Relationship between nitrogen and other chemical components in kikuyu grass from long-established pastures

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The relationship between nitrogen and other components in kikuyu grass (*Pennisetum clandestinum*) from long-established pastures was investigated. Leaf material, when compared to stem tissue, was higher in total organic nitrogen and protein content, but lower in non-protein organic nitrogen, nitrate, potassium, lignin, and non-structural carbohydrates. The *in vitro* digestibility of leaf material was higher than that of the stem. A high nitrogen content was associated with high protein and low lignin contents, which favours digestibility. However, it was also associated with a high nitrate content which tended to reduce the digestibility *in vitro*. The conditions under which the nutritional value is optimized without unduly sacrificing yield, should urgently be investigated.

Die verwantskap tussen stikstof en ander komponente in kikoejoegras (*Pennisetum clandestinum*) van lankgevestigde weidings is ondersoek. Blaarmateriaal het, in vergelyking met stingelmateriaal, meer totale organiese stikstof en proteïne, maar minder nie-proteïen organiese stikstof, nitraat, kalium, lignien en nie-strukturele koolhidrate bevat. Die *in vitro*-verteerbaarheid van blaarmateriaal was hoër as dié van die stingels. 'n Hoë stikstofinhoud is geassosieer met 'n hoë proteïen- en lae lignieninhoud, wat verteerbaarheid positief beïnvloed. Dit is egter ook geassosieer met 'n hoë nitraatinhoud, wat neig om verteerbaarheid *in vitro* te verlaag. Die toestande waarby die voedingswaarde geoptimiseer word sonder om onnodiglik opbrengs in te boet, moet dringend ondersoek word.

Keywords: Chemical composition, digestibility *in vitro*, kikuyu, nitrogen content.

Kikuyu (*Pennisetum clandestinum* Hochst) grass is one of the major pasture grasses used for summer grazing in the Natal Midlands and responds well to nitrogen fertilization. The grazing animal tends to recycle a considerable amount of nitrogen in the form of urine and faeces, especially in the case of dairy cattle receiving concentrates. In long-established pastures, this leads to an accumulation of nitrogen to levels unrelated to those due to annual fertilizer applications (Marais, Figenschou & Dennison, 1987). This may affect the whole pasture or sometimes only sections thereof. Annual production on these high-nitrogen pastures is often lower than expected when predictions are based on chemical analyses. Dugmore & Du Toit (1988) showed a significant negative correlation between the dry-matter intake and the NPN content of the herbage. Pattinson (1981) suggested that some nitrogenous component in kikuyu grass could be responsible for lower intake by the grazing animal in autumn. Furthermore, earlier work by this laboratory suggested that high levels of nitrate in high-nitrogen kikuyu could lower the digestibility of the grass (Marais, Therion, Mackie, Kistner & Dennison, 1988).

In order to obtain a better understanding of the factors controlling digestibility, especially in long-established kikuyu pasture containing elevated nitrogen levels, the relationship between nitrogen and other chemical components in kikuyu grass was investigated.

Samples were collected from a long-established kikuyu pasture used over many years as grazing for sheep on the Cedara Agricultural Research Station. The pasture was fertilized with a total of 250 kg N/ha per annum (as limestone ammonium nitrate) with the fertilizer nitrogen being applied in three equal dressings over the growing season (October, December and February). Forty-one grass samples (4-week growth stage) with nitrogen contents covering a wide range, were collected over the growing season (cutting height: 50 mm) and divided into leaf blade and stem + leaf sheath fractions. The samples were dried in a microwave oven and the leaf: stem ratios were determined. Samples were milled to pass a 1-mm sieve prior to chemical analysis.

Total organic nitrogen was determined by means of the macro-Kjeldahl procedure. Protein nitrogen was determined as described by Marais & Evenwell (1983). Non-protein organic nitrogen (NPON) was calculated as the difference between total nitrogen and protein nitrogen. Nitrate nitrogen was analysed by the colorimetric procedure of Cataldo, Haroon, Schrader & Young (1975). The total non-structural carbohydrates (TNC) were analysed by the procedure of Marais (1979). Lignin was determined by the procedure described by Ford, Morrison & Wilson (1979). Potassium was analysed after dry-ashing by means of atomic absorption spectroscopy.

Digestibility *in vitro* was determined by the two-stage procedure of Tilley & Terry (1963) as modified by Minson & McLeod (1972). Grass samples were analysed as such, or after extraction of a sample of known mass with water to remove soluble substances such as nitrate. In some instances, the extracts from high-nitrogen stem material were passed through an anion exchange column (Dowex 1, hydroxide form, 50—100 mesh, 4% cross-linked, 15 ml bed volume) to obtain a fraction containing cationic + neutral substances. Aliquots of the original extract and of the cationic + neutral fraction were passed through cation exchange columns (Dowex 50W, hydrogen form, 200—400 mesh, 8% cross-linked, 15 ml bed volume) to obtain two fractions containing anionic + neutral substances and neutral substances, respectively. To determine the effect of the three fractions on digestibility *in vitro*, aliquots of each fraction were separately added back to the original pre-extracted kikuyu material prior to digestion, at concentrations equal to those in the unextracted grass. The chemical composition of the whole sward was calculated from leaf and stem values taking the leaf: stem ratios into account.

Results presented in Table 1 show that the organic nitrogen content of the kikuyu sward was relatively high (mean 3,6%).

Kikuyu leaf material contained significantly ($P < 0,001$) more organic nitrogen than stem material, of which 77% was incorporated into protein compared to only 59% of the stem nitrogen in protein form. Most of the protein in the kikuyu sward is, therefore, concentrated in the leaf fraction (mean 3,15% N) and is a function of the total organic nitrogen content of the herbage (r = 0,6664, $P < 0,001$; Table 2).

Table 2 Relationship between total organic nitrogen (y) and chemical components (x) in kikuyu sward

x	Regression	r
Protein N	y = 1,039 + 1,011 x	0,6664***
NPON[a]	y = 2,510 + 1,008 x	0,7531***
Nitrate N	y = 2,376 + 3,023 x	0,6599***
TNC[b]	y = 5,080 – 0,223 x	–0,3121*
Potassium	y = –0,112 + 0,763 x	0,6105***

* $P < 0,05$.
*** $P < 0,001$.
[a] Non-protein organic nitrogen.
[b] Total non-structural carbohydrates.

The NPON content of the sward was high (mean 1,09%) and comprised on average about 30% of the total organic nitrogen of the sward. NPON content was positively correlated with the total organic nitrogen content of the sward (r = 0,7531, $P < 0,001$; see Table 2) and comprised about 42% of the total organic nitrogen of high-nitrogen kikuyu plants. The NPON is located mainly in stem tissue (mean 1,24%), in which it comprised up to 52% of the total organic nitrogen of high-nitrogen kikuyu grass.

The mean nitrate nitrogen content of the kikuyu sward was 0,4% (Table 1), which was double the concentration above which symptoms of nitrate toxicity could be expected in grazing animals (Wright & Davison, 1964). The nitrate level of kikuyu increased rapidly with an increase in total organic nitrogen level of the sward (Figure 1).

Leaf nitrate nitrogen levels did not exceed 0,45%, whereas nitrate reached levels of 1,3% in stem tissue of high-nitrogen plants. This confirms previous findings that stem tissue is the main site of nitrate accumulation in kikuyu grass (Marais, Figenschou & Dennison, 1987).

Table 1 Chemical composition and digestibility[a] *in vitro* of kikuyu herbage

	Leaf		Stem		Whole sward	
Component (%)	Range	Mean	Range	Mean	Range	Mean
Organic N	1,85 — 5,24	4,11	1,19 — 4,46	3,01	1,59 —4,89	3,60
Protein N	1,77 — 4,36	3,15	1,02 — 2,70	1,77	1,47 —3,53	2,52
NPON[b]	0,05 — 1,95	0,95	0,17 — 2,30	1,24	0,12 —2,04	1,09
Nitrate N	0,079— 0,450	0,174	0,036— 1,257	0,677	0,060—0,812	0,402
TNC[c]	3,90 — 8,98	6,29	5,38 — 9,59	7,15	4,59 —9,23	6,67
Lignin	3,23 — 5,42	4,34	3,92 — 6,56	5,27	3,56 —5,76	4,77
Potassium	2,39 — 4,82	4,05	4,01 — 7,04	5,82	3,04 —5,60	4,85
DMD[d] *in vitro* (extracted)	60,28 —67,72	64,26	51,62 —65,70	59,83	–	–
DMD *in vitro* (unextracted)	59,67 —68,59	64,49	46,89 —60,98	53,22	–	–

[a] Digestibility was determined either directly or after extraction of the sample with water to remove soluble digestion inhibiting substances.
[b] Non-protein organic nitrogen.
[c] Total non-structural carbohydrates.
[d] Dry-matter digestibility.

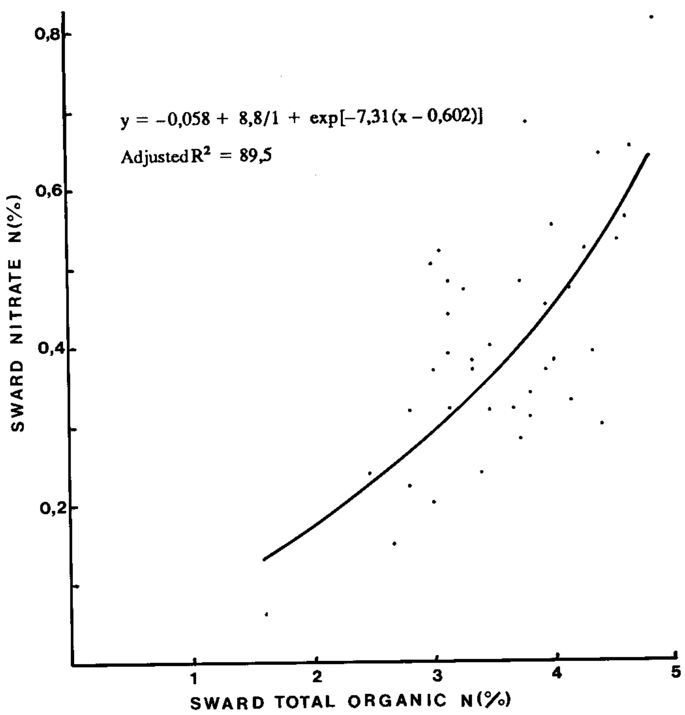

Figure 1 Relationship between the total organic nitrogen content and the nitrate nitrogen content of kikuyu sward.

The TNC level in the plant is controlled by the rate of carbohydrate production through photosynthesis and its rate of utilization for respiration and the synthesis of other plant components. The TNC content of pasture grasses, therefore, follow a diurnal pattern with minimum values at daybreak and reaching maximum values during the early afternoon (Smith, 1973). In the present investigation, grass samples were collected at 10h00, and TNC values are probably submaximal. Nevertheless, the values for whole sward given in Table 1 (mean 6,67%) were similar to those of other grasses of tropical origin (Smith, 1973), but were extremely low from an animal nutrition point of view. Stem TNC values were significantly ($P < 0,001$) higher than in leaf material.

In temperate grass species a negative correlation usually exists between nitrogen level and the non-structural carbohydrate content of the plant (Reid & Jung, 1965; Deinum, Van Soest & Van Es, 1968), while the nitrogen content has little or no effect on the non-structural carbohydrate level of tropical grasses (Ford & Williams, 1973). A significant negative correlation existed between the organic nitrogen content and the TNC content of kikuyu sward ($r = -0,3121$, $P < 0,05$; see Table 2). However, the decrease in TNC with an increase in nitrogen content was not as pronounced as in temperate grasses.

Kikuyu sward had a mean lignin content of 4,77%, which is slightly lower than the lignin values (5,5— 6,7%) for kikuyu grass reported from New Zealand (Bailey & Hunt, 1973). This could be due to the high nitrogen status of the kikuyu used in the present study. A high protein content in grasses is often associated with low cell wall and lignin contents (Ford & Williams, 1973). However, a negative but non-significant response of lignin in the sward to the organic nitrogen content of the grass was found ($r = -0,2250$).

Results presented in Table 1 show a remarkably high luxury uptake of potassium by kikuyu (mean value for leaf, 4,05% and for stem, 5,82%; see Table 1). A significant positive correlation existed between nitrogen and potassium in the sward ($r = 0,6105$, $P < 0,001$; see Table 2). This is in agreement with the findings of Ben–Zioni, Vaadia & Lips (1970; 1971) that potassium acts as a counter ion for nitrate uptake in the plant. High potassium values are probably also due to the recycling of potassium by the grazing animal. It is noteworthy that the potassium content of kikuyu reached levels almost ten times higher than the requirements for beef and dairy cattle (Reid & Jung, 1974).

Differences between the digestibilities of leaf and stem in tropical grasses are often small. Laredo & Minson (1973) reported slightly higher digestibilities (mean, 3,2 digestibility units higher) for stems compared to leaf material of low-nitrogen kikuyu (N < 2%) and several other tropical grasses. However, results presented in Table 1 indicate a mean leaf digestibility *in vitro* of 64% compared to only 53% for the stem tissue. Prior extraction of the sample with water increased the digestibility of stem material to 60%, indicating the presence of a water-extractable digestion inhibitor in kikuyu stems. The increase in *in vitro* digestibility of the sample, due to the extraction of soluble substances, could be counteracted by the addition of an amount of nitrate equivalent to the amount in the extract (Marais, 1980). In rumen digests, nitrate is partly converted to nitrite, which has been shown to reduce the digestibility by inhibiting the growth of some of the major cellulolytic rumen bacteria (Marais, Therion, Mackie, Kistner & Dennison, 1988). This suggests the possible involvement of nitrate in low digestibility of high-nitrogen kikuyu. Poor correlations existed between both leaf and stem nitrate and the digestibility of the tissue (leaf, $r = 0,2966$, $P > 0,05$; stem, $r = 0,1276$, $P > 0,05$). However, the increase in *in vitro* digestibility of the kikuyu stem material after aqueous extraction of soluble substances was closely correlated with nitrate content ($r = 0,9159$, $P < 0,001$; see Table 3).

In the present investigation, the potassium content of kikuyu stem material was also correlated with the increase in digestibility of stem material after aqueous extraction ($r = 0,6308$, $P < 0,001$; see Table 3). This was probably due to the close correlation between stem potassium and stem nitrate ($r = 0,6372$, $P < 0,001$) and not due to a direct effect of potassium on digestion *in vitro*. This was confirmed by the fact that the digestibility of pre-extracted kikuyu samples was not decreased by adding back to the digest the cationic fraction of the aqueous extract containing the potassium (Table 4). The reduction of digestibility by only the anionic fraction (containing the nitrate), further points to the possible involvement of nitrate in reduced digestibility.

Results presented in Figure 2 show that digestibility was little affected at low levels of nitrate, while at nitrate levels in excess of 0,3%, digestion was markedly affected. The small differences between the digestibility of extracted and unextracted leaf samples and the poor correlation between leaf nitrate and the increase in digestibility after aqueous extraction ($r = -0,0472$, $P > 0,05$), were probably due to the relatively low levels of nitrate (mean 0,17%) in leaf material which did not affect digestion. Of the 41 samples analysed, only four leaf samples contained slightly more than 0,3% nitrate.

Table 3 Relationship between chemical components (x) in kikuyu herbage and (1) the digestibility *in vitro* of pre-extracted grass or (2) the increase in digestibility *in vitro* after aqueous extraction (y)

Component x		Regression	
1. Digestibility; pre-extracted sample			
Leaf	Leaf protein N	y = 57,83 + 2,049 x	0,5338***
	Leaf lignin	y = 71,48 – 1,648 x	–0,3783*
Stem	Stem protein N	y = 49,30 + 5,91 x	0,5927***
	Stem lignin	y = 77,62 – 3,358 x	–0,5060***
2. Increase in digestibility after extraction			
Stem	Stem nitrate	y = –1,574 + 9,896 x	0,9159***
	Stem potassium	y = –6,93 + 2,067 x	0,6308***

* $P < 0{,}05$.
*** $P < 0{,}001$.

Table 4 The effect of the cationic, anionic and neutral fraction of a water extract of high-nitrogen kikuyu grass on the digestibility of water-extracted grass[a]

Treatment prior to digestion *in vitro*	Dry-matter digestibility *in vitro*
Control (unextracted)	55,27 ± 0,59
Extracted with water	61,40 ± 0,13
Neutral fraction replaced	61,11 ± 0,12
Cationic + neutral fraction replaced	61,30 ± 0,33
Anionic + neutral fraction replaced	51,25 ± 0,83

[a] The fractions were added back to the extracted grass prior to digestion *in vitro*.

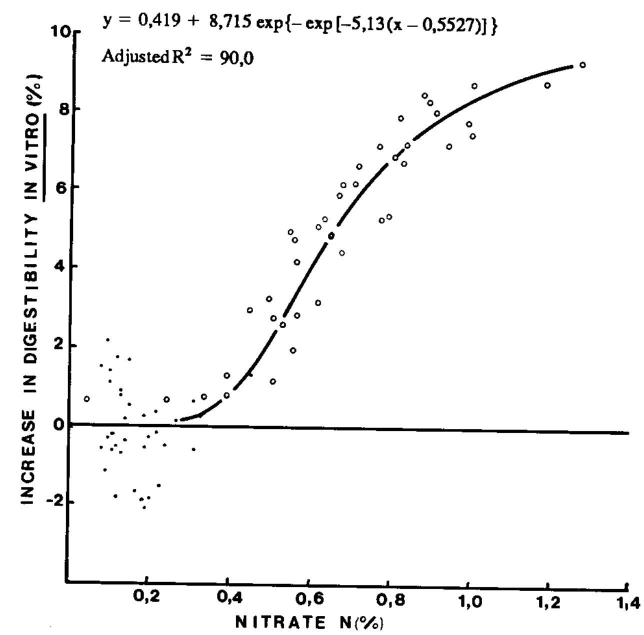

Figure 2 Relationship between the increase in dry matter digestibility *in vitro* after aqueous extraction of the sample and the nitrate content of kikuyu leaves (•) and stems (o).

In contrast to nitrate, high protein levels seem to improve the digestibility of kikuyu grass. The protein contents of both leaf and stem tissue were positively correlated with the digestibility *in vitro* of the tissue after extraction of soluble substances (leaf, $r = 0{,}5338$, $P < 0{,}001$; stem, $r = 0{,}5927$, $P < 0{,}001$; Table 3). However, without extraction the correlations were poorer, especially in the stem tissue containing high levels of nitrate (leaf, $r = 0{,}4936$, $P < 0{,}01$; stem, $r = 0{,}2596$, $P > 0{,}05$) suggesting that the positive effect of protein on digestion is masked by the negative effect of the soluble inhibitor of digestion (possibly nitrate).

The NPON fraction seems to have little effect on digestibility. The NPON content of both leaf and stem was poorly correlated with digestibility *in vitro* of pre- and unextracted grass (leaf, extracted: $r = -0{,}1237$, $P > 0{,}05$; unextracted: $r = -0{,}0665$, $P > 0{,}05$; stem, extracted: $r = 0{,}2927$, $P > 0{,}05$; unextracted: $r = -0{,}0329$, $P > 0{,}05$).

Both leaf and stem non-structural carbohydrates were poorly correlated with digestibility *in vitro* of the respective tissue (leaf, $r = 0{,}1730$, $P > 0{,}05$; stem, $r = 0{,}1929$, $P > 0{,}05$). However, the generally low non-structural carbohydrate content of kikuyu could seriously influence the efficiency of nitrogen incorporation into microbial protein in the rumen and limit the apparent retention of nitrogen and gross energy by the animal (Marais, Figenschou & Woodley, 1990). Due to the negative correlation between plant organic nitrogen and TNC content, elevated nitrogen levels in kikuyu could further lower the efficiency of nitrogen and energy metabolism in the rumen.

The higher stem lignin probably contributed substantially to the observed lower digestibility of stem material compared to leaf tissue. When the effect of nitrate on stem digestibility was removed by pre-extraction with water, stem digestibility *in vitro* was negatively correlated with lignin in the stem ($r = -0{,}5060$, $P < 0{,}001$; see Table 3). Leaf lignin was similarly associated with leaf digestibility *in vitro* ($r = 0{,}3783$, $P < 0{,}05$).

Nitrogen, which increases kikuyu yield, also had a major effect on the chemical composition of both leaf and stem tissue. This, in turn, influenced digestibility either positively or negatively. A high-nitrogen content was associated with elevated plant protein and slightly-reduced lignin contents, which both led to improved digestibility *in vitro*. Simultaneously, both nitrate and potassium concentrations were increased while the non-structural carbohydrate content was decreased. The potassium and non-structural carbohydrates did not seem to have any effect on digestibility *in vitro* but could affect animal nutrition by influencing magnesium levels in the blood and nitrogen retention by the animal, respectively. In contrast, a high nitrate content was associated with a marked reduction in digestibility *in vitro*. Nitrate concentrations increased rapidly with an increase in nitrogen status of kikuyu grass, but only concentrations above 0,3% seemed to be associated with a reduction in digestibility. It can be deduced from Figures 1 and 2 that deleterious concentrations are reached when the organic nitrogen content of the grass is higher than approximately 3,3%. However, caution must be exercised when interpreting *in vitro* digestibility results, since the quantitative effect of plant factors affecting digestion could be different *in vitro*, especially in tropical grasses (Olubajo, Van Soest & Oyenuga, 1974).

Due to its higher protein but lower nitrate, potassium and lignin contents, kikuyu leaf material appeared to have a higher nutritional value than stem tissue. As kikuyu matures the yield increases, but the leaf : stem ratio decreases, thereby probably reducing the nutritional value of the sward. Since both maturity and high nitrogen content seem to be negatively associated with nutritional value but positively associated with yield, the conditions under which the nutritional value is optimized without unduly sacrificing yield should urgently be further investigated.

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