Fertilizer sulphur as a factor affecting voluntary intake, digestibility and retention time of pangola grass (*Digitaria decumbens*) by sheep

BY M. C. REES AND D. J. MINSON

Division of Tropical Crops and Pastures, CSIRO Cunningham Laboratory, St Lucia, Queensland 4067, Australia

(*Received* 19 *December* 1975 – *Accepted* 13 *April* 1977)

1. Pangola grass (*Digitaria decumbens*) was grown with or without sulphur fertilizer, cut at seven stages of growth, dried and given to sheep with or without a supplement of 0·6 g S as sodium sulphate/d. Voluntary intake and digestibility of dry matter (DM) and retention time in the reticulo-rumen, of various components of the grasses were determined.

2. Grass grown on the S-fertilized plots had a mean S content of 1·57 g/kg DM compared with 1·03 g/kg DM for the control but there was no difference in the level of other elements. S fertilizer increased the level of acid-detergent fibre and lignin.

3. In the absence of a S supplement, fertilizer S increased voluntary intake by 10·5 %, DM digestibility by 0·008 and reduced the period food DM was retained in the reticulo-rumen by 18·5 %. Fertilizer S had no effect on rumen pH or volatile fatty acid concentration in rumen fluid, but decreased the molar proportions of propionic and butyric acids and increased those of acetic, isobutyric, isovaleric and caproic acids.

4. Supplementary S had little effect on the voluntary intake and DM digestibility of the S-fertilized grass but increased the voluntary intake and DM digestibility of the control grass by 22 % and 0·036 respectively ($P < 0·01$). Thus the voluntary intake and DM digestibility of S-fertilized grass was lower than that of the control grass when the determinations were made with sheep supplemented with S.

5. It was concluded that S fertilizer increased nutritive value by overcoming a simple S deficiency in the diet when diets contained less than 1·7 to 1·8 g S/kg DM but this benefit was offset by a decrease in the content of digestible neutral-detergent solubles and an increase in the lignin content of the food.

Sulphur fertilizers have been shown to increase both pasture yield and the concentration of S in the forage (Andrew, 1975). Where forages are deficient in S, feeding S supplements increases voluntary intake and dry matter (DM) digestibility (Kennedy & Siebert, 1972; Rees, Minson & Smith, 1974). By increasing the level of S in the plant, S fertilizer will overcome any primary effect of a S deficiency in the same way as feeding a S supplement. S fertilizer will also alter plant growth rate and plant composition and this may affect the nutritive value of forages in addition to that due to a simple increase in S level. This secondary effect was first shown in a preliminary study by Rees *et al.* (1974) who found that the voluntary intake by sheep of immature pangola grass (*Digitaria decumbens*) grown with application of S fertilizer was increased by 44% and DM digestibility by 5%. A simple S deficiency accounted for 63% of the difference in voluntary intake and all the difference in digestibility.

This paper describes a study with sheep of the effect of S fertilizer on the nutritive value of pangola grass at different stages of growth.

EXPERIMENTAL

Diets

Pangola grass was grown as a pure sward with 0 or 66 kg fertilizer-S/ha in 1971–72 and 0 or 116 kg fertilizer-S/ha in 1972–73 on a recently-cleared lateritic podzolic soil at Beerwah in south eastern Queensland (Thompson, 1958). S was applied as 'flowers of S'. There were two field replicates of both the control and S-fertilized plots to reduce any difference between sites. Grasses from the two replicates were mixed together before feeding. The control and the S-fertilized plots were 0·2 and 0·1 ha respectively and with two replicates the experiment covered a total area of 0·6 ha. Nutrients (kg/ha) other than S were applied as recommended by Andrew & Bryan (1955): lime 550, potassium as potassium chloride 53, copper carbonate 8, zinc carbonate 8, sodium tetraborate 4; sodium molybdate 500 g/ha. Phosphorus at 222 kg/ha was applied in the first year and an additional 240 kg P/ha in the second year as commercial grade monocalcium phosphate to the control plots which received no S. For S-fertilized plots the less expensive diammonium hydrogen phosphate, fertilizer grade, containing 30 g S and 210 g nitrogen/kg, was used. S was applied as elemental S and fertilizer grade $(NH_4)_2HPO_4$. A total of 246 kg N/ha was applied to each treatment during the establishment year and 100 kg N/ha applied after each harvest as ammonium nitrate or $(NH_4)_2HPO_4$.

The grass was cut and removed on 2 November 1971 and the regrowth on the two replicates cut 6 weeks later using a tractor-mounted reciprocating mower, mixed together and dried in a batch drier with an inlet temperature of 100°. The dried grass was chopped into approximately 20–40 mm lengths, and samples were analysed as previously described (Rees & Minson, 1976). The plots were subdivided, and the further regrowth was harvested and dried after 6 or 10 weeks.

In the second year, the grass was removed in August and all the regrowth harvested after 6 weeks. The plots were subdivided into three and the regrowth harvested after 6, 10 or 15 weeks.

From this cutting programme there were fourteen different grasses consisting of seven stages of regrowth each at two levels of S fertilizer. The statistical significance of any effect of fertilizer S on yield and composition was determined by an analysis of variance with six degrees of freedom for error. This analysis makes the assumption that the regrowths were independent which is not strictly correct.

Determination of voluntary intake and digestibility

Six-toothed Merino wether sheep weighing between 32 and 40 kg were used to measure voluntary intake and digestibility in a 17 d experimental period which included a 7 d preliminary period followed by a 10 d measurement period. The sheep were drenched on the third day of the preliminary period with thiobendazole (Merck Sharp & Dohme (Aust.) Pty Ltd, Sydney) to reduce any effects of internal parasites. The feeding study consisted of five periods each of 17 d using the same twenty-eight sheep. In every period each grass was fed to two sheep so that at the end of the five periods each grass had been fed to ten sheep. The grasses were allocated to the sheep at random except that no regrowth was fed to the same sheep more than once. In each period one sheep on each of the fourteen grasses was drenched daily with 54 ml water containing 0·6 g S as sodium sulphate. The sheep were kept in individual metabolism pens (Minson & Milford, 1968) and faeces collected in canvas bags attached to the animal by a harness (Weston, 1959). The sheep were weighed at the beginning and end of the 10 d measurement period. Metabolic size ($W^{0.75}$, kg) was calculated from the mean body-weight. Voluntary intake and digestibility of the fourteen grasses were determined as previously described (Rees & Minson, 1976). Sulphate-S was determined in blood plasma samples by the method of Bird & Fountain (1970).

The statistical significance of the effect of fertilizer, supplementary S, regrowth stage and their interactions on voluntary intake and digestibility were determined by analysis of variance. Regressions were also calculated relating the increase in voluntary intake and DM digestibility caused by supplementary S to the content of S in the fourteen diets.

Table 1. *Mean yield of dry matter (DM) and composition (g/kg DM) of pangola grass grown with and without sulphur fertilizer and harvested at seven stages of growth*†

(Mean values with their standard error for seven samples)

Treatment of grass ...	Control	S-fertilized	SEM
DM yield (kg/ha)	4040	5280	212 **
Leaf lamina (% total)	15·0	21·6	1·0 **
S	1·03	1·57	0·04 ***
Nitrogen	17·7	16·3	0·5 NS
Phosphorus	2·3	2·1	0·1 NS
Calcium	4·6	4·9	0·1 NS
Magnesium	1·8	1·6	0·1 NS
Potassium	7·1	6·6	0·2 NS
Sodium	3·5	3·4	0·1 NS
Copper (μg/kg DM)	6	7	0·3 NS
Zinc (μg/kg DM)	41	44	3 NS
Total ash	55	53	1 NS
NDF	656	678	7 NS
ADF	361	385	4 **
NDS	289	270	6 NS
Hemicellulose	294	292	5 NS
Cellulose	303	311	4 NS
Lignin	59	74	3 **

NDF, neutral-detergent fibre (Van Soest & Wine, 1967); ADF, acid-detergent fibre (Van Soest, 1963); NDS, neutral-detergent-soluble organic matter (Van Soest & Wine, 1967); NS, not significant ($P > 0·05$). Difference between values was significant: ** $P < 0·01$, *** $P < 0·001$.

† For details, see p. 6.

Food retention in the reticulo-rumen

The retention time of each of the fourteen grasses in the reticulo-rumen was determined with a separate group of fistulated sheep using the technique described by Minson (1966). Each grass was fed to the same two wethers, each fitted with an 80 mm rumen fistula. The apparent retention times of different fractions of the diet were calculated by dividing the weight of each fraction in the reticulo-rumen by the hourly consumption of each fraction. The rumen contents were analysed for pH, total volatile fatty acids (VFA) concentration (Annison, 1954) and molar proportions of the individual VFA (Stobbs & Brett, 1972). The significance of the effects of fertilizer S throughout the seven stages of regrowth was determined by an analysis of variance.

RESULTS

Composition of diet

The mean yields of DM from the control and S-fertilized plots were 4000 and 5300 kg/ha per harvest respectively, while the concentrations of S in the grass were 1·03–1·57 g/kg DM respectively. There was little difference in the concentrations of N, P, calcium, magnesium, K, sodium, copper, zinc, total ash, hemicellulose and cellulose (see Table 1). The amounts of leaf lamina (% total), acid-detergent fibre (ADF; Van Soest, 1963) and lignin appeared to be increased by fertilizer S (see Table 1).

Voluntary intake

When S supplements were omitted the mean intake of the S-fertilized grass was 10·5% higher than that of the control (Table 2). Feeding an S supplement increased voluntary intake of the control by 21·8% ($P < 0·01$) and the S-fertilized grass by 3·1% ($P > 0·05$)

Table 2. *Voluntary intake of dry matter* (DM) (*g/kg metabolic size* (*body-weight*$^{0.75}$) *per d*) *by S-supplemented* (0·6 *g S as sodium sulphate/d*) *and unsupplemented sheep, and apparent digestibility of pangola grass* (*Digitaria decumbens*) *grown with and without S fertilizer*

(Mean values for samples cut at seven stages of growth each fed to five animals)

Treatment of grass†	S supplement (g/d)	Voluntary intake	Digestibility: DM	Organic matter	NDS	Hemi-cellulose	Cellulose	Lignin
Control	0	44·0	0·533	0·548	0·612	0·578	0·541	−0·023
	0·6	53·6	0·569	0·581	0·622	0·629	0·607	−0·045
S fertilized	0	48·6	0·541	0·551	0·568	0·616	0·588	0·039
	0·6	50·1	0·541	0·557	0·562	0·628	0·605	0·048
SE ‡		0·9	0·005	0·005	0·005	0·010	0·010	0·022
Statistical significance of effect of:								
Fertilizer (F)		NS	NS	NS	***	NS	*	**
Supplement (S)		***	***	**	NS	**	**	NS
Regrowth (R)		***	***	***	**	***	***	**
Interaction F×S		***	**	*	NS	NS	**	NS
F×R		*	*	NS	*	NS	NS	NS
R×S		NS	NS	NS	NS	NS	NS	NS

NDS, neutral-detergent solubles (Van Soest & Wine, 1967); NS, not significant ($P > 0·05$), * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details of treatments, see p. 6.

‡ Based on thirty-five values (five sheep × seven regrowths).

(Table 2). The increase in voluntary intake (I) due to the S supplement was correlated ($r - 0·70$, $P < 0·01$) with the S content of the grass (S):

$$I = 20·6 - 11·6S. \quad (1)$$

Digestibilities

The DM digestibility of the grass on the S-fertilized plots was only slightly higher than that of the control with a mean difference of 0·008 ($P > 0·05$, Table 2). When the grasses were supplemented with Na_2SO_4 the mean DM digestibility of the S-fertilized grass was unchanged but with the control grasses there was a mean increase of 0·036 ($P < 0·01$, Table 2). The increase in DM digestibility (D) due to the S supplement was correlated ($r - 0·72$, $P < 0·01$) with the S content of the grasses (S)

$$D = 0·090 - 0·055S. \quad (2)$$

Organic matter (OM) digestibilities followed the same trends as the DM digestibilities (Table 2).

The digestibility of the cell contents, measured as neutral-detergent-soluble organic matter (NDS; Van Soest & Wine, 1967) was reduced by the application of S fertilizer (Table 2). The digestibility of the NDS did not vary when supplemented with Na_2SO_4 (Table 2).

The digestibility of the hemicellulose of the S-fertilized grass was higher than that of the control with a mean difference of 0·038 ($P < 0·10$, Table 2) but when the grasses were supplemented with Na_2SO_4 there was no difference in hemicellulose digestibility. The digestibility of hemicellulose decreased from 0·68 in the 6-week regrowths to 0·48 in the 15-week regrowth ($P < 0·01$).

Table 3. *Mean retention time (h) of various dietary components in the reticulo-rumen and molar proportions of volatile fatty acids (VFA) in the rumen of sheep given pangola grass (Digitaria decumbens) grown with and without fertilizer S*

Treatment of grass ...	Control	S-fertilized	SEM	Mean of control and S-fertilized	Relative retention times
Dietary component					
Dry matter	29·7	24·2	1·1 *	27·0[a]	1·04
Organic matter	28·7	23·2	1·1 *	26·0[b]	1·00
NDS	16·7	15·7	0·7 NS	16·2[c]	0·62
Hemicellulose	32·1	26·2	1·5 *	29·2[df]	1·12
Cellulose	33·4	23·8	1·7 **	28·6[abd]	1·10
Lignin	50·7	41·1	2·1 *	45·9[e]	1·77
NDF	34·3	26·1	1·6 *	30·2[f]	1·16
VFA	Molar proportions of VFA (mmol/mol)				
Acetic acid	676	710	12·7 NS		
Propionic acid	203	179	6·5 *		
Isobutyric acid	3	5	0·4 *		
Butyric acid	110	92	7·1 NS		
Isovaleric acid	2	6	1·1 *		
Valeric acid	5	5	0·4 NS		
Caproic acid	1	2	0·8 NS		
Total VFA (mmol/l)	90·7	87·6	3·7 NS		
Rumen pH	6·5	6·5	0·1 NS		

Mean values with the same superscript letter were not significantly different.
NDS, neutral-detergent solubles (Van Soest & Wine, 1967); NDF, neutral-detergent fibre (Van Soest & Wine, 1967); NS, not significant ($P > 0·05$); difference between mean values statistically significant, * $P < 0·05$; ** $P < 0·01$.

The digestibility of the cellulose of the S-fertilized grass was higher than that of the control with a mean difference of 0·047 ($P < 0·05$, Table 2). Supplementation with Na_2SO_4 had little effect on the digestibility of cellulose in the S-fertilized grass but increased the digestibility of the control grass by 0·066 ($P < 0·01$). Cellulose digestibility decreased from 0·63 for 6-week regrowths to 0·44 in the 15-week regrowth.

The digestibility of lignin in the grasses was increased by fertilizer S but there was no significant effect of supplemental S (Table 2).

Retention time for food in the reticulo-rumen

S-fertilizer reduced the mean period during which DM and OM of pangola grass were retained in the reticulo-rumen of sheep by 5·5 h (Table 3). The retention times of the NDS, hemicellulose, cellulose, lignin and neutral-detergent fibre (NDF; Van Soest & Wine, 1967) were shorter for the S-fertilized grass (Table 3). The NDS fraction was retained for a shorter time than the food OM ($P < 0·01$) while hemicellulose and lignin were retained in the reticulo-rumen longer than the OM ($P < 0·05$, Table 3). For lignin this increase in retention time was 77% more than found for the food OM. VFA concentration and pH of the rumen contents were similar for S-fertilized and control grass but there were differences in the proportions of the different VFA; S fertilizer increased the proportions of acetic, isobutyric and isovaleric acids but decreased the proportions of propionic and butyric acids in rumen fluid (Table 3).

S-fertilizer increased the mean level of blood sulphate from 2·6 to 3·3 μmol/l for unsupplemented sheep. Feeding a Na_2SO_4 supplement increased the mean levels of blood sulphate to 3·9 and 4·0 μmol/l for sheep given control and S-fertilized grass.

DISCUSSION

S fertilizer changed the nutritive value of pangola grass in two ways; first by overcoming a simple S deficiency in the diet of the sheep and secondly by changing the type of plant grown and hence the composition and nutritional characteristics of the carbohydrate fractions. These two independent pathways will be considered separately.

The S-fertilized grass contained 1·57 g S/kg DM and this appeared adequate to meet the requirements of the sheep used, since supplementary S had no effect on digestibility, and only a small effect on voluntary intake of the grass. However, with the control grass the mean S level was 1·03 g/kg DM and the S supplement increased DM digestibility by 0·036 and voluntary intake by 22%. These increases are smaller than those previously reported by Rees *et al.* (1974), a difference that is probably caused by the higher mean level of S in the control grasses in our present work since the size of the increase in voluntary intake and digestibility is correlated with the S level of the grass (equations nos 1 and 2). The grasses studied by Rees *et al.* (1974) contained 0·9 g S/kg DM. Using equations nos 1 and 2, the estimated increase in voluntary intake and DM digestibility was 10·2 g/kg $W^{0.75}$ and 0·042 respectively compared with the observed values of 12·4 g/kg $W^{0.75}$ and 0·054 respectively.

From equations nos 1 and 2 it can be calculated that increasing the level of S in the grass will only increase voluntary intake and digestibility when the grasses contain less than 1·7–1·8 g S/kg DM. This value is higher than the value of 1·0 suggested by Downes, Langlands & Reis (1975), a difference that is possibly due to the young stage of growth of the pangola grass included in our study compared with those reviewed by Downes *et al.* (1975). The S requirements of 1·7–1·8 g/kg DM are in general agreement with those reported by Bray (1965), Rendig & Weir (1957) and Langlands, Sutherland & Playne (1973).

Feeding a S supplement increased the DM digestibility of the low-S grass by increasing the digestibility of the hemicellulose and cellulose of the cell wall. However, S supplementation had no effect on the digestibility of either the lignin or the NDS of the cell contents. Fertilizer S had very little effect on either DM or OM digestibility although the level of S in the unfertilized grass was inadequate as indicated by a response to supplemental S. This apparent failure of fertilizer S to improve digestibility was caused by the fertilizer altering the growth form of the plant, increasing the proportion of leaf and lignin but decreasing the level and digestibility of NDS in the plant. These factors caused a reduction in digestibility which was balanced by the increase associated with the S-induced improvement in digestibility of the hemicellulose and cellulose fractions (Table 2).

The voluntary intake of food is usually inversely related to the period the food is retained in the reticulo-rumen (Thornton & Minson, 1972) and in this study the retention time of the S-fertilized grass was 18·5% less than the control value. This difference in retention time of food in the reticulo-rumen was more than sufficient to account for the observed 10·5% higher voluntary intake of the S-fertilized grass without the need to evoke a difference in palatability. This shorter retention time was due to a reduction in the period the different dietary components (except NDS) were retained in the reticulo-rumen (Table 3). The longer retention time of the control grass was probably due to a lack of S for maximum activity of rumen micro-organisms.

Although fertilizer S had no effect on the pH of the rumen fluid there were significant changes in the molar proportions of the different VFA. S fertilizer decreased the proportion of propionic and butyric acid while increasing the proportion of acetic, isobutyric and isovaleric acids. These differences were probably due to differences in the level of S in the control and S-fertilized grass since similar differences in proportions have been found when grass-meal diets were supplemented with different levels of Na_2SO_4 (L'Estrange, Upton & McAleese, 1972). The branched-chain VFA isobutyric and isovaleric are essential nutrients for many rumen bacteria (Bentley, Lehmkuhl, Johnson, Hershberger & Moxon, 1954, Bryant & Doetsch, 1954) and the longer retention time of cellulose in the rumen and reduced cellulose digestibility of the low-S grasses was probably due to the poorer supply of branched-chain VFA to the rumen bacteria.

Moir (1970) reported that diets giving blood sulphate levels of 1·5 μmol/l contained adequate levels of S. However, in our study sheep given the S-deficient control diets had blood sulphate levels of 2·6 μmol/l. The feeds used in our study contained many young regrowths and it is possible that when eating this type of material the critical blood sulphate level should be higher than 2·6 μmol/l. Further work on this subject is required.

It is concluded that S fertilizer increased the nutritive value of pangola grass by overcoming a simple S deficiency in the diet of the sheep and this effect exceeded the adverse effects of S fertilizer on the digestibility of the NDS fractions of the grass and the increase in lignin content. The relative importance of these two opposing effects will probably be different in sheep and cattle due to their different S requirements (Kennedy & Siebert, 1972) and further work is required before these conclusions can be applied to pastures grazed by cattle.

The authors with to thank Dr F. W. Smith for advice with the fertilizer rates and Messrs J. Anderson, J. Biggs, L. B. Currell and G. A. Taylor for help with the preparation and feeding of the diets. We also thank Messrs D. J. Brett and A. D. Johnson for help with the chemical analysis and Messrs G. F. Maywald, C. Ross and P. G. Tuckett for technical assistance.

REFERENCES

Andrew, C. S. (1975). In *Sulphur in Australasian Agriculture*, p. 196 [K. D. McLachlan, editor]. Sydney: Sydney University Press.
Andrew, C. S. & Bryan, W. W. (1955). *Aust. J. agric. Res.* **6**, 265.
Annison, E. F. (1954). *Biochem. J.* **57**, 400.
Bentley, O. G., Lehmkuhl, A., Johnson, R. R., Hershberger, T. V. & Moxon, A. L. (1954). *J. Am. Chem. Soc.* **76**, 5000.
Bird, P. R. & Fountain, R. D. (1970). *Analyst, Lond.* **95**, 98.
Bray, A. C. (1965). Ph.D. Thesis, Univ. Western Australia, Nedlands.
Bryant, M. P. & Doetsch, R. N. (1954). *Science, NY* **120**, 944.
Downes, A. M., Langlands, J. P. & Reis, P. J. (1975). In *Sulphur in Australian Agriculture*, p. 117 [K. D. McLachlan, editor]. Sydney: Sydney University Press.
Kennedy, P. M. & Siebert, B. D. (1972). *Aust. J. agric. Res.* **23**, 45.
Langlands, J. P., Sutherland, H. A. M. & Playne, M. J. (1973). *Br. J. Nutr.* **30**, 537.
L'Estrange, J. L., Upton, P. K. & McAleese, D. M. (1972). *Ir. J. agric. Res.* **11**, 127.
Minson, D. J. (1966). *Br. J. Nutr.* **20**, 765.
Minson, D. J. & Milford, R. (1968). *J. agric. Sci., Camb.* **71**, 381.
Moir, R. J. (1970). In *Symposium: Sulphur in Nutrition*, p. 165. Westport: The AVI Publishing Company.
Rees, M. C. & Minson, D. J. (1976). *Br. J. Nutr.* **36**, 179.
Rees, M. C., Minson, D. J. & Smith, F. W. (1974). *J. agric. Sci., Camb.* **82**, 419.
Rendig, V. V. & Weir, W. C. (1957). *J. Anim. Sci.* **16**, 451.
Stobbs, T. H. & Brett, D. J. (1972). *Proc. Aust. Soc. Anim. Prod.* **9**, 297.
Thompson, C. H. (1958). Divl Rep. Div. Soils CSIRO no. 15/57.
Thornton, R. F. & Minson, D. J. (1972). *Aust. J. agric. Res.* **23**, 871.
Van Soest, P. J. (1963). *J. Ass. off. agric. Chem.* **46**, 825.
Van Soest, P. J. & Wine, R. H. (1967). *J. Ass. off. agric. Chem.* **50**, 50.
Weston, R. H. (1959). *Aust. J. agric. Res.* **10**, 865.

Printed in Great Britain