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

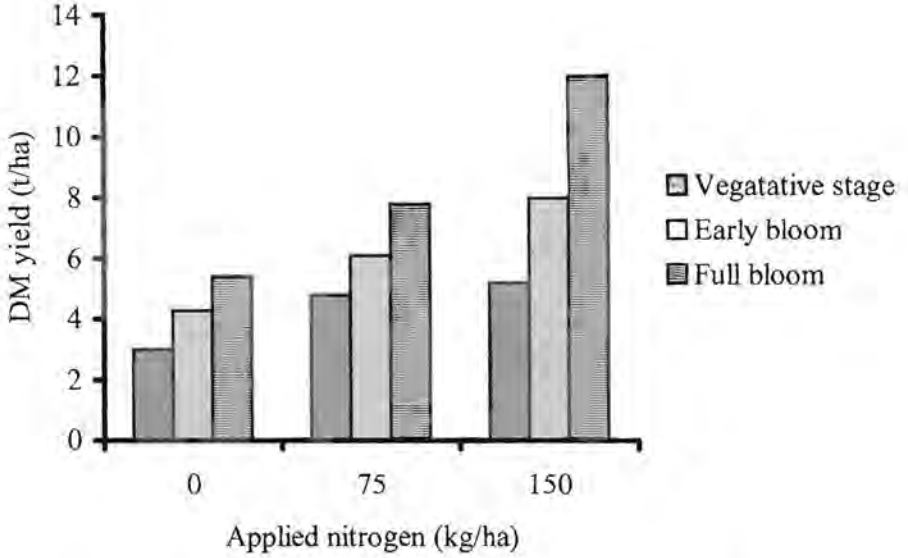

Figure 13 The influence of three N treatments on the DM yield of *P. maximum* cv Gatton during three stages of maturity in summer

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.

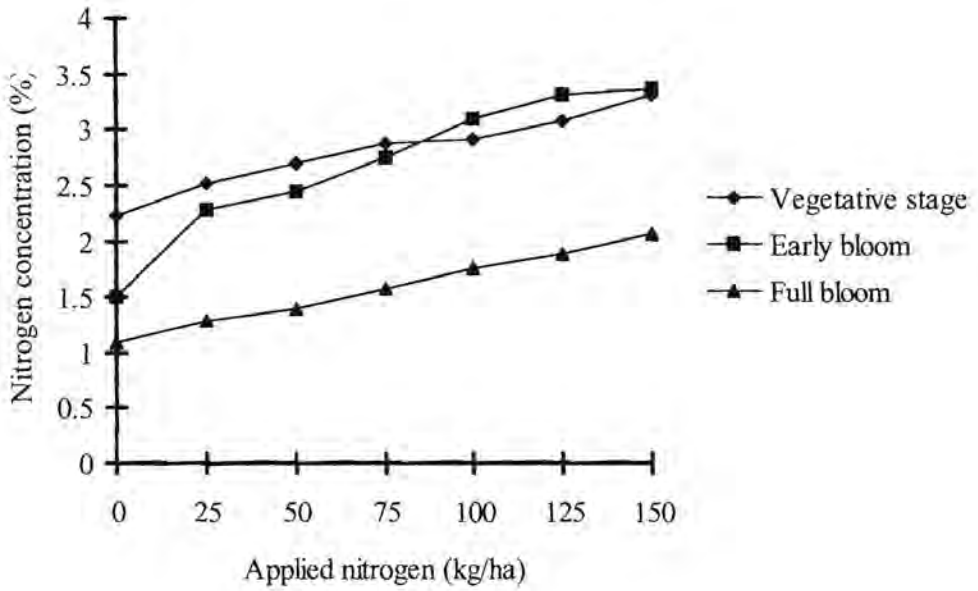

Figure 14 The influence of N fertilization on the N concentration of *P. maximum* cv Gatton during three stages of maturity in the summer months

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.

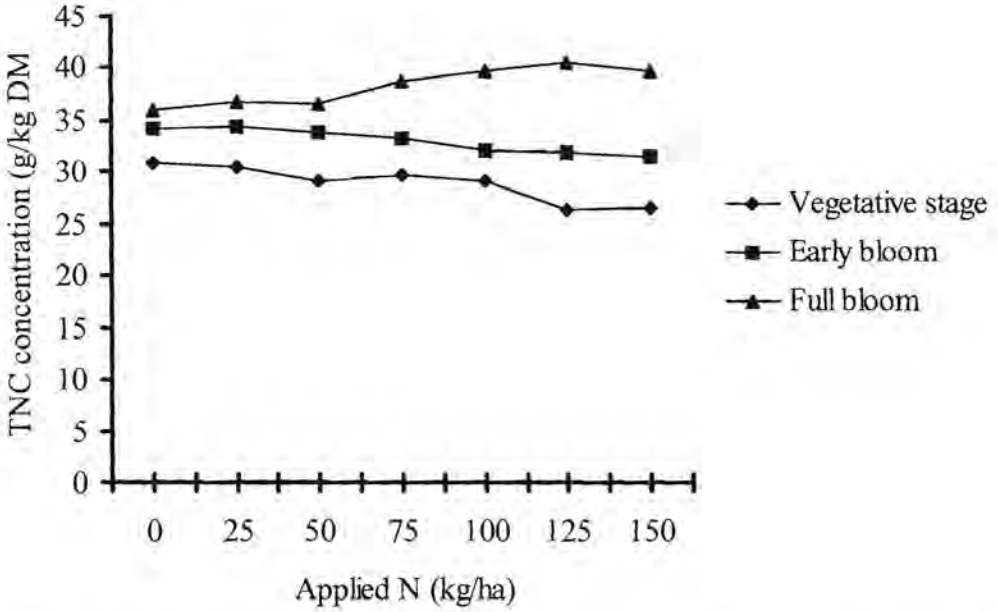

Figure 15 The influence of N fertilization on the TNC concentration of *P.maximum* cv Gatton at three maturity stages in the summer growing period

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.

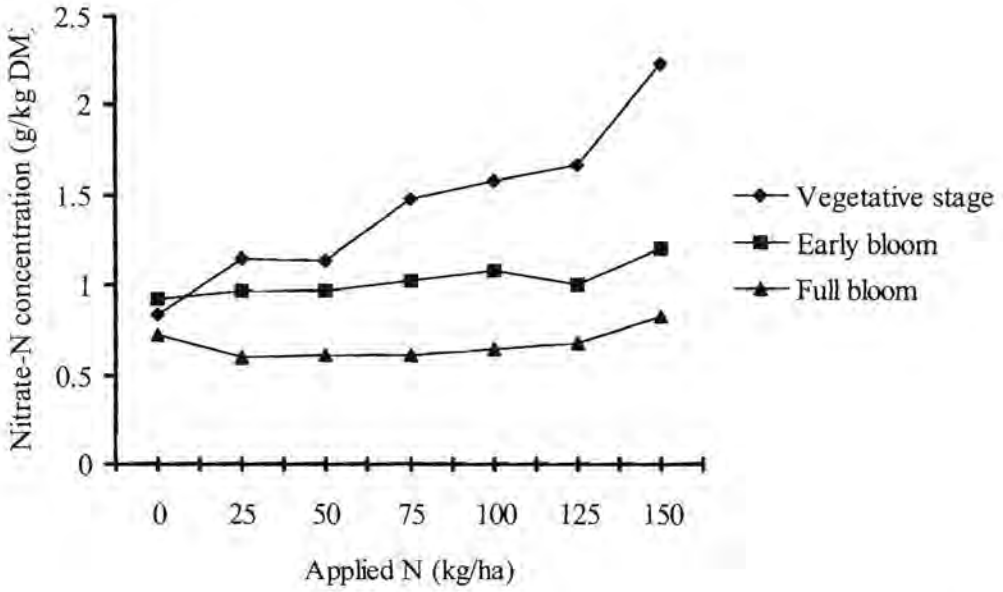

Figure 16 The influence of N fertilization and stage of maturity on the nitrate concentration of *P. maximum* cv Gatton during the summer months

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.