertilized grass was grazed by sheep, increasing levels of N had no marked effect on the rumen pH, but increased the levels of rumen ammonia as well as of total volatile fatty acid production in the rumen. With advancing stage of maturity pH was increased, while rumen ammonia decreased rapidly. Total volatile fatty acids also tend to decrease as the grass matured.

The disappearance of OM in both the stomach and small intestine weren't significantly altered by increasing levels of N fertilization or advanced stages of maturity.

CHAPTER 4

THE INFLUENCE OF NITROGEN FERTILIZATION AND STAGE OF MATURITY ON THE DRY MATTER YIELD AND QUALITY OF *PANICUM MAXIMUM* CV GATTON DURING SUMMER

4.1 Abstract

An experiment was conducted to evaluate the influence of nitrogen (N) fertilization and stage of maturity on the quality of *Panicum maximum* cv Gatton during summer. Seven N treatments, namely 0, 25, 50, 75, 100, 125 and 150 kg N/ha and three stages of maturity (vegetative stage, early bloom and full bloom) were evaluated using different parameters.

Each plot was fertilized at the start of the summer growing period with the appropriate amount of N. Samples were taken at the beginning of each maturity stage and dry matter (DM) concentration and yield was then determined. Treatments were evaluated in terms of DM yield, N concentration, total nonstructural carbohydrates (TNC), nitrate-nitrogen (NO_3-N), acid detergent lignin (ADL), neutral detergent fibre (NDF) and *in vitro* organic matter digestibility (IVOMD).

Dry matter yield, N and NO_3-N concentration increased with increasing levels of N fertilization. DM yield increased from 3 t/ha to 5.2 t/ha during the vegetative stage and from 5.4 t/ha to 12 t/ha during the full bloom stage. Nitrogen concentration increased from 1.6% to 2.92% and NO_3-N increased from 0.83 g/kg/DM to 2.33 g/kg/DM. In contrast, TNC concentration decreased with increasing levels of N fertilization. With advancing maturity DM yield and TNC concentration increased, while N and NO_3-N concentration decreased. Nitrogen fertilization had no effect on ADL concentration or IVOMD, while both of these parameters were strongly influenced by maturity.

4.2 Introduction

With the growing human population in South Africa, less and less land is available for animal production. In these limited areas, there is a growing interest in intensive grassland production (Salette, 1970). Grassland production can be intensified by numerous management techniques (Wiedenfeld *et al.*, 1985). One of the best known techniques is N fertilization. It is known from the literature that N fertilization will increase dry matter (DM) yield (Quinlan *et al.*, 1981; Singh *et al.*, 1987) and the N concentration of plants (Vincente-Chandler *et al.*, 1959; Morrison and Russell, 1980). The stage of maturity at harvest is another important management consideration that can play an important role in the quality of forage (Steenekamp, 1995).

Panicum maximum is indigenous to the subtropical areas of Southern Africa and occurs mainly in the subhabitat under trees (Smit and Rethman, 1989 as quoted by Pieterse *et al.*, 1997). It is a perennial tufted grass and can reach heights of one to two metres. In experiments conducted by Rodel and Boutlwood (1971), it was found that *P. maximum* was one of the highest yielding tufted grasses.

Not much work has been done on *P. maximum* cv Gatton in South Africa. This paper describes the influence of N fertilization and stage of maturity on the DM yield, N concentration, total nonstructural carbohydrates (TNC), nitrate-nitrogen (NO_3-N), acid detergent lignin (ADL), neutral detergent fibre (NDF) concentration as well as the *in vitro* organic matter digestibility (IVOMD) of *P. maximum* cv Gatton during summer.

4.3 Materials and methods

A small plot experiment was conducted during the summer months, on a three-year-old stand of *P. maximum* cv Gatton, which was established on a deep red Hutton soil on the Hatfield Experimental Farm of the University of Pretoria at an altitude of 1372 m. Maximum temperatures varied between 18 and 32 °C with an annual rainfall of ± 700-mm, occurring mainly in the summer.

The trial was conducted as a simple factorial with seven levels of N, three stages of maturity and three replications. A randomized block design was used in the layout. The N levels were 0, 25, 50, 75, 100, 125 and 150 kg N /ha and each N level was harvested at three stages of maturity, namely:

Vegetative stage ..... 20 - 30 cm high
Early bloom..... 30 - 40 cm high
Full bloom..... ..... 60 - 90 cm high

Each plot was 2m x 5m or 0.001 ha.

Nitrogen, in the form of limestone ammonium nitrate (LAN) (28 % N), was applied in November. All plots were also fertilized with 300 kg/ha KCl, on the basis of soil analysis, in order to prevent any potassium deficiencies.

DM yield was determined on several occasions by harvesting an area of 1 x 5m in the middle of each plot and determining the oven dry DM concentration.

Hand clipped samples were taken at each maturity stage. The grass samples were clipped around 08h00 and frozen immediately. At the end of the sampling period, the samples for each maturity stage were weighed, freeze-dried, ground and stored in glass bottles for further analysis.

The following analyses were conducted. Dry matter content was determined according to AOAC (1980). All results were calculated on a dry matter basis.

Total N was determined by Kjeldahl procedures and included both organic and inorganic N.

Total nonstructural carbohydrates were determined using the technique described by Marais (1979). The TNC was analyzed as reducing sugars after quantitative hydrolysis to monosaccharides by means of carefully controlled acid hydrolysis procedure (Marais, 1979). The reducing sugars formed during hydrolysis were determined quantitatively by the modified Nelson-Somogyi method (Morrison and Boyd, 1966). This procedure is not suitable for samples containing starch, but can be used for analyzing temperate (C_3) grasses, storing fructose and sub-tropical (C_4) grasses, storing glucose.

For the determination of NO_3-N, the analysis was based on the procedures described by Cataldo *et al.* (1975). These are based on the nitration of salicylic acid under highly acidic conditions and the calorimetric determination of the resulting coloured complex which absorbs maximally at 410 nm in basic (pH > 12) solutions.

Acid detergent lignin was determined using the procedures described by Goering and Van Soest (1970) while NDF was determined using the procedures described by Robertson and Van Soest (1981).

In vitro organic matter digestibility of the samples was determined by making use of the technique as described by Tilley and Terry (1963) and modified by Engels and Van der Merwe (1967).

4.4 Statistical analysis

For this trial, the GLM (General Linear Modules) procedure by Statistical Analysis Systems (SAS, 1995), was used to analyze the data.

All main effects (treatment, replication and period) as well as all possible first-order interactions were included in the initial model. Interactions that did not make a significant ($p > 0.05$) contribution to the variance, were omitted in subsequent analyses.

Models were tested for the dependent parameters namely DM yield, TNC, N, NDF, ADL, NO_3-N and IVDOM.

Significance of difference between least square means was determined by Bonferoni's test (Van Ark, 1981).

4.5 Results and discussion

4.5.1 Dry Matter Yield

Dry matter yield, as calculated for three N treatments (0, 75 and 150 kg N/ha) during three stages of maturity (vegetative stage, early bloom and full bloom), is presented in Figure 13

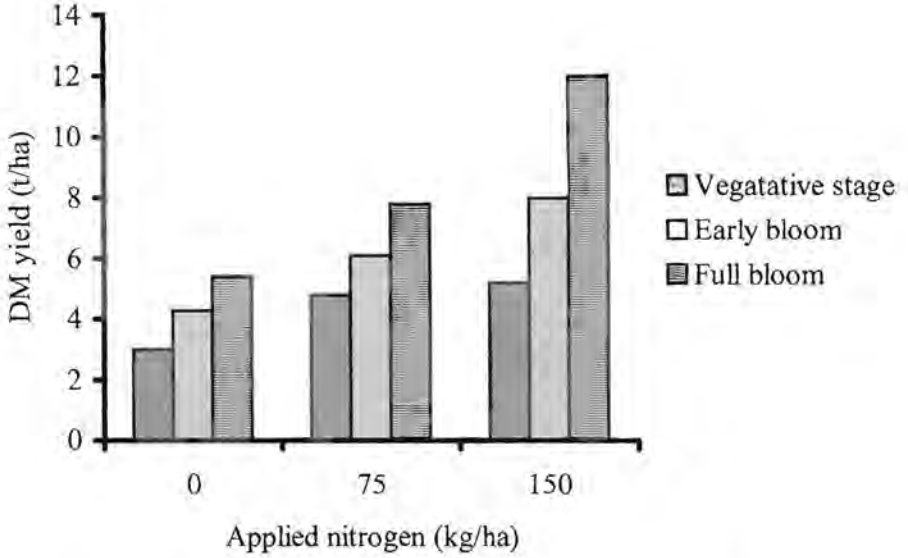


Figure 13 The influence of three N treatments on the DM yield of *P. maximum* cv Gatton during three stages of maturity in summer

Dry matter yield increased significantly ($p \leq 0.05$) with increasing levels of N fertilization. Dry matter yield increased during the vegetative stage from 3 t/ha on the control (0 kg/ha N) to 5.2 t/ha on 150 kg/ha N treatment. During the full bloom stage DM yield increased from 5.4 t/ha on the control to 12 t/ha on pastures fertilized with 150 kg/ha N. This represents an increase of between 73 and 126%. Dry matter yield averaged 6.2 t/ha over the summer months at different maturity stages and N fertilization levels. This value corresponds well with values reported by Hall *et al.* (1982) in the USA, on pastures fertilized with 0, 75 and 150 kg/ha N, who found the following means: *P. virgatum* – 6.19 t/ha, *Andropogon gerardi* – 6.29 t/ha and *Sorghastrum mutans* - 5.59 t/ha. Cook and Mulder (1984) in a trial with nine different tropical grasses, reported an increase in DM yield with increasing levels of N fertilization. In the latter trial *P. maximum* had an average annual DM yield of about 10 t/ha when grass was fertilized with 25 kg N/ha and about 11.4 t/ha when fertilized with 50 kg N /ha.

Dry matter yield did not only increase with increasing levels of N fertilization, but it also increased significantly ($p \leq 0.05$) with advancing maturity. DM yield increased from a mean of 4.3 t/ha during the vegetative stage to a mean of 8.4

t/ha during the full bloom stage. Similar increases were also reported by Forwood *et al.* (1988), for Caucasian Bluestem.

4.5.2 Nitrogen concentration

In Figure 14 the influence of seven levels of N fertilization and three stages of maturity on the N concentration of *P. maximum* cv Gatton is illustrated.

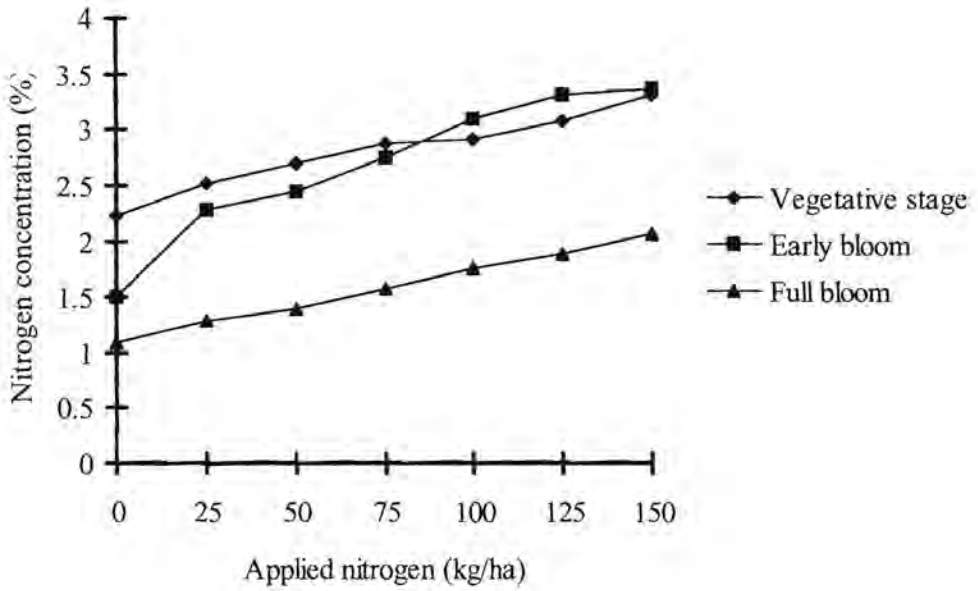


Figure 14 The influence of N fertilization on the N concentration of *P. maximum* cv Gatton during three stages of maturity in the summer months

Nitrogen concentration of plants increased with increasing levels of N fertilization. The mean value for plants receiving no N was 1.6 % N and this differed significantly ($p \leq 0.05$) from the mean value of 2.92 % N for plants receiving 150 kg N /ha. Saibro *et al.* (1978) found with *Phalaris tuberosa* that N concentration also increased with increasing levels of N fertilization. These authors found that the N concentrations at three stages of maturity (seed, inflorescence and vegetative stage), were much higher than the generally accepted 1.5% N

deficiency level of plants. It varied between 2.8 and 4.6% N. These values correspond well with values found in this trial for the vegetative stage. Numerous other citings support the findings in this trial. Vincente-Chandler *et al.* (1959), Wilman (1980), and Van Niekerk *et al.* (1993) all found an increase in N concentration of plants as N fertilization levels were increased. According to Nowakowski (1962), N fertilization increased the organic N concentration of herbage, including the proportion in soluble form, such as peptides, amides and amino acids, as opposed to proteins.

As plants matured the N concentration declined, but the N concentration of plants receiving 150 kg N/ha was still significantly ($p \leq 0.05$) higher from those receiving no N. With advancing maturity plants develop more stems and flowers. These plant parts are generally lower in N than green leaves, which might explain the lower N concentration with advancing maturity (Fleischer *et al.*, 1983). Forwood *et al.* (1988) and Steenekamp and Rethman (1995) also reported a decrease in N concentration of plants as they mature.

4.5.3 Total nonstructural carbohydrates

The influence of N fertilization on the TNC concentration of plants during three stages of maturity is illustrated in Figure 15.

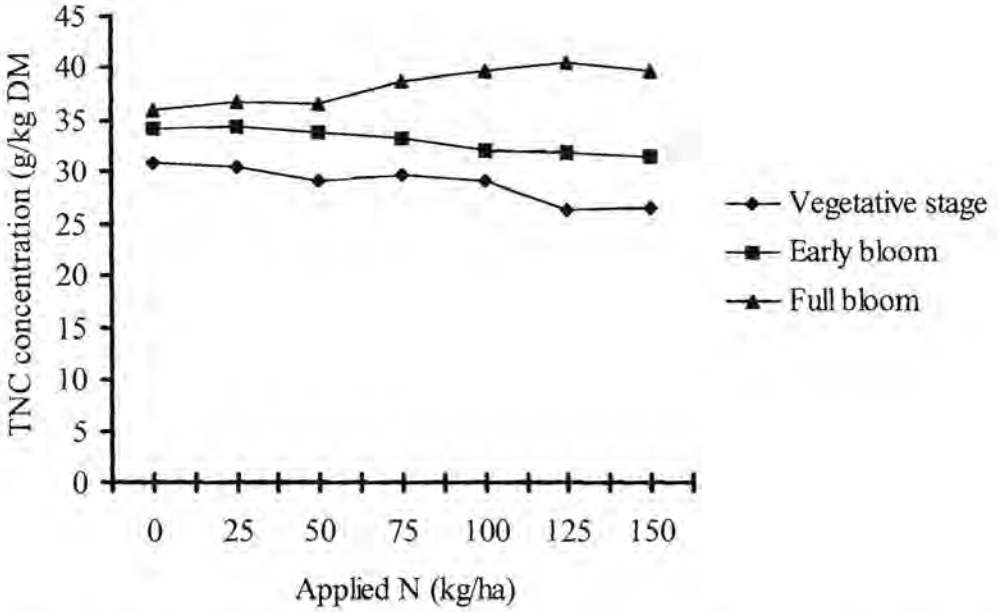


Figure 15 The influence of N fertilization on the TNC concentration of *P.maximum* cv Gatton at three maturity stages in the summer growing period

Total nonstructural carbohydrates (TNC), including reducing sugars, non-reducing sugars, fructan and starch, seem to be the primary reserves of grasses (White, 1985). Carbohydrates produced in excess of the needs of the plant are translocated to and accumulated in the stems as fructosans (Smith, 1973). Nitrogen fertilization has an inverse effect on the TNC reserves of plants. As N fertilization levels increase, TNC concentration of plants decreased significantly ($p \leq 0.05$) during the vegetative and early bloom stages, while TNC increased significantly ($p \leq 0.05$) during the full bloom stage (Fig. 3). A decrease from 34.1 g/kg DM to 31.5 g/kg DM was found in the vegetative stage, while TNC declined from 30.9 g/kg DM to 26.5 g/kg DM during the early bloom stage and increased from 35.89 g/kg DM to 39.64 g/kg DM in the full bloom stage. George *et al.* (1989) found that in Switchgrass a similar decrease in TNC concentration of plants occurred when N fertilization levels were increased. According to Smith (1973), this decrease is probably due to the acceleration of herbage growth rate, which is promoted by N fertilization. Nowakowski (1962) reported similar findings and concluded that TNC declined because the sugars were used in the vigorous growth of leaves, which resulted from the high N fertilization levels. The use of

sugars eventually takes place at the expense of carbohydrate reserves in the form of fructosan. During the full bloom stage, growth of leaves was much slower and the plant has already taken up most of the N from the soil. This may explain the increase in TNC concentration during the full bloom stage.

As plants mature, TNC concentration increased significantly ($p \leq 0.05$), from a mean of 32.98 g/kg DM during the vegetative stage to a mean of 38.27g/kg DM during full bloom. Percentage TNC is greatly influenced by the ratio of stem to leaf tissue. With advancing maturity more stems develop and the TNC percentage increases. This is because TNC, and especially fructosan, is stored in the stems (Smith, 1973).

4.5.4 Nitrate-Nitrogen

In Figure 16 the influence of N fertilization and stage of maturity on the NO_3-N concentration of plants is presented.

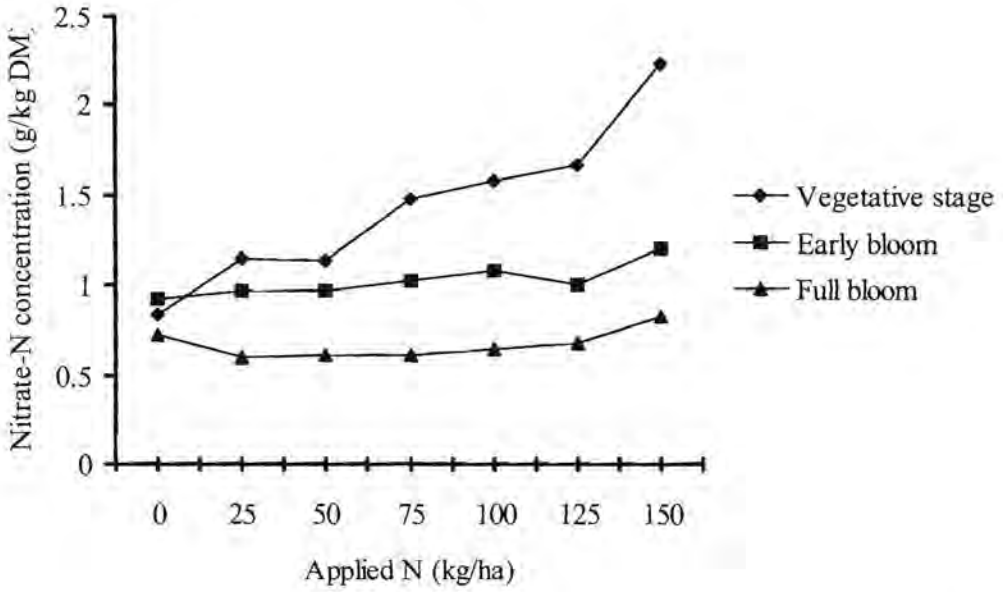


Figure 16 The influence of N fertilization and stage of maturity on the nitrate concentration of *P. maximum* cv Gatton during the summer months

Nitrogen fertilization had a strong influence on the NO_3-N concentration of herbage. As the N fertilization levels increased, the NO_3-N concentration of plants also increased. This increase is especially evident in the vegetative stage where NO_3-N increased significantly ($p \leq 0.05$) from a low value of 0.83 g/kg DM to a high value of 2.33 g/kg DM. According to various authors the safe amount of nitrate in plants for animals is 0.5 – 0.75 g/kg DM (Prins, 1984). This value may be exceeded when N fertilization is high. With a low TNC concentration and high NO_3-N concentration, the plants may be toxic to animals. Nitrate is the form in which most of the chemically combined N is absorbed by plants. When NO_3-N accumulates, it implies that the rate of assimilation has not kept pace with the rate of uptake (Madison and Kenneth, 1963). At maturity, NO_3-N concentration declined significantly ($p \leq 0.05$) and during full bloom there was almost no difference in NO_3-N concentration between the different treatments.

One explanation for the decline in NO_3-N as plants mature, would be the influence of age. As plants grow older, more stem, fruits and seeds develop, while the proportion of leaves decline. Fruits and seeds contain very little NO_3-N, so the NO_3-N concentration in other parts of the plant tends to be diluted. Another explanation for the decline in NO_3-N concentration as plants mature, is that the N supplying power of the soil usually diminishes as plants approaches maturity, permitting the plants to assimilate most of the NO_3-N that accumulated when more was available (Madison and Kenneth, 1963).

4.5.5 *In vitro* digestibility

In Table 19 the influence of N fertilization and stage of maturity on the IVOMD of *P. maximum* cv Gatton is tabulated.

Table 19 ***In vitro*** **organic matter digestibility of *P. maximum* cv Gatton as influenced by N fertilization and stage of maturity during summer**

kg N/ha	Vegetative stage	Early bloom	Full bloom	Mean
0	$76.13^{a}{}_{1}$	$69.72^{a}{}_{2}$	$59.2^{a}{}_{3}$	**68.35**
25	$73.29^{ab}{}_{1}$	$72.45^{a}{}_{1}$	$60.86^{a}{}_{2}$	**68.87**
50	$73.64^{ab}{}_{1}$	$69.38^{a}{}_{2}$	$60.89^{a}{}_{3}$	**67.97**
75	$70.69^{b}{}_{1}$	$69.33^{a}{}_{1}$	$60.00^{a}{}_{2}$	**66.67**
100	$71.57^{b}{}_{1}$	$70.92^{a}{}_{1}$	$61.26^{a}{}_{2}$	**67.92**
125	$71.56^{b}{}_{1}$	$72.02^{a}{}_{1}$	$58.09^{a}{}_{2}$	**67.22**
150	$71.63^{b}{}_{1}$	$69.55^{a}{}_{1}$	$59.98^{a}{}_{2}$	**67.05**
Mean	**72.64**	**70.48**	**60.04**	

Values in the same column and in the same row with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).

Nitrogen fertilization did not have a large influence on the IVOMD of *P. maximum* cv Gatton. It only had a significant influence on IVOMD during the vegetative stage and then a significant depressing effect (with the control having the highest digestibility). There was no statistical difference between grass fertilized with different levels of N during the early and full bloom stages. These findings correspond well with findings in the literature, which reported N fertilization as having little or no influence on the digestibility of grass. Van Niekerk *et al.* (1993) found with *P. maximum* cv Gatton that there was no change in digestibility when N fertilization levels were increased. Minson *et al.*, 1967 as quoted by Wilson (1973), also found no change in digestibility with increasing levels of N fertilization.

In vitro digestibility decreased significantly ($p \leq 0.05$) as plants matured. During the vegetative stage the average digestibility was 72.67 % and this decreased to an average of 60.04 % in mature plants. Numerous literature citations support this finding. Cherney *et al.* (1992) found that perennial grass decreased in

digestibility from 79.7 % to a low of 44.2 %. This decrease was even greater than that found in this trial.

The digestibility of grass is also influenced by the stem to leaf ratio (McDonald *et al.*, 1992). In very young grass the stem is more digestible than the leaves, but with advancing maturity the digestibility of the stem declines rapidly, while that of the leaves decreases slowly. As plants mature the stem comprises an increasing proportion of the total herbage and hence has a much larger influence on the digestibility of the whole plant (McDonald *et al.*, 1992). Twidwell *et al.* (1988), found, for instance, that leaf blades comprised 47 % of total forage at first harvest, but declined to 26 % of total forage in later harvests.

The digestibility of grass is also influenced by lignin concentration. According to Biblack and Buxton (1992), lignin is thought to be the major chemical constituent contributing to lower digestibility in mature forages. (Also see section on ADL).

4.5.6 Acid detergent lignin

The influence of N fertilization and stage of maturity on the ADL concentration of *P. maximum* cv Gatton is presented in Table 20.

Table 20 **Acid detergent lignin concentration of *P. maximum* cv Gatton, as influenced by N fertilization and stage of maturity during summer**

kg N/ha	Vegetative stage	Early bloom	Full bloom	Mean
0	$3.98^{a}{}_{2}$	$3.88^{a}{}_{2}$	$5.09^{a}{}_{1}$	**4.32**
25	$4.11^{a}{}_{2}$	$4.03^{a}{}_{2}$	$5.01^{a}{}_{1}$	**4.38**
50	$4.06^{a}{}_{2}$	$4.12^{a}{}_{2}$	$5.03^{a}{}_{1}$	**4.40**
75	$4.01^{a}{}_{2}$	$4.28^{a}{}_{2}$	$5.01^{a}{}_{1}$	**4.43**
100	$4.16^{a}{}_{2}$	$4.17^{a}{}_{2}$	$5.16^{a}{}_{1}$	**4.50**
125	$4.24^{a}{}_{2}$	$4.17^{a}{}_{2}$	$5.09^{a}{}_{1}$	**4.50**
150	$4.13^{a}{}_{2}$	$3.95^{a}{}_{2}$	$5.19^{a}{}_{1}$	**4.42**
Mean	**4.10**	**4.09**	**5.08**	

Values in the same column and in the same row with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).

Increasing levels of N fertilization did not have any significant ($p > 0.05$) effect on the ADL concentration of the grass in this trial. Vincente-Chandler *et al.* (1959) found an increase in ADL concentration as N fertilization levels were increased. Waite (1970) found that the comparatively low levels of N fertilization normally applied to temperate grasses, did not affect the ADL levels of such species.

Although stage of maturity had no effect on the ADL concentration of plants between the vegetative and early bloom stages, ADL concentration increased sharply during the full bloom stage. During the first two stages, plants had an average ADL concentration of 4.10 g/kg DM which increased to a mean of 5.08 g/kg DM during the full bloom stage.

Acid detergent lignin can, as noted earlier, depress the digestibility of plants. Acid detergent lignin may effect digestibility negatively because of its inert nature, but also seems to interact with hemicellulosic polysaccharides, pectic polysaccharides and the remaining cellulosic fraction to limit cell wall degradability (Hatfield, 1989). Jung and Vogel (1986) found that ADL is

especially bound to the hemicellulose fraction of the cell wall and inhibited the digestion of this fraction.

4.5.7 Neutral detergent fibre

Table 21 illustrates the influence of N fertilization and stage of maturity on the NDF concentration of *P. maximum* cv Gatton.

Table 21 Neutral detergent fibre concentration of *P. maximum* cv Gatton as influenced by N fertilization and stage of maturity during summer

kg N/ha	Vegetative stage	Early bloom	Full bloom	Mean
0	$58.69^{a}{}_{3}$	$63.52^{a}{}_{2}$	$72.58^{a}{}_{1}$	**64.93**
25	$57.62^{ab}{}_{2}$	$58.12^{c}{}_{2}$	$71.72^{ab}{}_{1}$	**62.49**
50	$57.92^{ab}{}_{3}$	$60.80^{b}{}_{2}$	$71.23^{abc}{}_{1}$	**63.32**
75	$56.48^{b}{}_{2}$	$58.32^{c}{}_{2}$	$70.48^{bc}{}_{1}$	**61.76**
100	$57.00^{ab}{}_{2}$	$58.12^{c}{}_{2}$	$69.98^{c}{}_{1}$	**61.70**
125	$55.73^{bc}{}_{3}$	$59.03^{bc}{}_{2}$	$69.04^{c}{}_{1}$	**61.27**
150	$54.37^{c}{}_{1}$	$57.25^{c}{}_{2}$	$65.98^{d}{}_{1}$	**59.20**
Mean	**56.83**	**59.31**	**70.14**	

Values in the same column and in the same row with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).

Increasing levels of N fertilization tended to depress the NDF concentration of plants with between four and seven percentage units. This tendency was more marked in the early and full bloom stages than in the vegetative stage. Rouquette *et al.* (1972) found that with *P. coloratum* L., increasing levels of N increased the cell wall concentrations, which then resulted in a significant decrease in NDF concentration.

Van Niekerk *et al.* (1993) also reported a decrease in NDF concentration with increasing levels of N fertilization in *P. maximum* cv Gatton.

With advancing maturity, NDF increased from 56.83 % to 70.14 %. Cherney *et al.* (1992) also found a similar NDF increase in their trial on perennial grass, namely 40.0 % to 62.7 %.

4.6 Conclusion

Nitrogen had both positive and negative effects in this trial. Nitrogen fertilization increased the DM yield from a mean of 4.30 t/ha on pasture fertilized with 0 kg N/ha to 8.4 kg/ha on pastures fertilized with 150 kg N/ha. Nitrogen fertilization also increased the N concentration of plants from 1.6 % to 2.92 %.

Nitrogen fertilization also increased the NO_3-N concentration of plants from a low value of 0.83 when no N was applied, to a high of 2.33 g/kg when grass was fertilized with 150 kg N/ha. This increase occurred during the vegetative stage, shortly after N application. After the first stage, the increase in NO_3-N was less marked. The increase in NO_3-N is not desirable and care must be taken not to apply too high a level of N, or to utilize the pasture too soon after applying N, in order to prevent nitrate poisoning of animals.

With the increase of N fertilization levels, the TNC concentration of plants decreased.

In this trial N fertilization had no effect on either ADL concentration or IVOMD, but it did decrease the NDF concentration of plants with up to 7 percentage units. This is an advantage to the animals, since less cell wall means more cell content, which is more digestible. With advancing maturity the quantity and quality of the grass changed. Dry matter yield increased from 4.3 t/ha to 8.4 t/ha. This was an increase of almost 95 %. With this increase in yield, however, the quality declined. Nitrogen concentration decreased from 2.81 % during the vegetative stage to 1.58 % during full bloom. The TNC concentration, however, increased, while the NO_3-N concentration decreased. Both NDF and ADL concentration increased as plants matured, causing the IVOMD to decrease significantly.

CHAPTER 5

THE INFLUENCE OF NITROGEN FERTILIZATION AND STAGE OF MATURITY ON INTAKE, RUMEN CONDITIONS AND ORGANIC MATTER DISAPPEARANCE IN SHEEP GRAZING *PANICUM MAXIMUM* CV GATTON DURING SUMMER.

5.1 Abstract

A study was conducted to investigate the influence of three levels of nitrogen fertilization (0, 75 and 150 kg/ha) and three stages of maturity (vegetative, early bloom and full bloom) on different quality parameters of *Panicum maximum* cv Gatton as well as the disappearance of the fertilized grass, in the digestive tract of grazing sheep.

It was found that N fertilization increased the N concentration of plants significantly ($p \leq 0.05$) from 1.78 g/kg DM to 2.75 g/kg DM, while N fertilization decreased the neutral detergent fibre (NDF) concentration significantly ($p \leq 0.05$) from 63.40 to 59.40 %. Although the acid detergent lignin (ADL) concentration seemed to increase from 3.48 to 3.75 g/kg DM and *in vitro* digestible organic matter (IVDOM) seemed to decrease from 81.08 to 79.58 % when N fertilization levels increased, these changes were not significant ($p > 0.05$).

Nitrogen fertilization did not have a significant influence on rumen pH and all values were above 6. Nitrogen fertilization significantly increased the NH_3-N concentration of the rumen while stage of maturity decreased the NH_3-N concentration.

Nitrogen fertilization tends to increase the volatile fatty acid concentration of the rumen, while it was decreased by the stage of maturity.

Nitrogen fertilization did not appear to have a significant influence on the flow of OM through the digestive tract of the sheep.

5.2 Introduction

It is known that nitrogen fertilization will increase the DM yield (Quinlan *et al.*,1981), nitrogen concentration and nitrate-N concentration of a grass, but decreases the carbohydrate concentration. Stage of maturity will increase DM yield, lignin and carbohydrate concentration and decrease nitrogen and nitrate concentration. This is also true for *P. maximum* cv Gatton. The increase in DM yield with increasing levels of nitrogen fertilization is, for instance, especially positive, since areas for animal husbandry in South Africa are limited (Salette, 1970).

All the above plant parameters are influenced by nitrogen fertilization and stage of maturity. The question arises: What is the influence of this fertilized grass in different stages of maturity, on the grazing animal.

This chapter describes the influence of nitrogen fertilization and stage of maturity on voluntary intake, different rumen conditions and the passage of digesta through the digestive tract of grazing animals.

5.3 Materials and Methods

For this experiment nine paddocks of three different sizes (0.04 ha, 0.08 ha and 0.16 ha) were used. The camps were situated on the Hatfield Experimental Farm of the University of Pretoria, South Africa. The area received an average annual summer rainfall of ± 700-mm and is located at an altitude of 1372 masl. The pastures used were established in the summer of 1988 on a deep red Hutton soil. For this trial, the camps were mown at the beginning of February. After mowing, 0, 75 and 150 kg of N per ha were applied to the different paddocks. This was done as follows: The 0.04 ha camps received 150 kg N / ha, the 0.08 ha camps received 75 kg N / ha and those of 0.16 ha received no N. All the camps received 300 kg of KCl /ha, on the basis of soil analysis, to prevent any K deficiencies during the investigation. No P fertilizer was applied because the phosphorous status of the soil was relatively high (± 30 ppm).

Each N fertilization level was evaluated at three stages of maturity, namely vegetative stage, early bloom and full bloom.

For the partial digestion experiment, five Döhne merino wethers, were used. Each animal was fitted with a rumen cannula and T-shaped cannula in the abomasum as well as in the terminal ileum. Each animal was also fitted with a harness and faeces bag. Because the experiment was conducted in the field and not in metabolism cages, each animal was also fitted with a peristaltic pump and two marker bags on it's back (Corbett *et al.*, 1976). The animals were allowed free access to water during the trial. Before the start of the experiment, animals were adapted to the specific pasture for seven days. After the adaptation period, Cr-EDTA was prepared according to the procedure of Binnerts *et al.* (1968), and continuously infused (240 mg Cr/day) by a peristaltic pump fitted to the back of each animal, into the rumen of the sheep. Yb acetate, dissolved in distilled water, was also infused (100 mg Yb/day) into the rumen.
Cr-EDTA and Yb acetate were infused into the rumen via different infusion lines from two separate marker bags. The reason for this was that if Cr-EDTA and Yb acetate were mixed, a precipitate might be formed on standing (Siddons *et al.*, 1985). The technique of Faichney (1980) was used to estimate, via the two markers, digesta flow in the digestive tract.
The infusion of markers was done for four days, starting with a prime dose, before sampling started on day five (Faichney, 1980). Samples were taken from the rumen, abomasum and ileum. Samples were collected every 12 hours as follows:

Day 5	06:00	and	18:00
Day 6	09:00	and	21:00
Day 7	12:00	and	00:00
Day 8	15:00	and	03:00

A complete collection of faeces was also done during the four days of sampling.

5.3.1 Treatment of collected samples

Each rumen sample was filtered and the pH was recorded after filtration. Twenty ml of each rumen sample was acidified with 4 ml 0.5 M H_2SO_4 for ammonia analysis, while another 20 ml was preserved with 2 ml of a 10 % NaOH solution

for volatile fatty acid analysis. After these treatments, the samples were frozen and kept for further analyses. Fifty ml of each abomasum and ileum sample was stored frozen without any added preservatives. Faeces were collected twice in every 24-h period. The two collections were mixed together, weighed and a 10 % sample was stored frozen. Faeces were collected for four days. Each sheep's samples, collected over the four day sampling period, were stored together and analyzed as one sample for each sheep.

Five sheep, fitted with oesophageal fistulas, were used to obtain a sample of the grass selected by the grazing animals. Ten samples were taken from each camp, five at the beginning and five at the end of a sampling period. The first and second samples were analyzed separately and a mean value was then calculated from the values obtained.

Oesophageal collected samples were obtained by fasting animals for three hours. The animals were then put to the pasture for 45 minutes while samples were collected in oesophageal bags fastened around their necks. Most of the saliva was squeezed out of the samples using a double layer of cheesecloth, before they were frozen.

5.3.2 Preparation of samples

All the samples were thawed overnight. Separation of the soluble and particulate matter of the thawed samples of the rumen, abomasum and ileal digesta was achieved by high-speed centrifugation at 5000 rpm for 20 minutes. The clear centrifuge of all the samples were stored in glass bottles in the refrigerator.

Abomasum samples were dried at 55 ^{0}C for 48 hours, while ileum samples were freeze - dried. The dried abomasum and ileum samples were milled and stored in bottles.

Samples of the dried faeces samples were also milled and stored in bottles.

5.3.3 Analytical methods

Dry matter (DM) of abomasum and ileum samples were determined by drying 5 ml of the samples for 24 hours at 100 ^{0}C. Dry matter of faeces was also obtained by drying a small sample for 24 hours at 100 ^{0}C.

Organic matter of all the samples was determined by ashing the dried samples at 550 ^{0}C for 3 hours.

Total N in abomasum, ileum and faeces samples were determined by the Kjeldahl procedure and ammonia-N in the rumen by the use of an Auto Analyzer II. The concentration of N in the rumen samples was calculated as follows:

$$\text{mmol N / litre in the test sample} = \text{mmol N / litre in the standard} \times \frac{\text{peak height for sample}}{\text{peak height for standard}}$$

Corrections must be made for dilution of the samples e.g. the addition of the sulphuric acid solution as preservative.

$$\text{mmol N / litre} \times 14 = \text{mg N / litre rumen digesta.}$$

Volatile fatty acids were determined by means of injecting the "supernatant" into a gas chromatograph. The Carlo Erba 4200 gas chromatograph with flame ionisation detector was used, fitted with a 20 m glass column (3mm internal diameter) packed with 60/80 Carbopack c/0.3 % carbowax 20 M /0.1 % H_3PO_4. The column was conditioned overnight at 153 ^{0}C and a flow of ±15 ml N per minute.

ADL was determined using methods described by Goering and Van Soest (1970), while NDF was determined by the methods of Robertson and Van Soest (1981). *In vitro* digestibility of the oesophageal samples was determined by making use of the technique described by Tilley and Terry (1963) and modified by Engels and Van der Merwe (1967).

5.4 Statistical analysis

The GLM (General Linear Modules) procedure by Statistical Analysis Systems (SAS, 1995), was used to analyze the data.

All main effects (treatment, replication and period) as well as all possible first-order interactions were included in the initial model. Interactions that did not make a significant ($p > 0.05$) contribution to the variance, were omitted in subsequent analysis.

Models were tested for the dependent parameters namely N, NDF, ADL, IVDOM, intake, rumen pH, volatile fatty acids, rumen ammonia (NH_3-N) and N and organic matter (OM) disappearance in the rumen and small intestine.

Significance of difference between least square means was determined by Bonferoni's test (Van Ark, 1981).

5.5 Results and Discussion

5.5.1 Intake study

Five oesophageal fistulated animals were used to collect samples of the grass selected by grazing animals during the partial digestibility study. The results of the quality parameters of the collected grass are presented in Table 22.

Table 22 Quality parameters of *P. maximum* cv Gatton fertilized with N during different stages of maturity, collected from oesophageal fistulated animals during summer

Parameters	kg N/ha	Vegetative	Early Bloom	Full Bloom
N (g/kg DM)	0	$1.78^{a}{}_{3}$ (±0.1)	$1.70^{a}{}_{3}$ (±0)	$1.70^{a}{}_{3}$ (±0)
	75	$2.28^{a}{}_{2}$ (±0.1)	$1.88^{b}{}_{2}$ (±0.1)	$1.98^{b}{}_{2}$ (±0.1)
	150	$2.75^{a}{}_{1}$ (±0.3)	$2.13^{b}{}_{1}$ (±0.1)	$2.03^{b}{}_{1}$ (±0.1)
NDF (%)	0	$63.40^{b}{}_{1}$ (±1.3)	$66.50^{a}{}_{12}$ (±0.7)	$64.85^{ab}{}_{1}$ (±1.2)
	75	$63.38^{b}{}_{1}$ (±2.1)	$68.30^{a}{}_{1}$ (±1.4)	$63.85^{b}{}_{1}$ (±1.8)
	150	$59.40^{b}{}_{2}$ (±2.0)	$65.55^{a}{}_{2}$ (±2.0)	$65.75^{a}{}_{1}$ (±1.1)
ADL (%)	0	$3.48^{ab}{}_{1}$ (±0.6)	$4.78^{a}{}_{1}$ (±1.0)	$5.25^{a}{}_{12}$ (±0.5)
	75	$3.78^{b}{}_{1}$ (±0.4)	$5.23^{a}{}_{1}$ (±0.5)	$5.73^{a}{}_{1}$ (±0.5)
	150	$3.75^{b}{}_{1}$ (±0.4)	$5.03^{a}{}_{1}$ (±0.2)	$4.90^{a}{}_{2}$ (±0.3)
IVDOM (%)	0	$81.08^{a}{}_{1}$ (±2.8)	$72.55^{b}{}_{1}$ (±0.8)	$65.48^{c}{}_{1}$ (±3.3)
	75	$76.3^{a}{}_{2}$ (±2.4)	$69.05^{b}{}_{1}$ (±3.2)	$61.88^{c}{}_{12}$ (±6.3)
	150	$79.58^{a}{}_{1}$ (±1.5)	$71.78^{b}{}_{1}$ (±2.7)	$59.63^{c}{}_{2}$ (±2.7)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$)
The values in brackets indicate standard deviation.

Selected pasture had a high N concentration for all N treatments. As N fertilization increased, the N concentration of selected material also increased significantly ($p \leq 0.05$) from a mean of 1.73 g/kg to 2.30 g/kg. This represents a crude protein concentration 10.81 % and 14.38 % respectively. Various writers also found a strong increase in the crude protein concentration of plants with increasing levels of N fertilization. Van Niekerk *et al.* (1993) found that N fertilization of *P. maximum* cv Gatton, increased the crude protein concentration from 11.25 to 16.9 %. This was a slightly greater increase than found in this experiment. Messman *et al.* (1991) reported an increase in crude protein, during the late boot stage, from 10.79 to 15.6 % when Bromegrass (*Bromus inermis L.*) was fertilized with 0 and 89 kg N/ha respectively.

Where no N fertilization was applied, the N concentration did not decline significantly ($p > 0.05$) as the plants matured, but when the grass was fertilized with either 75 or 150 kg N/ha, the nitrogen concentration declined significantly from 2.28 to 1.98 g/kg and from 2.75 to 2.03 g/kg respectively. Although the N concentration of plants fertilized with 150 kg N/ha declined with age, it never reached the low values seen in grass receiving no N. These results correlate well with results reported by Fleischer *et al.* (1983), who also found that N concentration decreased as plants mature. The reason for this is probably the decline in the proportion of leaves and the increase in amount of stem as plants mature, since leaves contain more N than stems.

Nitrogen fertilization did not appear to have a large influence on the NDF concentration of plants. Although it seemed as if N fertilization depressed the NDF concentration slightly during the vegetative stage, this decrease was not significant ($p > 0.05$). During the early bloom stage there was a significant ($p \leq 0.05$) decrease of NDF as N fertilization was increased, while there were no significant ($p > 0.05$) changes during the full bloom stage. The findings in this trial contrast with those by Panditharatne *et al.* (1986) who found that NDF of orchardgrass increased with high levels (345 kg N/ha) of N fertilization. Rouquette *et al.* (1972) reported, however, that, on a whole plant basis, NDF was reduced by 5 percentage units when *P. coloratum* L. was fertilized with different levels of N ranging from 28 to 560 kg N per ha.

As plants matured, the NDF concentration increased significantly from the vegetative to the early bloom stages, but then decreased significantly to the full bloom stage. This is in contrast with what Cherney *et al.* (1992) found with perennial grasses, where an increase in NDF concentration from 40 to 62.7 % was reported. Rouquette *et al.* (1972) also reported an increase in NDF concentration of the whole plant, with advancing maturity.

Acid detergent lignin was not significantly changed by N fertilization. Although the ADL values increased as N fertilization level increased, this increase was not significant ($p > 0.05$). This is in contrast with Vicente-Chandler *et al.* (1959), who

reported that increasing levels of N fertilization did, in fact, increase the ADL concentration of pastures.

With advancing maturity the ADL concentration of plants in this study increased significantly from 3.67 to 5.29 g/kg. Cell wall concentrations increase with advancing maturity, and since lignin is part of this cell wall, it will also increase with age (McDonald *et al.*, 1992).

Minson (1971) reported that lignin had a digestibility of 5.5 %. This in part explains the decrease in IVDOM from 78.99 to 62.33 % with advancing maturity. According to Van Soest (1982), lignin is the chemical component in forage cell wall, which is most commonly associated with the reduced digestibility of fibre. Therefore, the older the plants are, the more lignin is formed and the more the IVDOM will decrease.

When plants are fertilized with increasing levels of N fertilization, no significant change occurred in the IVDOM of plants. Minson and Milford (1967) reported that when grass was fertilized with N just before the winter, it had no effect on the IVDOM of the pasture. Wilson (1973) also reported that N fertilization did not alter the digestibility of grasses.

5.6 Partial digestibility study

5.6.1 Rumen conditions

5.6.1.1 Rumen pH

Table 23 represents the rumen pH of animals as measured during the different maturity stages of the trial periods.

Table 23 Rumen pH of animals which grazed *P. maximum* cv Gatton at three stages of maturity that was fertilized with three levels of N during the summer

kg N/ha	Vegetative	Early Bloom	Full Bloom
0	$5.95^{b}{}_{1}$ (±0.1)	$6.16^{a}{}_{1}$ (±0.2)	$6.20^{a}{}_{1}$ (±0.1)
75	$6.02^{b}{}_{1}$ (±0.1)	$6.22^{a}{}_{1}$ (±0.1)	$6.23^{a}{}_{1}$ (±0.1)
150	$6.08^{b}{}_{1}$ (±0.1)	$6.26^{a}{}_{1}$ (±0.1)	$6.30^{a}{}_{1}$ (±0.1)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$)
The values in brackets indicate standard deviation.

Normal rumen pH is kept between 5.5 and 6.5 (McDonald *et al.*, 1992). Except for one treatment during the vegetative stage when the pH was 5.95, none of the pH values dropped below 6. Nitrogen fertilization seems to increase the rumen pH slightly, but statistically this increase was not significant ($p > 0.05$). These findings are in contrast with that found by Krysl (1986), who found an increase in rumen pH with increasing levels of fertilization.

The rumen pH of grazing animals increased significantly ($p \leq 0.05$) as the grass matured. This increase was evident on all N treatments. As grasses mature, chewing time on the harder plant parts increases and, therefore, more saliva is produced. Saliva has a buffering effect on the volatile fatty acids in the rumen (Krysl *et al.*, 1987). This phenomenon explains the increase in rumen pH as plants mature.

5.6.1.2 Rumen ammonia

In Table 24 the rumen ammonia levels, as influenced by N fertilization and stage of maturity, are presented.

Table 24 **Rumen ammonia concentration (mg/100 ml rumen fluid) of animals grazing *P. maximum* cv Gatton fertilized with different levels of N and grazed at different stages of maturity during summer**

kg N/ha	Vegetative	Early Bloom	Full Bloom
0	$26.93^{a}{}_{1}$ (±9.1)	$12.47^{b}{}_{1}$ (±4.4)	$8.10^{b}{}_{1}$ (±2.9)
75	$30.88^{a}{}_{1}$ (±9.5)	$15.33^{b}{}_{1}$ (±3.2)	$13.80^{b}{}_{1}$ (±5.4)
150	$34.38^{a}{}_{1}$ (±9.4)	$19.05^{b}{}_{1}$ (±3.1)	$12.28^{b}{}_{1}$ (±3.6)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).
The values in brackets indicate standard deviation.

Shortly after N fertilization (during the vegetative stage) the NH_3-N concentration was at its highest for all three treatments. Although not statistically meaningful, there is a tendency for the NH_3-N to increase with increasing levels of N fertilization. In work done by McIntyre (1970) who reported that forage providing sheep with 37.3 g N/day, resulted in a rumen NH_3-N level of between 20 – 30 mg/100 ml rumen fluid. These values correlate well with the levels reported in this trial, during the vegetative stage. According to Morrison and Russel (1980) the optimum NH_3-N level for fermentation in the rumen should be 5 mg NH_3–N per 100 ml rumen fluid. This value is, however, not enough to stimulate food intake or to ensure optimum microbial activity (Morrison and Russell, 1980). A somewhat higher value than 5 mg /100 rumen fluid would, therefore, appear more desirable. In this trial, however, there was no danger of too low NH_3-N values, rather too high NH_3-N values, especially during the vegetative stage. These high values, ranging from 26 to 34 mg /100 ml rumen fluid, can have a negative influence on animal performance, especially in fast growing animals who have a high energy requirement (Van Niekerk, 1997). The reason for this is that only the feed protein converted to microbial protein, as well as the proportion escaping degradation in the rumen, will eventually be utilized in the small intestine as protein. The remaining proportion of the NH_3-N released in the

rumen, will be absorbed and converted to urea. This is an energy consuming process and puts strain on the energy reserves of the body (Van Niekerk, 1997). As plants mature, there is a significant ($p \leq 0.05$) decline in the rumen NH_3-N concentration. Although the NH_3-N concentration decreased between 60 and 70 % as plants matured, values still ranged between 8 and 13 mg/100ml rumen fluid. The rumen NH_3-N concentration was, therefore, still high enough to ensure optimum rumen bacterial activity.

5.6.1.3 Volatile fatty acids

Volatile fatty acids (VFA) in the rumen are produced by the breakdown of polysaccharides by the rumen bacteria (McDonald *et al.*, 1992). In Table 25 the volatile fatty acids produced in this way, are presented.

Table 25 **Different volatile fatty acids (mmol/100 ml rumen fluid) produced in the rumen of sheep grazing *P. maximum* cv Gatton fertilized with different levels of N, and at different stages of maturity, during the summer**

Parameters	kg N /ha	Vegetative	Early bloom	Full bloom
Total volatile fatty acids	0	$24.63^{a}{}_{2}$ (±1.5)	$21.03^{a}{}_{1}$ (±3.1)	$22.20^{a}{}_{1}$ (±2.8)
	75	$24.45^{a}{}_{1}$ (±1.3)	$21.90^{b}{}_{1}$ (±1.6)	$21.35^{b}{}_{2}$ (±1.4)
	150	$25.13^{a}{}_{1}$ (±1.2)	$20.80^{b}{}_{1}$ (±2.6)	$18.98^{c}{}_{2}$ (±2.3)
Acetic acid	0	$17.93^{a}{}_{2}$ (±1.3)	$14.93^{a}{}_{1}$ (±2.1)	$15.70^{a}{}_{1}$ (±1.9)
	75	$17.58^{a}{}_{1}$ (±0.8)	$15.43^{b}{}_{1}$ (±1.2)	$15.68^{b}{}_{2}$ (±1.7)
	150	$18.15^{a}{}_{1}$ (±0.8)	$15.00^{b}{}_{1}$ (±1.9)	$13.55^{c}{}_{2}$ (±1.6)
Propionic acid	0	$4.25^{a}{}_{2}$ (±0.3)	$3.88^{a}{}_{1}$ (±2.0)	$4.23^{a}{}_{1}$ (±0.7)
	75	$4.40^{a}{}_{1}$ (±0.4)	$4.13^{b}{}_{1}$ (±0.4)	$3.73^{b}{}_{1}$ (±0.4)
	150	$4.60^{a}{}_{1}$ (±0.6)	$3.83^{b}{}_{1}$ (±0.4)	$3.78^{b}{}_{1}$ (±0.6)
Butyric acid	0	$2.00^{a}{}_{2}$ (±0.2)	$1.70^{a}{}_{1}$ (±6.0)	$1.73^{a}{}_{1}$ (±0.4)
	75	$1.95^{a}{}_{1}$ (±0.2)	$1.88^{ab}{}_{1}$ (±0.3)	$1.55^{b}{}_{1}$ (±0.1)
	150	$1.88^{a}{}_{1}$ (±0.3)	$1.60^{b}{}_{1}$ (±0.2)	$1.33^{b}{}_{1}$ (±0.2)
A : P*	0	$4.25^{a}{}_{1}$ (±0.3)	$3.884^{a}{}_{1}$ (±0.2)	$3.75^{a}{}_{1,2}$ (±0.4)
	75	$4.03^{c}{}_{1}$ (±0.3)	$3.75^{bc}{}_{1}$ (±0.3)	$4.23^{a}{}_{1}$ (±0.8)
	150	$3.98^{a}{}_{1}$ (±0.6)	$3.98^{a}{}_{1}$ (±0.1)	$3.68^{a}{}_{2}$ (±0.4)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$)
The values in brackets indicate standard deviation.

*A : P = ratio of acetic acid to propionic acid

Volatile fatty acids in the rumen are produced by the breakdown of polysaccharides by the rumen bacteria. On a fibre based diet acetate is usually the dominant VFA, while it will be less dominant on a grain based diet (Michell, 1974).

Total volatile fatty acid concentrations in the rumen increased significantly ($P \leq 0.05$) during the vegetative stage when grass was fertilized with increasing levels

of N. This increase was, however, not evident during the early and full bloom stages. During these two stages the VFA concentration decreased, although the decrease was not significant during the full bloom stage.

Except for the full bloom stage, the acetic acid concentration increased slightly during the vegetative and early bloom stages as N fertilization increased. This increase was not, however, significant ($p > 0.05$).

Propionic acid also increased while butyric acid decreased with increasing levels of N fertilization. As was the case with acetic acid, neither of these changes were significant ($p > 0.05$).

From this trial it did not seem as if N fertilization had a very large influence on the VFA production in the rumen, except during the vegetative stage.

Although the values differ widely, the tendency was for most of the VFA in the rumen to decrease as the plants matured.

When no N was applied, the A:P ratio tended to decrease with advancing maturity, although this decrease was not significant ($p > 0.05$). When N was applied, the A:P ratio increased significantly ($p \leq 0.05$) or stayed the same. With increasing levels of N fertilization, no significant ($p > 0.05$) change was observed.

5.7 Organic matter disappearance

The following Table shows the disappearance of OM in the different parts of the digestive system of animals grazing on *P. maximum* cv Gatton fertilized with different levels of N and at different stages of maturity.

Table 26 OM disappearance in the stomach and small intestine of sheep grazing *P. maximum* cv Gatton fertilized with N at different stages of maturity

Parameters	kg N/ha	Vegetative	Early Bloom	Full Bloom
OM disapp. in the stomach (g/d)	0	$189.13^{b}{}_{1}$ (±93.1)	$937.5^{a}{}_{1}$ (±112)	$193.65^{b}{}_{1}$ (±166)
	75	$520.43^{a}{}_{1}$ (±99)	$851.8^{a}{}_{1}$ (±100)	$318.05^{a}{}_{1}$ (±100)
	150	$510.75^{a}{}_{1}$ (±85.6)	$597.0^{a}{}_{1}$ (±366)	$167.25^{b}{}_{1}$ (±57.8)
OM disapp. as % of total OMI	0	$18.53^{b}{}_{2}$ (±10.8)	$55.9^{a}{}_{1}$ (±6.6)	$25.2^{b}{}_{1}$ (±18.1)
	75	$43.90^{a}{}_{1}$ (±7.4)	$55.6^{a}{}_{1}$ (±6.1)	$33.8^{a}{}_{1}$ (±8.4)
	150	$44.0^{ab}{}_{1}$ (±6.7)	$48.2^{a}{}_{1}$ (±17.4)	$23.9^{b}{}_{1}$ (±9.9)
OM disapp. in the small intestine (g/d)	0	$389.23^{a}{}_{1}$ (±185)	$205.68^{a}{}_{1}$ (±101)	$151.88^{a}{}_{1}$ (±88.3)
	75	$160.75^{a}{}_{1}$ (±123)	$217.05^{a}{}_{1}$ (±148)	$100.23^{a}{}_{1}$ (±159)
	150	$298.18^{a}{}_{1}$ (±124)	$163.98^{a}{}_{1}$ (±205)	$164.40^{a}{}_{1}$ (±118)
OM disapp. as % of Total OMI	0	$35.15^{a}{}_{1}$ (±11.8)	$13.0^{a}{}_{1}$ (±6.6)	$23.18^{a}{}_{1}$ (±16.9)
	75	$13.78^{a}{}_{1}$ (±9.9)	$13.75^{a}{}_{1}$ (±8.5)	$11.58^{a}{}_{1}$ (±17.0)
	150	$25.75^{a}{}_{1}$ (±10.9)	$16.35^{a}{}_{1}$ (±19.6)	$21.28^{a}{}_{1}$ (±13.9)

Values in the same row and in the same column with different superscripts (a-b) and subscripts (1-3) respectively are significantly different ($p \leq 0.05$).
The values in brackets indicate standard deviation.

Both feed and animal factors affect the rate of passage of feed from the rumen, the predominant factors being feed intake, the chemical (Mir *et al.*, 1991) and physiological nature of the diet, the physiological state of the animal and the climatic conditions under which the animal "lives" (Faichney and Black, 1984). Although there are very large differences between the individual values, there are, with the exception of one or two values, no real statistical differences between the disappearance of OM in the stomach of sheep when they consume grass fertilized with different levels of N. The only statistical difference noted was

that, when *P. maximum* cv Gatton received no N, a decrease in disappearance of OM from the rumen was observed during the vegetative and full bloom stages.

Rate of digestion of food refers to the quantity of food that can be digested per unit of time. It is essentially a function of time. The speed of digestion is determined by the composition of the diet, its quality, deficiencies, excesses and availability of nutrients. Generally soluble components such as sugars, are fermented very rapidly, while less soluble substrates are digested more slowly (Van Soest, 1982). The more slowly components are digested, the longer will be the retention time. Meissner *et al.* (1992) reported that more OM would disappear in the stomach when the retention time was longer.

High levels of N fertilization caused the crude protein (CP) concentration as well as sometimes the lignin concentration of pastures to increase, while soluble carbohydrates decreased because of the rapid growth of plants. Such inbalances can cause disturbances in the rumen of the animal (Nowakovski, 1962).

The main N product when protein breaks down in the rumen is ammonia (Nowakovski, 1962). This process, however, needs energy which microbes obtain by fermenting soluble components, such as soluble carbohydrates. When there is a shortage of energy, microbial growth can be limited (Steinhour and Clark, 1980). With fewer microbes in the rumen, less of the de-aminated protein will be built into microbial protein. This process will also take longer, and more OM can, therefore be absorbed in the stomach parts.

The disappearance of OM in the small intestine corresponds well with the amounts absorbed in the rumen. The more OM absorbed in the rumen, the less will disappear in the small intestine and *visa versa*. This finding corresponds well with the finding of Van Niekerk, (1997) who reported that the elevated disappearance of OM in the small intestine indicates that a part of the OM not absorbed in the stomach has reached the small intestine and was absorbed there.

5.8 Conclusion

In this trial it was found that N fertilization had a positive influence on most of the parameters studied. Nitrogen fertilization increased the N concentration of plants from as little as 1.72 % to a high of 2.3 % when grass was fertilized with 0 and 150 kg N /ha respectively. With advancing maturity the N concentration declined significantly. Although the differences were not always significant, it seemed as if N fertilization had a slight depressing effect on NDF concentration of plants, while stage of maturity increased NDF concentration significantly. Nitrogen fertilization did not have a significant effect on ADL concentration, while ADL concentration was increased by stage of maturity.

No change in IVDOM was found when levels of N fertilization were increased, but IVDOM was decreased, as plants grew older.

When sheep grazed the fertilized grass, N fertilization did not seem to have a significant effect on the rumen pH, while rumen pH was slightly increased as pastures matured.

Rumen ammonia concentration was high when sheep grazed pastures during the vegetative stage, shortly after N fertilization was applied. As the pasture matured the high levels of NH_3-N declined significantly to more acceptable levels.

Total volatile fatty acid concentration in the rumen was increased by N fertilization during the vegetative stage, but no change was found during the more mature stages. Total volatile fatty acid concentration was significantly decreased by stage of maturity.

Although the individual values differ, it is evident from this trial that a larger proportion of the OM disappearance, postulated as a percentage of OM intake, was absorbed in the rumen when grass was fertilized with 75 kg N/ha. A corresponding lower amount of OM as a percentage of OM intake disappeared in the small intestine when grass was fertilized with 75 kg N/ha. Larger amounts of OM disappeared in the small intestine when grass was fertilized with either 0 or 150 kg N/ha.

CHAPTER 6

GENERAL CONCLUSION

According to this experiment it is clear that N fertilization and stage of maturity have a marked effect on a variety of qualitative and quantitative parameters *of P. maximum* cv Gatton. It must, however, be kept in mind that these effects are not always positive and that furthermore, this grass is produced with one goal in mind, namely to feed animals. One must, therefore, not only strive for maximum grass production per hectare, but the nutritional value should be optimized to sustain healthy animal production.

An examination of the factors influencing DM yield and nutritional value of *P. maximum* cv Gatton, indicates that factors (such as N fertilization or stage of maturity) should not be considered in isolation.

During both the summer and autumn trials, DM yield increased as the grass matured, as well as when the level of N fertilization was increased. Up to 150 kg N/ha, as was used in this trial, DM yield continued to increase. It is therefore suggested that DM yields could be increased even further during both summer and autumn, if the levels of N fertilization were increased. The N levels should, however, not be too high, because DM yield is not the only parameter that is important in terms of animal production.

With and increase in N fertilization levels, the N concentration in plants increased rapidly immediately after N application, but decreased with maturity. This was observed in both the summer and autumn, although the individual values for the summer were higher than those in autumn. Although N concentration decreased with advancing maturity, the values for *Panicum*, receiving no N were always lower than that receiving extra N. This was evident in both summer and autumn. The generally accepted N deficiency level of plants is 1,5 % N and lower. In both summer and autumn the N concentrations were much higher than this value

when *Panicum* was fertilized with 75 – 100 kg N/ha. This was also the case during the vegetative and early bloom stages. During the summer a N application of 75 kg N/ha and more could also sustain this minimum suggested level at the full bloom stage. During autumn, not even 150 kg N/ha ensured a minimum concentration of 1,5 % N in the grass.

Total nonstructural carbohydrates can easily be digested in the rumen and are used as a source of energy by the animal. This is, therefore, an important factor, which can influence animal production. These results indicated that increasing levels of N fertilization tended to decrease the TNC concentration, mainly because of the vigorous growth of *Panicum* that goes hand in hand with N fertilization. With maturation, growth rate of a grass and the production of leaves decline, and stems and flowers increase respectively. This is accompanied by an increase in the TNC concentration of such a grass.

While TNC content declined with increasing levels of N fertilization, the NO_3-N concentration of *Panicum* increased very rapidly shortly after N was applied. This was true for both summer and autumn growth periods. This is a undesirable trait, because NO_3-N at high concentrations is poisonous to animals. It is, therefore, recommended that animals must not be allowed to graze newly N-fertilized *Panicum* during the vegetative stage.

Nitrogen fertilization decreased the NDF concentration of *Panicum* in both summer and autumn, but as the grass matured, the NDF concentration increased markedly. *Panicum* should, therefore, be utilized before the NDF concentration is too high. Early bloom proved to be the optimal stage.

In this experiment it was found that N fertilization did not have a significant influence on the ADL concentration of *Panicum*. As *Panicum* grew older, however, the ADL concentration increased significantly. This was found to be true for both summer and autumn, although the ADL concentration increased to a higher value in summer than in autumn during the full bloom stages.

Both NDF and ADL are correlated with IVDOM. As a grass matured, both NDF and ADL increased, while IVDOM decreased. As a result a significant decrease in the digestibility of the grass was observed during the full bloom stages in both summer and autumn.

Increasing levels of N fertilization had little effect on IVDOM in both summer and autumn.

It is important to examine the influence of N fertilization and stage of maturity on both plants and animals, since animal production remains the main objective. The rumen pH of grazing animals was not altered with increasing levels of N fertilization. This was evident for both summer and autumn. Rumen pH was, however, increased when the animals grazed a more mature grass. Because of the high concentrations of NO_3-N in *Panicum* in the vegetative stage, the rumen ammonia-N levels were very high in both summer and autumn. A concentration of 5 mg ammonia-N per 100 ml of rumen fluid is enough to satisfy the needs of the rumen micro-organisms. When sheep grazed *Panicum* fertilized with 150 kg N/ha during the vegetative stage, the rumen ammonia level was almost 8 times higher than the suggested level. This concentration decreased drastically as *Panicum* matured, although these values were still higher than the suggested 5 mg per 100 ml rumen fluid.

Total volatile fatty acids produced in the rumen were increased by increasing levels of N fertilization in both summer and autumn. While VFA's increased in autumn, these acids decreased in summer as *Panicum* matured.

The DM intake of animals decreased as the levels of N fertilization increased, but also decreased as the plants matured. This was evident in both summer and autumn.

Although individual values differ widely, it was found that the best flow of digesta through the digestive tract of sheep was when *Panicum* was fertilized with 75 kg N/ha, both during summer and autumn.

It is evident from the above that one cannot take only one parameter into account when making a decision on how much fertilizer to use or when to utilize a grass. From this experiment it is recommended that *P. maximum* cv Gatton should be fertilized with 75 to 100 kg N /ha/year and be utilized by sheep during the early bloom stage. This will not necessarily ensure the maximum production of such grass, but will ensure optimum animal production.

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