VARIATION IN OXALATE, MAJOR CATIONS, AND DRY MATTER DIGESTIBILITY OF 47 INTRODUCTIONS OF THE TROPICAL GRASS SETARIA

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ABSTRACT

Oxalate was found in all 47 introductions of setaria examined. There were large differences between introductions in oxalate concentration; introductions from Kenya were low in oxalate concentration whereas those from southern Africa were high in oxalate.

Under the conditions of low potassium fertilization of the experiment sodium concentration was low in introductions from Kenya, but high (up to 1.80%) in introductions from southern Africa. There was little variation in potassium concentration. A correlation coefficient of $r = 0.93$ between oxalate content and the concentration of potassium and sodium indicated that there is little variation in oxalate concentration which is not dependent on the uptake of these two ions.

There was a significant positive correlation ($r = 0.50$) between oxalate concentration and estimated dry matter digestibility (DMD) of the 47 introductions. However, when DMD was adjusted for percentage of oxalate, low in energy value, there was still a varietal range of nine units (50.9-60.3) in adjusted DMD.

Dry matter yield was positively correlated with oxalate concentration. It was concluded that selection for low oxalate concentration would be likely to result in associated decreases in dry matter yield and possibly in DMD.

INTRODUCTION

High levels of soluble oxalate have been reported in pasture grasses in the *Setaria sphacelata* complex, and are responsible for occasional deaths of cattle grazing this grass (Jones *et al.* 1970). Oxalate concentration increases with increasing levels of nitrogen and potassium fertilisation (Jones and Ford 1972, Smith 1972) and in cv. Kazungula is linearly related to cation concentration in the plant (Jones and Ford 1972). The same authors have postulated that the high excess cation concentration in setaria is probably the reason for the accumulation of high levels of oxalate.

Screening a collection of setarias to detect accessions low in oxalate is hence complicated by variation due to uptake of cations. Low concentrations of oxalate could be due to low uptake of cations; alternatively low oxalate concentration could be associated with the production of alternative organic anions other than oxalic acid to maintain ionic balance, as suggested by Smith (1972). An accession with low oxalate concentration associated with a high excess cation concentration might be expected to maintain low levels of oxalate even under conditions of high potassium and nitrogen application. Hence in screening setarias for low oxalate concentration it is necessary to consider the overall oxalate-cation balance to determine the basis of any variation which might exist.

High levels of oxalate are also undesirable because oxalate has a very low energy value (Roughan and Slack 1973). As 90% of the oxalate in setaria is soluble (Jones and Ford 1972) it would appear in the digestible fraction in both *in vivo* and *in vitro* measurements of DMD (dry matter digestibility). Consistent varietal differences in DMD have been reported in setaria (Hacker and Minson 1972) but if the introductions with high DMD owe their superiority to high oxalate concentration, they may have little advantage in energy value.

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TABLE 1

Introductions of setaria, their chromosome numbers and area of origin

Group	C.P.I.†	Chromosome number (2n)	Area of origin*
A	15899, 32711	36, 45	Tanzania
B	15898	36	Tanzania
	16802, 19907, 19915		South Africa
	32436		Uruguay
C	28709, 32232, 32233	18	Kenya
	32846, 33139		
D	16729, 32657, 32658	18	Rhodesia
	32659, 32660		
E	28655, 32230, 32728, 32847	36, 45, 54	Kenya
	32848, 32850, 32880, 32883		
	32930, 33145, 33452, 33453		
F	16728	45	South Africa
G	16413, 17150	72, 90	Nigeria
	28777, 29307		South Africa
H	15847	18, 36, 45, 54	South Africa
	32759		New Guinea
	32714		N. Tanzania
	32231, 32856, 32925, 32929		Kenya
	32934, 32935, 32936, 32938		
	33130, 33144		

*Introductions from outside Africa were not indigenous. Kenya and Rhodesian introductions are all from known collection sites. Kazungula strains derive from the Zambesi-Chobe River area of Zambia, South African high polyploids from Transvaal (Gildenhuys 1951).
†C.P.I.—Commonwealth Plant Introduction Number.

The present paper describes a study of variation in oxalate concentration, concentration of major cations, dry matter digestibility and dry matter yield in setaria. Relationships between oxalate concentration and major cations are determined in order to indicate accessions which might balance excess cations with an alternative anion. Through a study of variation in oxalate and cation concentration, DMD and dry matter yield it was hoped that suitable accessions could be detected for incorporation in a breeding programme.

MATERIALS AND METHODS

Forty-seven introductions of setaria were grown in six replicates on a meadow podzolic soil at Samford, south-east Queensland. Each introduction was represented by four genotypes planted on a 40 cm square; all genotypes were clonally multiplied so that the replicates were genetically identical, and the plots within replicates were contiguous. The first experimental harvest was taken in July 1966 after six weeks regrowth and then four further four week regrowth harvests were taken; the final harvest was retained for chemical and DMD analysis. Dry matter yield data for fourteen introductions in this experiment have previously been published (Hacker 1972).

The 47 introductions were classified into eight groups on the basis of phenotype, origin and chromosome number. Group A included both accessions of the robust *S. splendida*. Group B included accessions of *S. anceps* cv. Kazungula and other southern African tetraploids which were morphologically similar. Group F was a single accession of the Bua River variety, morphologically quite similar to Group B. The East African accessions, characteristically less robust than Groups A, B and F, were classified into three groups. Group C included *S. anceps* cv. Nandi,

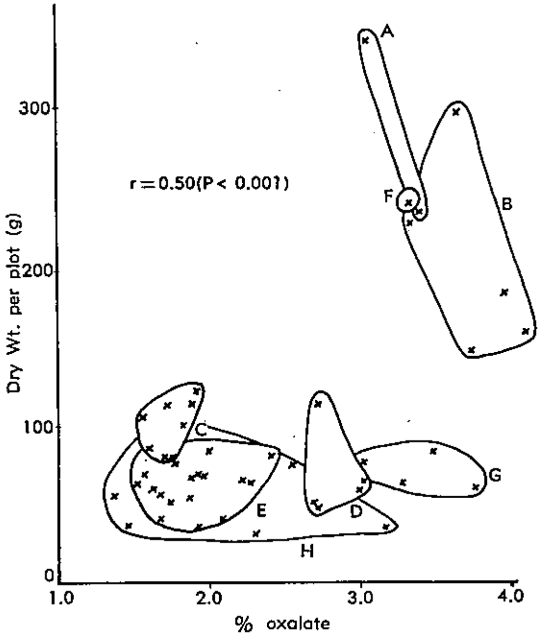

FIGURE 1
The relationship between oxalate (expressed as % anhydrous oxalic acid in the dry matter) and dry weight in 47 accessions of setaria.

accessions derived from Nandi through intravarietal selection, and other similar diploids from the Nandi district. Group E consisted of all accessions exhibiting frost tolerance—their origin was geographically confined to the Aberdares Mts., and although representing at least two species (*S. anceps*, *S. trinervia* and possibly *S. sphacelata sensu stricto*) they tended to be similar in their growth habit and leaf erectness. The remainder of the East African accessions (*S. anceps* and *S. trinervia*) were included in Group H, these being accessions which could not be readily classified. Group D was a distinctive group of diploids (*S. anceps*) from Rhodesia with broad basal leaves and strongly flattened shoot bases. Group G included the accessions with well developed rhizomes. These were two accessions of the du Toits kraal cultivar from South Africa (*Setaria* sp.) and two Nigerian accessions of *S. anceps*.

The six replicates were dried, weighed and then bulked and sub-sampled for analysis of total oxalate, following the method of Moir (1953), K, Na, Ca and Mg, and dry matter digestibility which was estimated by the *in vitro* technique of Minson and McLeod (1972). The *in vitro* results were adjusted to *in vivo* values with setaria samples of known *in vivo* dry matter digestibility. For fourteen introductions representative of the apparent range in morphological variation the six replicates were bulked into two groups each of three replicates for analysis of variance.

RESULTS

Oxalate

Highest concentrations were recorded in *S. splendida*, Kazungula, Bua River and octaploid and decaploid groups (Groups A, B, F and G). All introductions from Kenya (Groups C, E and H) were comparatively low in oxalate (<2.5 per

TABLE 2

Cations and oxalate concentrations (% DM) and estimated DMD in 47 introductions of setaria

Group	Number of Intro-ductions	K Mean	K range	Na Mean	Na range	Ca Mean	Ca range	Mg Mean	Mg range	Oxalate* Mean	Oxalate* range	DMD Mean	DMD range	Adjusted DMD Mean	Adjusted DMD range
A	2	1.55	(1.50-1.60)	1.43	(1.37-1.48)	0.18	—	0.25	(0.24-0.30)	2.21	(3.03-3.40)	63.3	(62.0-64.6)	57.9	(56.3-59.5)
B	5	1.48	(1.30-1.65)	1.59	(1.42-1.80)	0.22	(0.17-0.26)	0.21	(0.19-0.24)	3.73	(3.32-4.07)	60.0	(58.8-61.8)	53.8	(52.1-55.2)
C	5	1.79	(1.67-1.97)	0.06	(0.05-0.10)	0.24	(0.22-0.26)	0.30	(0.27-0.32)	1.72	(1.56-1.91)	56.5	(54.8-57.6)	53.3	(51.3-54.1)
D	5	1.64	(1.37-1.80)	0.82	(0.41-1.02)	0.25	(0.24-0.25)	0.24	(0.21-0.28)	2.81	(2.67-2.99)	57.8	(55.5-58.9)	53.0	(50.9-54.1)
E	12	1.78	(1.60-2.20)	0.07	(0.05-0.20)	0.20	(0.15-0.24)	0.26	(0.22-0.32)	1.80	(1.51-2.41)	59.7	(57.0-64.1)	56.3	(53.5-60.3)
F	1	1.32	—	1.42	—	0.18	—	0.19	—	3.32	—	60.1	—	54.6	—
G_1	2	3.03	(2.95-3.11)	0.14	(0.06-0.22)	0.23	(0.19-0.26)	0.30	(0.27-0.32)	3.50	(3.26-3.74)	58.7	(58.0-59.3)	52.2	(52.0-53.3)
G_2	2	1.51	(1.50-1.51)	1.11	(1.02-1.19)	0.19	(0.19-0.20)	0.19	(0.18-0.20)	3.24	(3.02-3.46)	59.5	(58.3-60.7)	54.1	(53.2-54.9)
H	13	1.75	(1.52-2.27)	0.06	(0.05-0.08)	0.24	(0.17-0.36)	0.29	(0.26-0.37)	2.03	(1.37-3.14)	58.4	(55.8-63.2)	54.6	(52.6-59.0)
Total	47	1.76	(1.30-3.11)	0.43	(0.05-1.80)	0.22	(0.15-0.36)	0.26	(0.18-0.37)	2.35	(1.37-4.07)	58.9	(54.8-64.6)	54.7	(50.9-60.3)
LSD ($P = 0.05$) between introductions†		0.57				0.05		0.05		0.53		2.3		2.0	

*Expressed as anhydrous oxalic acid.
†based on fourteen introductions only.

cent) with the exception of two introductions from the Mt. Marsabit area (CPI 32934, 32936). Group D, from Rhodesia, was intermediate in oxalate concentration.

There was a highly significant positive correlation between oxalate concentration and dry matter yield for this harvest (Figure 1). The most vigorous varieties were in Groups A, B and F. Group G, and to a lesser extent D combined a low yield with a high oxalate concentration but in no accession was a high yield associated with a low oxalate concentration. The high dry matter yield of Groups A, B and F was consistent throughout the two-year period of this experiment.

Cations

Frequency distribution for sodium concentration was markedly bi-modal, and an analysis of variance was not attempted. Groups C, E and H were very low in sodium (<0.20 per cent) whereas Groups A, B and F had sodium levels above 1.37 per cent. Group D was again intermediate. Group G included the low sodium introductions (from West Africa) and high sodium introductions (from South Africa) and has therefore been split into G_1 and G_2 respectively in Table 2.

High values for potassium concentration were obtained in Group G_1, but otherwise there was little difference between groups. There was some variation between groups for concentration of magnesium, with low concentrations in Groups B, F and G_2, but little variation between groups for calcium concentration.

Digestibility

DMD of the 47 introductions ranged from 54.8 to 64.6%. Ranking order for the nine Groups was A, F, B, E, G_2, G_1, H, D, C.

Correlations between cations and oxalate concentration

Sodium and potassium concentrations were negatively correlated ($r = -0.42$, $P < 0.01$). There was a highly significant correlation between oxalate and sodium concentration ($r = 0.82$, $P < 0.001$), but no significant correlation between oxalate and potassium concentration ($r = 0.07$). When the 47 introductions were divided into two groups, sodium accumulators and sodium non-accumulators, there was a positive correlation ($r = 0.86$, $P < 0.001$) between oxalate and potassium in those introductions which did not accumulate sodium (Table 3).

TABLE 3

Correlation coefficients for Na, K and oxalate in setarias which accumulate sodium and those that do not accumulate sodium

	Number of introductions	Na and oxalate	K and oxalate
Sodium accumulators	15	0.86***	—0.14 N.S.
Sodium non-accumulators	32	0.34 N.S.	0.86**
Total	47	0.82***	0.07 N.S.

**P < 0.01
***P < 0.001

The relationship between sodium, potassium and oxalate was clarified when the two cations (summed as milli-equivalents %) were plotted against oxalate (Figure 2). The correlation was $r = 0.93$ ($P < 0.001$), and there was little improvement in the correlation when total cations (Na, K, Mg and Ca) were correlated with % oxalate ($r = 0.94$, $P < 0.001$). Fitted linear regressions were:

$y = -19.5 + 0.77x_1$ $x_1 = Na + K + Mg + Ca$ expressed as m-equivs %
$y = 9.33 + 0.71x_2$ $x_2 = Na + K$ expressed as m-equivs %

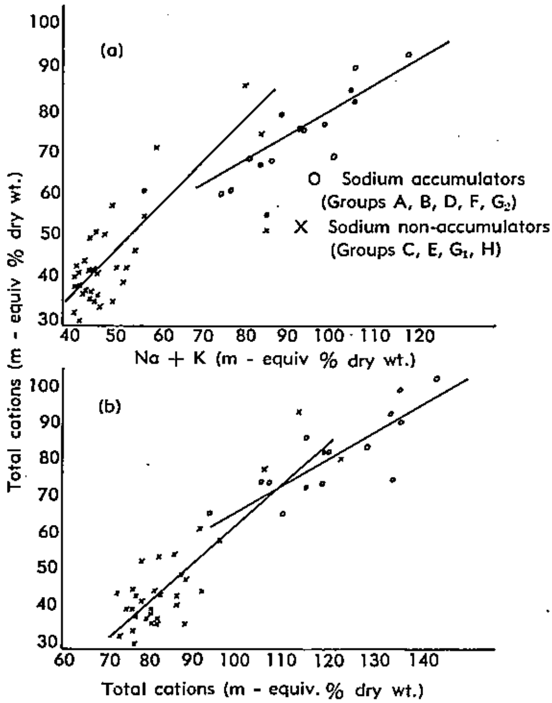

FIGURE 2
The relationship between oxalate (expressed as m-equiv. % dry wt. of anhydrous oxalic acid) and (a) potassium and sodium, and (b) total cations (K, Na, Mg, Ca) in setarias which accumulate sodium and those which do not.

where y is the concentration of oxalate, expressed as anhydrous oxalic acid. The best relationship was obtained by fitting separate linear regressions for those introductions which accumulated sodium and those which did not. Both for total cations and for Na and K differences between slopes were significant. Fitted regressions are now

Sodium accumulators
$y = 3.6 + 0.58x_1$
$y = 20.6 + 0.58x_2$
Sodium non-accumulators
$y = -30.6 + 0.89x_1$
$y = -6.1 + 1.02x_2$

The relationship between oxalate concentration and DMD

It is likely that in some introductions the oxalate ion is balanced by potassium and in others largely by sodium. Potassium oxalate and sodium oxalate percentages in the plant were calculated on the assumption that the ratio is in proportion to the ratio (in milli-equivalents %) of potassium to sodium in the plant. This will certainly be an over-simplification, but errors in terms of % dry weight are likely to be small. Total oxalate (as K_2 and Na_2 salts) was positively correlated with DMD (r = 0.36,

$P < 0.05$), but when DMD was adjusted for oxalate (by subtracting the calculated percentage of potassium oxalate and sodium oxalate) there was a significant correlation between DMD and adjusted DMD ($r = 0.58$, $P < 0.001$). It is apparent that accessions with the highest DMD tend to have high concentrations of oxalate but that this is not the only factor responsible for their high DMD values.

DISCUSSION

The results show that there is significant variation in oxalate accumulation in setaria, and that this is largely associated with area of origin. Introductions from southern and western Africa tend to accumulate more oxalate than those from Kenya when grown under similar conditions and under the fertilizer regime imposed. However, it is known that both nitrogen and potassium application have a pronounced effect on oxalate (Jones and Ford 1972; Smith 1972), and hence under different fertilizer management ranking for oxalate level could well differ. Nevertheless in a study by Jones *et al.* (1970) ranking for nine setarias was similar to that found in the present study, although growing conditions were such that almost double the concentration of oxalate was accumulated (2.78-4.16 per cent).

Breeding for low oxalate concentration *per se* should therefore be based on accessions from Kenya. However all the Kenya accessions were low yielding in comparison to those in Groups A, B and F, and it would appear that high oxalate concentration is intimately associated with high yield. The only group which combined low oxalate with moderately high yield was Group C, which included the cultivar Nandi.

The ability to accumulate sodium also appears to be genetically controlled. Setarias from Kenya were consistently very low in sodium concentration. Low sodium concentrations have been previously reported in setarias from Kenya (Hacker and Jones 1969) and Uganda (Long *et al.* 1969) whereas setarias from southern Africa (Hacker and Jones 1969) and Zaire (Laudelot *et al.* 1954; Germaine and Scaut 1960; Risopoulos 1966) accumulate sodium. However Germaine and Scaut report that *S. splendida* does not accumulate sodium, and *S. splendida* CPI 15899 also grown in the present study, does not accumulate sodium under nutrient conditions which favour potassium accumulation (Hacker and Jones 1969).

The Kenya introductions in this study did not include any of the Kazungula or *S. splendida* phenotype, so it was not clear whether the low sodium uptake of introductions from Kenya was associated with phenotypic grouping or area of origin. Only a single introduction of the *S. splendida* phenotype (CPI 33084) was available from Kenya in nursery collections (Collection site Meru swamp (0°, 38°E); Boonman, personal communication) and this proved to be a sodium accumulator. Vegetative samples taken from a nursery row had a sodium concentration of 1.11%. This would suggest that phenotypic grouping is dominant to geographic origin with regard to sodium accumulation.

Although it is apparent that there is genetic variation in oxalate content in setaria, the good correlation of $r = 0.93$ between oxalate and Na and K indicates that the variation is intimately associated with cation uptake. The present study has shown that it is unlikely that any of the setarias produce an alternative anion to maintain ionic balance, and there would appear to be little justification in screening setarias for the production of an alternative anion (Smith 1972). Under conditions which lead to accumulation of excess cations, particularly Na and K, any setaria is likely to accumulate high concentrations of oxalate. The similarity in correlation coefficients and slope of regressions between oxalate and K + Na, and oxalate and total cations is consistent with the possibility that there is little or no variation in setaria in the proportion of oxalate present as magnesium or calcium oxalate.

Jones and Ford (1972) showed the effect of applied nitrogen and potassium in increasing sodium and potassium uptake in Kazungula and similarly increasing oxalate concentration. The regressions for oxalate and (Na and K) and oxalate and (Na, K, Mg, and Ca) quoted by Jones and Ford are very similar to those reported here as shown in Figure 3. Thus relationships derived in the field from different setarias grown under a similar level of nutrition compare closely with those derived from a single variety grown under different nutrient levels.

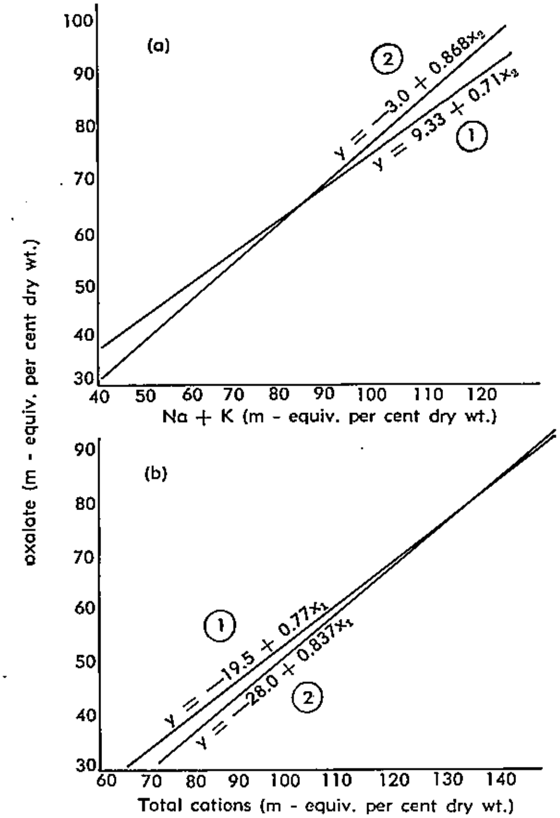

FIGURE 3

The relationship between oxalate (expressed as m-equiv. % dry wt. of anhydrous oxalic acid) and (a) potassium and sodium, and (b) total cations (K, Na, Mg, Ca). The regressions are from the present study (1) and that of Jones and Ford, 1972b (2).

The difference in slope between sodium accumulating and non-accumulating setarias for the regression of oxalate on total cations suggests that at high levels of total cations sodium accumulators would be lower in oxalate concentration. However this inference should be treated with caution as there was little overlap between the sodium accumulators and non-accumulators in oxalate concentration, and the plants were grown at exceptionally low levels of potassium.

Most field studies on setaria have indicated that setaria may accumulate much higher levels of K than reported here (for example van Wyck *et al.* 1951, Hacker and Jones 1969). However it would appear that varieties which are able to take up large amounts of potassium, for example Kazungula, Bua River, *S. splendida*, are those which accumulate Na when K is in short supply. The lack of an overall correlation between potassium and oxalate concentration in the present study is attributable to the narrow range of potassium concentrations;

with higher levels of applied potassium a greater divergence of potassium concentration would be expected and an improvement in the potassium-oxalate correlation.

The positive correlation between estimated concentration of sodium and potassium oxalate and DMD suggests that setaria introductions with high DMD values owe some of their superiority to high oxalate content. The method used for estimating proportion of sodium and potassium oxalate is likely to be an over-simplification, but errors are probably small. Selection for high DMD would be expected to result in an increase in oxalate, but also to an improvement in overall feeding value as oxalate contributed relatively little to the digestibility of the better introductions. Of particular note in this context is Group E which combined high DMD with a relatively low oxalate concentration.

In conclusion it would appear that there is a complex inter-relationship between cation concentration, oxalate concentration, dry matter yield and digestibility. At least under conditions of low potassium, highest yielding varieties are high in oxalate and high in DDM, and this is associated with excess sodium uptake. It would appear unlikely from the present study that breeding for low oxalate concentration may be successfully undertaken in setaria without sacrifices in yield and possibly in digestibility.

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REFERENCES

GERMAINE, R., and SCAUT, A. (1960)—Herbage and nutritional aspects of animal husbandry in the Congo. Proceedings of the Eighth International Grassland Congress. p. 371.

GILDENHUYS, P. J. (1951)—Grass Breeding. Pasture Research in South Africa; Progress Report No. 111; Government Printer, Pretoria.

HACKER, J. B. (1972)—Seasonal yield distribution in *Setaria*. *Australian Journal of Experimental Agriculture and Animal Husbandry*. **12**: 36.

HACKER, J. B., and JONES, R. J. (1969)—The *Setaria sphacelata* complex—A review. *Tropical Grasslands*. **3**: 13.

HACKER, J. B., and MINSON, D. J. (1972)—Varietal differences in *in vitro* dry matter digestibility in *Setaria*, and the effects of site, age and season. *Australian Journal of Agricultural Research*. **23**: 959.

JONES, R. J., and FORD, C. W. (1972)—Some factors affecting the oxalate content of the tropical grass *Setaria sphacelata*. *Australian Journal of Experimental Agriculture and Animal Husbandry*. **12**: 400.

JONES, R. J., SEAWRIGHT, A. A., and LITTLE, D. A. (1970)—Oxalate poisoning in animals grazing the tropical grass *Setaria sphacelata*. *Journal of the Australian Institute of Agricultural Science*. **36**: 41.

LAUDELOT, H., GERMAINE, R., and KESLER, W. (1954)—Premiers résultats sur la dynamique chimique des jachères herbacées et pâturages à Yangambi. *Ve Congres International de la Science des Sols. Leopoldville*. p. 312.

LONG, M. I. E., NDYANABO, W. K., MARSHALL, B., and THORNTON, D. D. (1969)—Nutritive value of grasses in Ankole and Queen Elizabeth National Park, Uganda. 4. Mineral Content. *Tropical Agriculture, Trinidad*. **46**: 201.

MINSON, D. J., and MCLEOD, M. N. (1972)—The *in vitro* technique: Its modification for estimating digestibility of large numbers of tropical pasture samples. Division of Tropical Pastures Technical Paper No. 8, C.S.I.R.O., Australia.

MOIR, K. W. (1953)—The determination of oxalic acid in plants. *Queensland Journal of Agricultural Science*. **10**: 1.

RISOPOULOS, S. A. (1966)—Management and use of grasslands. F.A.O. Pasture and Fodder Crop Studies No. 1.

ROUGHAN, P. G., and SLACK, C. R. (1973)—Simple methods for screening and quantitative estimation of oxalate content of tropical grasses. *Journal of the Science of Food and Agriculture*. **24**: 803-811.

SMITH, F. W. (1972)—Potassium nutrition, ionic relations and oxalic acid accumulation in three cultivars of *Setaria sphacelata*. *Australian Journal of Agricultural Research*. **23**: 969.

VAN WYCK, H. P. D., OOSTHUIZEN, S. A., and BASSON, J. D. (1951)—The nutritive value of South African feeds. II. Hay and Pasture Crops. Science Bulletin No. 298, Dept. of Agriculture, Union of South Africa.

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