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).

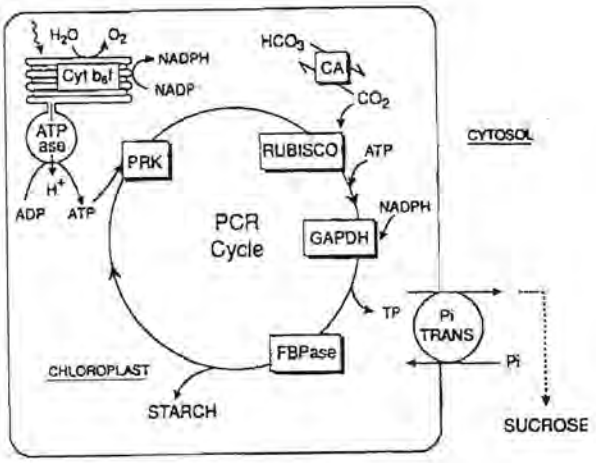


Figure 1 Simple schematic scheme of the C3 pathway (Furbank and Taylor, 1995)

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.

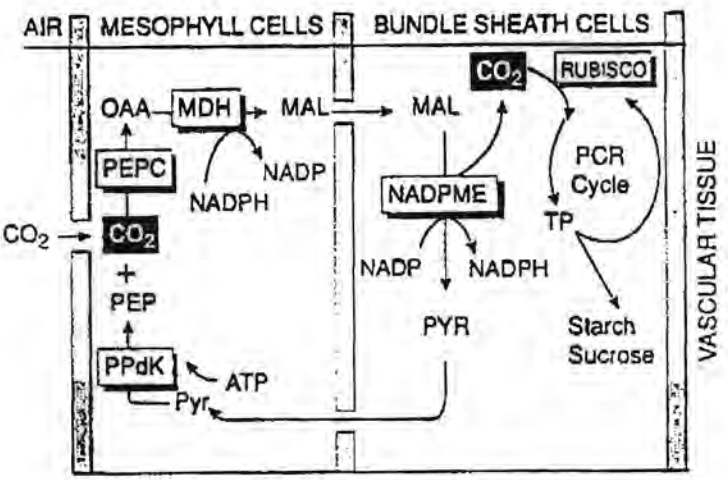
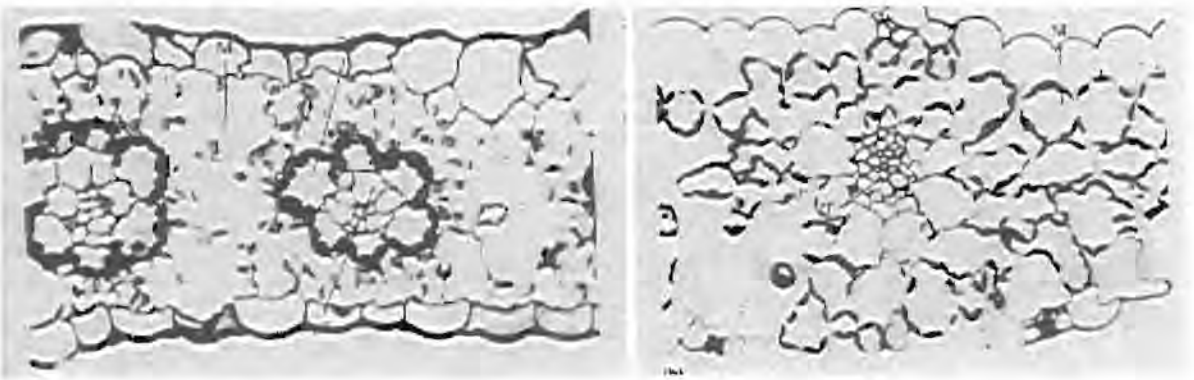


Figure 2 A Schematic scheme of the C4 photosynthetic assimilation cycle (Furbank and Taylor, 1995)

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).

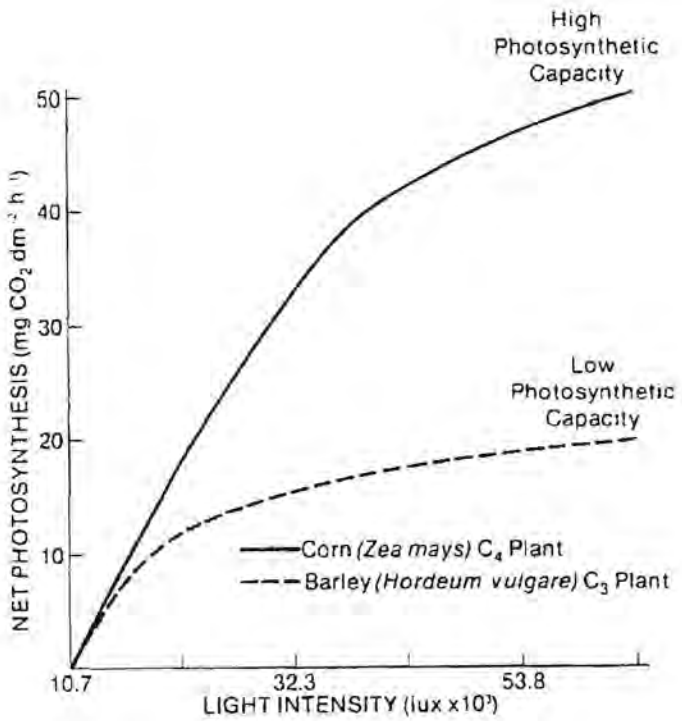


Figure 4 **Effect of light intensity on the nett photosynthetic rates of a C4 plant (corn) and a C3 plant (barley) (Taiz and Zeiger, 1991)**

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).

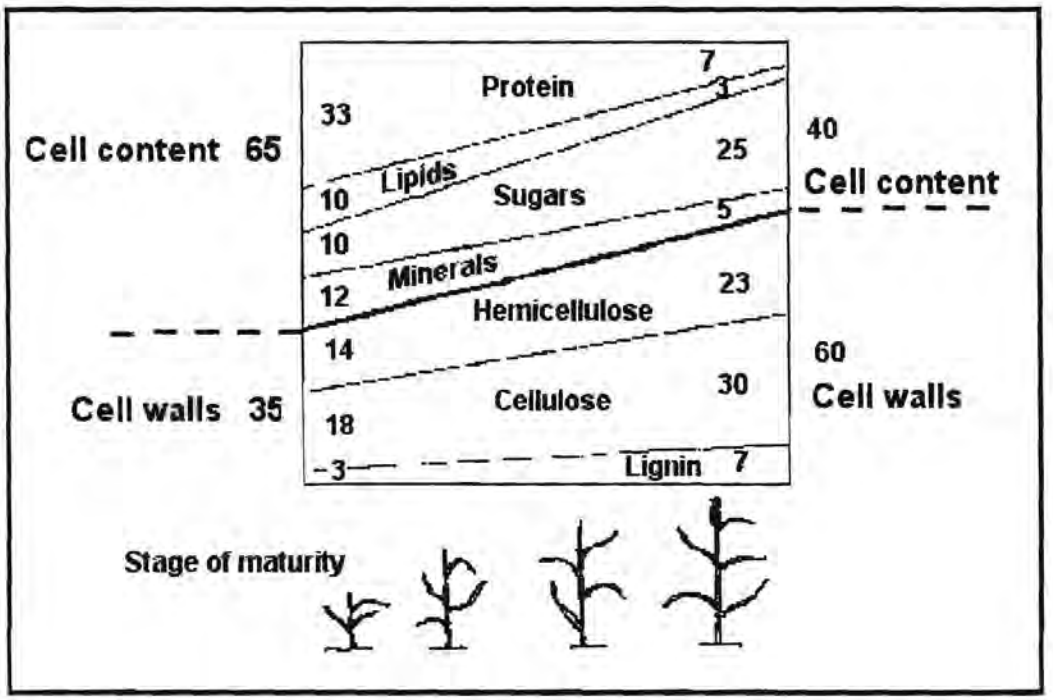


Figure 5 **A schematic scheme of the changes in chemical composition of plants as they go through different stages of maturity (Holmes, 1980 as quoted by Van Niekerk, 1997).**

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.

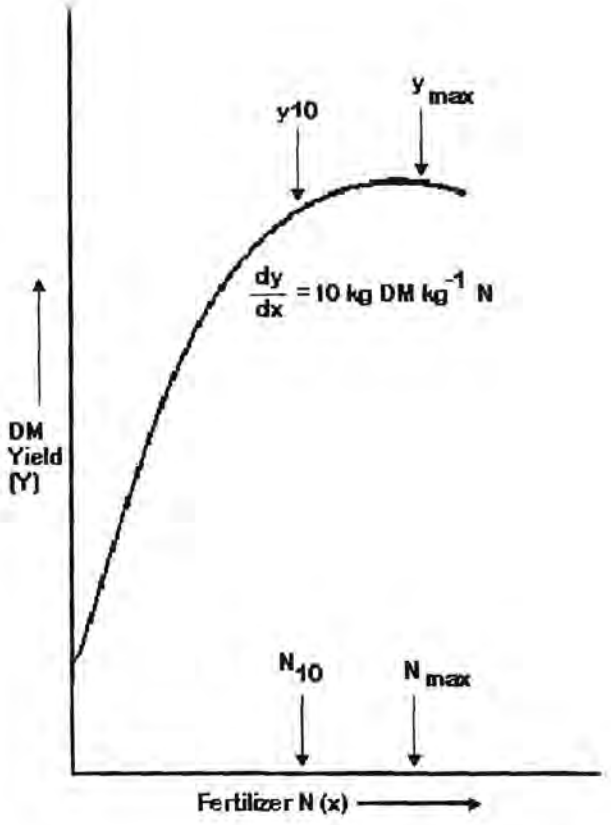


Figure 6: General form of the response of grassland to nitrogen fertilization (Morrison *et al.*, 1980)

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).

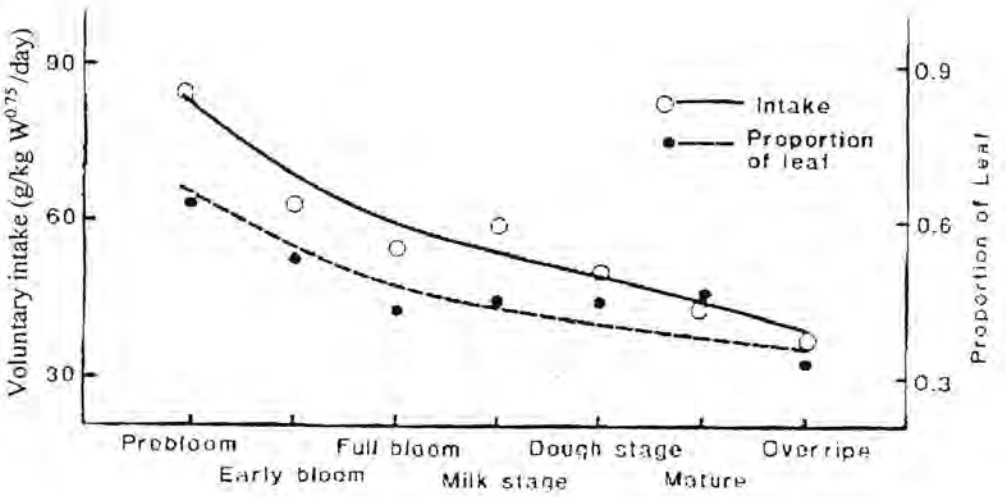


Figure 7 **Mean voluntary intake by sheep and proportion of leaf in four grasses cut at different stages of growth. (Data from Troelsen and Campbell (1969)**

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.

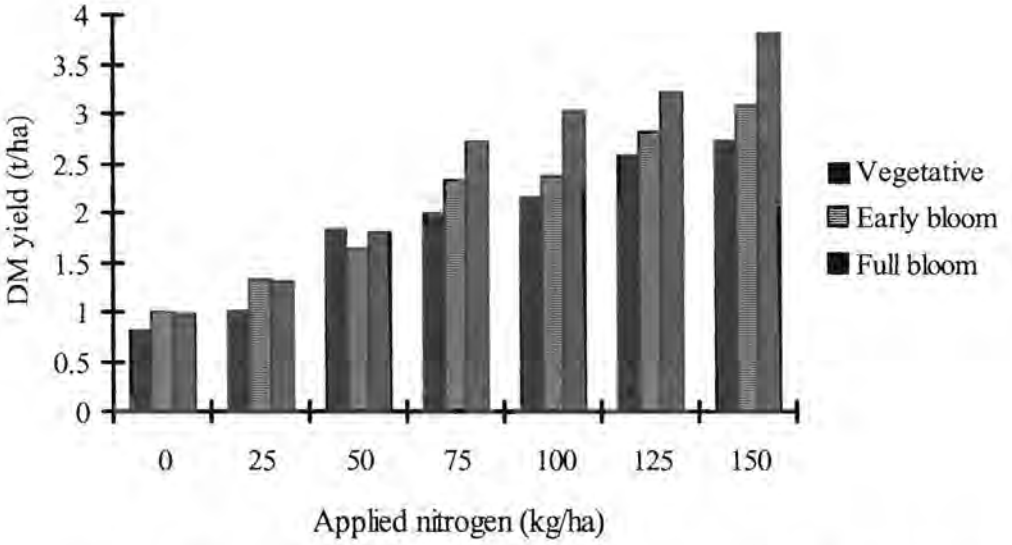


Figure 8 DM yield (t/ha) of *P. maximum* cv Gatton, as affected by level of N fertilization and stage of maturity during autumn

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.

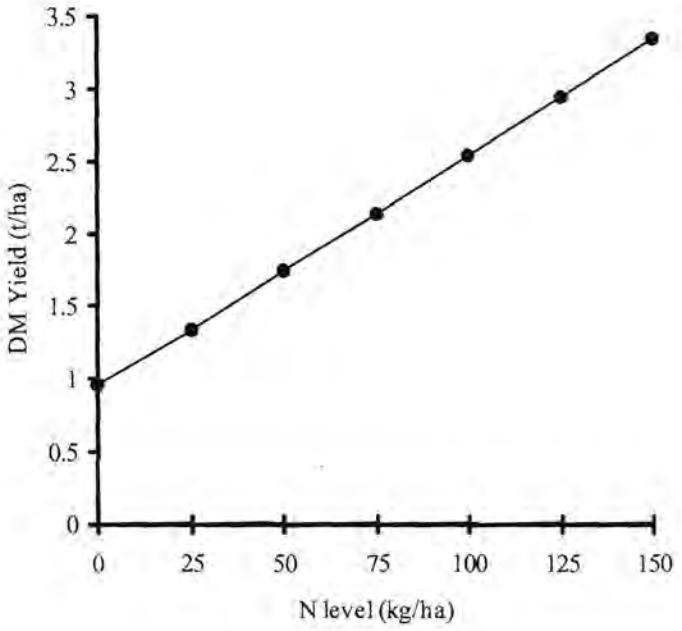


Figure 9 **The relationship between DM yield and N level of *P. maximum* cv Gatton ($Y = 0.016x + 0.94$, $r^2 = 0.71$)**

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.

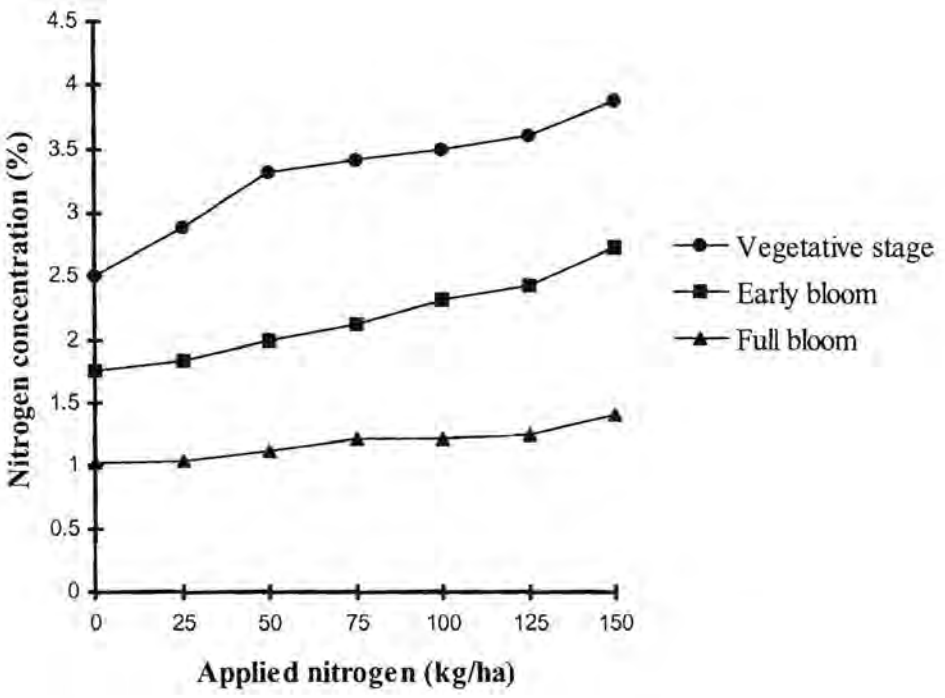


Figure 10 **Nitrogen concentration of *P. maximum* (cv. Gatton) during three stages of maturity as influenced by level of N fertilization**

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.

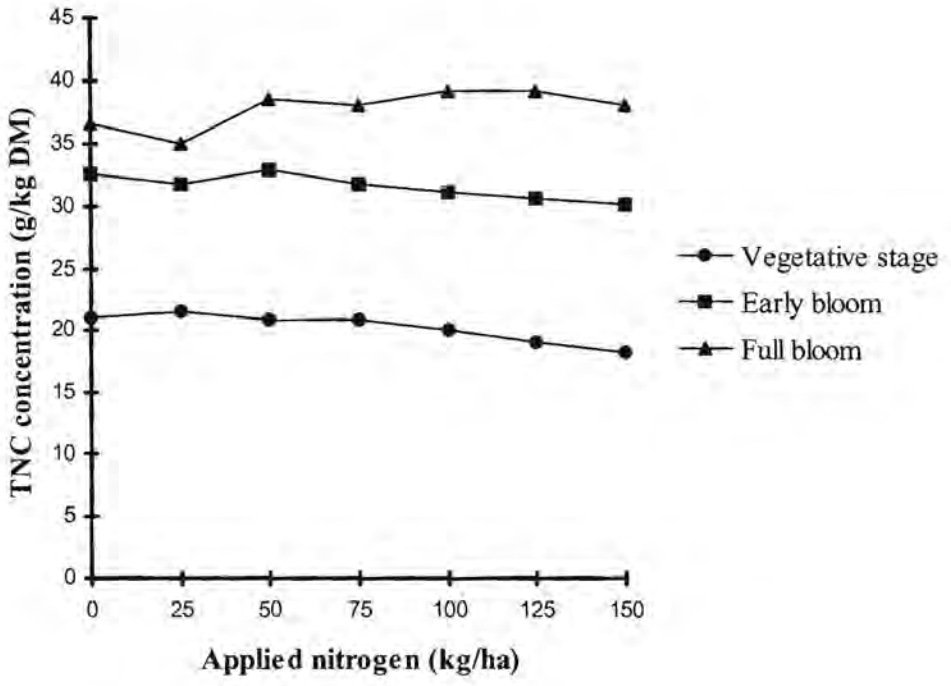


Figure 11 TNC concentration of *P. maximum* cv Gatton during three stages of maturity as influenced by level of N fertilization

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.

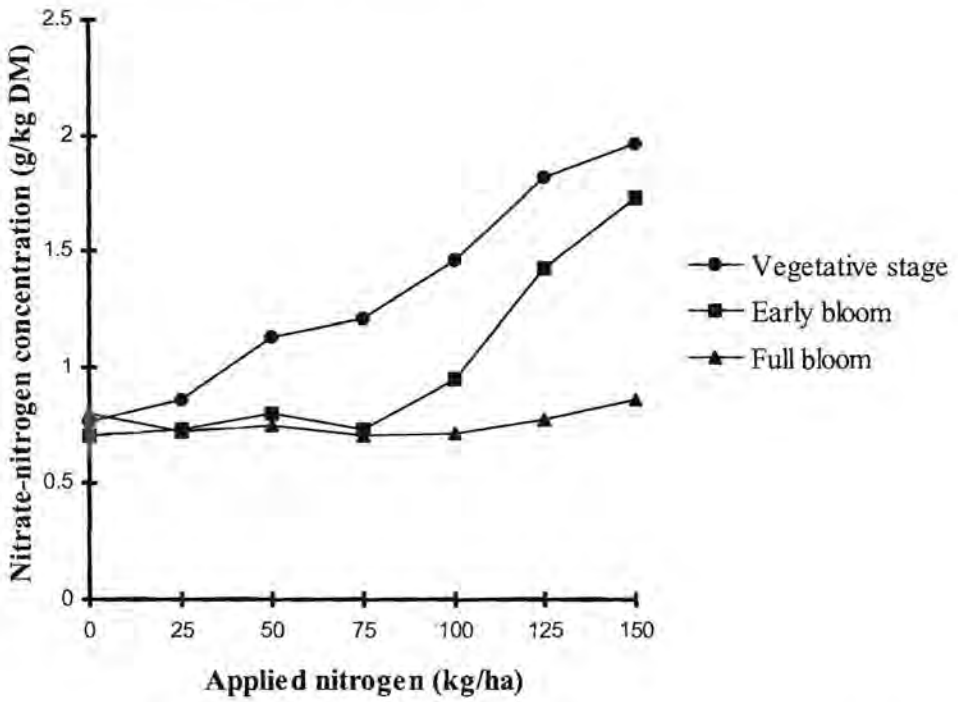


Figure 12 The NO_3-N concentration of *P. maximum* cv Gatton at three stages of maturity as influenced by level of N fertilization

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.