Published September, 1982

Forage Quality for Sheep and Chemical Composition Associated with Sulfur Fertilization on a Sulfur Deficient Site[1]

M. B. Jones[2], V. V. Rendig[3], D. T. Torell[2], and T. S. Inouye[3]

ABSTRACT

Improvements in forage quality and yield have resulted from application of S fertilizer. The purpose of this study was to relate forage composition changes associated with S fertilization to quality, as measured by lamb growth, efficiency of feed eaten by lambs, in vitro digestibility, and blood serum sulfate levels. Five rates of S were applied on a Sutherlin soil series (Ultic Haploxerolls) in each of 2 successive years to two sets of plots, one set seeded to subclover (*Trifolium subterraneum* L.) (designated "clover-grass"), the other to ryegrass (*Lolium multiflorum* L.). The ryegrass plots were also fertilized with N, and all plots received applications of P and K. The forage was harvested as hay, ground, pelleted, and fed to lambs in two feeding trials with an average duration of 60 days.

Sulfur concentrations ranged from 0.13 to 0.24% in the clover-grass and from 0.09 to 0.22% in ryegrass. Protein ranged from 10.3 to 13.1% in the clover-grass, and from 6.3 to 8.0% in the ryegrass. The N/S ratios ranged from 8 to 13 for the clover-grass and from 6 to 13 for ryegrass.

Application of S to the soil increased the average daily gains (ADG) of lambs for the two trials, from 141 to 186 g for the clover-grass, and from 32 to 84 g for the ryegrass. Ninety percent of the maximum ADG (defined as a critical value) was obtained at 0.19% S in the clover. A critical value is not given for ryegrass since ADG increased with each increment of S added, including the highest level of S applied. This was likely due to N being more limiting than S in the diet of the lambs. Blood serum sulfate-S in the lambs was more closely related to % S in clover–grass forage (r^2 = 0.76) than to % S in ryegrass (r^2 = 0.50). A good relationship (r^2 = 0.90) was found between serum sulfate-S level and sulfate-S intake for both forages. Blood sulfate was related to growth rate of lambs by a negative exponential function, with estimated critical values of 30 and 39 μg/ml, respectively, for the ryegrass and the clover-grass hay pellets.

An average of 11 kg feed/kg gain was required for sheep on clover-grass with zero S applied. This dropped to nine where S was applied. On ryegrass this ratio was 48 on the zero S treatment, but it dropped to an average of 18 with S applied.

Additional index words: **Sulfur concentrations, N/S ratios, Protein, Lamb gains, Blood sulfate, Digestibility, Subterranean clover, (*Trifolium subterraneum* L.), Ryegrass (*Lolium multiforum* L.).**

IMPROVEMENTS in quality as well as in yield, as a result of applications of S fertilizers, have been reported. Rendig and Weir (14) found that lambs fed alfalfa (*Medicago sativa* L.) hay containing 0.25% S made better gains than lambs fed unfertilized alfalfa containing 0.14% S. Results of previous studies (13) in this same geographic area indicated that S application at the rate of 77 kg/ha more than doubled hay yields. The hay with the higher S concentration was found to be more digestible. Also, blood serum sulfate was related to S intakes from the hay plus that from any S-containing supplements. In subsequent studies (15) it was found that alfalfa plants grown in nutrient solutions containing low levels of S had lower concentrations of sugars, and higher concentrations of amide N compared with plants having adequate S levels. Studies on subclover (*Trifolium subterraneum* L.) (7) have shown that S fertilization also resulted in higher concentrations of sugars and proteins and higher in vitro digestibility. Rees and Minson (12) determined the effects of S on feeding quality of pangola grass (*Digitaria decumbens* Stent.) by comparing the digestibility, voluntary intake, and retention time by sheep fed hay receiving no S fertilizer. They attributed 63% of the increase in digestibility and intake to the increased concentration of S in hay per se, and 37% to secondary effects that results from influences of S on other plant components.

[1] Contribution of Univ. of California, Dep. of Agronomy and Range Science, Land, Air and Water Resources, Animal Science, and the Hopland Field Station. This study was supported in part by a grant from the Sulphur Institute. Received 16 Oct. 1981.

[2] Agronomist and specialist in animal science, respectively, Univ. of California, Hopland, CA 95449.

[3] Professor and staff research associate, Dep. of Land, Air and Water Resources, Univ. of California, Davis, CA 95616.

Table 1. Forage characteristics resulting from S fertilization of subclover-grass and N-fertilized ryegrass.

Annual treatments		Yield		S		SO_4-S		N		N/S ratios	
1978	1979	1978	1979	1978	1979	1978	1978	1978	1979	1978	1979
kg S/ha		T/ha		%		ppm		%			
Subclover-grass:											
0	0	3.11	2.92	0.14	0.13	180	275	1.64	1.68	11.7	12.9
11	--	4.95	-	0.16	--	263	--	1.93	--	12.1	-
22	22	5.39	4.43	0.17	0.15	300	506	1.98	1.71	11.6	11.4
45	45	4.68	4.96	0.18	0.18	340	633	1.98	2.08	11.0	11.6
90	90	5.53	4.26	0.20	0.20	382	909	2.02	2.10	10.1	10.5
--	179	-	4.67	--	0.24	--	1,250	--	1.98	-	8.3
Ryegrass:											
0	0	8.56	4.64	0.09	0.09	163	203	1.09	1.14	12.1	12.7
11	--	8.90	-	0.11	--	206	--	1.09	--	9.9	--
22	22	10.20	5.00	0.17	0.12	323	372	1.02	1.14	6.0	9.5
45	45	9.83	7.50	0.17	0.16	345	918	1.28	1.06	7.5	6.6
90	90	11.58	6.21	0.21	0.20	604	1,167	1.27	1.20	6.0	6.0
--	179	-	7.45	--	0.22	--	1,506	--	1.25	--	5.7

The National Research Council (11) states the S requirements for mature ewes to be 0.14 to 0.18% of diet dry matter and for young lambs 0.18 to 0.26%.

The purpose of this study was to evaluate the effects of increasing concentrations of S in forage resulting from S fertilizer on the quality of two annual grassland forage types fed as pellets, with a view towards establishing critical values for plant S content. Quality was measured by weight gains of lambs, which was then related to total forage S, sulfate-S, the N/S ratio, and blood serum sulfate-S.

METHODS

A 4.5-ha pasture with Sutherlin soil series (Ultic Haploxerolls) was fertilized uniformly in October 1977 with concentrated superphosphate (24% P, 0% S, 14% Ca) at 50 kg P/ha and with KCl (51% K) at 105 kg K/ha. The pasture was next divided into two parts. A mixture of three cultivars of subclover (Mt. Barker, Woogenellup, and Geraldton) were sown to 2.7 ha at the rate of 40 kg/ha, and annual ryegrass (*Lolium multiflorum* var. Wimmara) was seeded at the rate of 60 kg/ha to the remaining 1.8 ha. Nitrogen was applied as urea to the ryegrass at the rate of 100 kg/N ha in October 1977 and again in February 1978. On 9 Dec. 1977, 0.5 kg/ha paraquat (1,1'-dimethyl-4, 4'-bipyridinium ion) was sprayed on the 2.7 ha of subclover pasture to reduce the grass component. By spring 1978 the pasture was about 60% subclover, 25% grass (*Festuca megalura* Nutt. and *Hordeum leporinum* Link.), and 15% was filaree (*Erodium* sp.). The mixture will be designated as clover-grass.

The clover-grass and ryegrass sections were each subdivided into five parts and randomly treated with 0, 11, 22, 45, and 90 kg S ha as gypsum in October 1977 in order to produce forage with a range of S concentrations. In May 1978 the forage from the 10 plots was cut with a swather, dried in the field, baled, weighed, ground, and pressed into pellets (Diameter = 8 mm, length = 20 mm). The pelleting reduced selectivity by the lambs.

In September 1978, 100 lambs weighing about 31 kg were divided into 10 groups. The groups were fed ad lib from the 10 lots of pellets, which were weighed daily as distributed. A surplus was kept before the animals, which was weighed back weekly. Water was always available. Each group had a 24 m^2 area inside the barn with an equal space outside in which they could move freely. Weights were taken at the beginning and every 14 days with animals on full feed and water. The trial ended on the 61st day. Blood serum samples were taken from each lamb at the end of the trial for sulfate determination.

In 1978-1979 the experiment was repeated with the following changes: No further additions of P, K, or subclover seed were made, 25 kg/ha ryegrass seed was sown on the grass plots, and S was reapplied but at rates double those used the previous year, to give a greater range in S concentrations. Eighty lambs were divided among the 10 treatments, with four replications of two lambs, each held in pens 1 m square, thus eight lambs were fed pellets from each of the 10 treatments for 57 days.

To obtain a composite sample for analyses, a few pellets were collected from each of the portions provided daily to the animals. The methods described by Johnson and Nishita (5) were used to determine total S and sulfate S in the plant material and sulfate S in blood serum. The Kjeldahl method modified to include nitrate (3) was used to determine N. Forage digestibility was determined by the Tilly and Terry method (18). All analyses were run in duplicate.

Simple correlation coefficients (r) were calculated, and scatter diagrams were made to show relationships between measured variables. Where appropriate the relative average daily gain (RADG) (y) was plotted against forage or blood composition (x) using the Mitscherlich equation, $y = a(1-be^{-cx})$ where a = maximum RADG, b = a-y/a when x = o, c = coefficient that determines the shape of the curve, and R^2 values are given as a measure of goodness of fit. The RADG was obtained by dividing all average daily gains by the ADG from the highest S treatment on clover-grass each year (235 g/day in 1978 and 166 g/day in 1979). Data for ADG, feed consumption, feed efficiency, and blood serum sulfate-S obtained in 1979, when the lambs were divided into four replications for the feeding trial, were subjected to an analysis of variance.

RESULTS AND DISCUSSION

The data on forage production and S concentrations shown in Table 1 indicate S deficiency where no S was applied, and fertilizer S increased plant concentrations of S and sulfate S. Martin and Matocha (9) specified a "critical nutrient range" of 0.16 to 0.20% S for subclover, which is much above values found here in the clover-grass forage samples from zero S plots, or those receiving low rates of S applications. In other studies in this same geographic area Jones and Martin (6) and Jones et al. (8) obtained a critical sulfate-S value of less than 200 ppm for subclover forage yields. The low concentration of S (0.09%) in the ryegrass would indicate severe deficiency by comparison with other data reported in the literature (4).

Correction of a moderate S deficiency by applica-

Table 2. Effects of S fertilization on the feeding value of subclover-grass and N-fertilized ryegrass hay for lambs.

Annual treatments		ADG		In vitro digestibility		Feed consumed		Feed efficiency		Serum blood SO_4-S	
1978	1979	1978	1979	1978	1979	1978	1979	1978	1979	1978	1979
kg S/ha		g/day		%		kg/hd/day		kg feed/kg gn		g/ml	
Subclover-grass											
0	0	163	118	69	70	1.67	1.47	10	12	26	28
11	-	189	--	69	--	1.75	--	9	--	31	--
22	22	183	174	69	70	1.63	1.63	9	9	32	37
45	45	181	179	69	71	1.67	1.62	9	9	33	44
90	90	235	179	70	71	1.77	1.64	7	9	45	54
--	179	--	166	--	70	--	1.47	--	9	--	76
Ryegrass:											
0	0	46	17	58	61	1.54	1.06	34	62	20	21
11	--	33	--	60	--	1.11	--	34	--	20	--
22	22	92	60	63	61	1.34	1.34	15	22	22	28
45	45	99	76	65	61	1.32	1.22	13	16	26	64
90	90	144	81	68	67	1.56	1.60	11	20	39	74
--	179	--	90	--	66	--	1.42	--	16	--	67
L.S.D. (0.05)			33				1.32		2		6

Average gain for 57 days in 1978 and 63 in 1979.

tions of fertilizer has been shown to increase the concentraton of N in the forage, if available N was present (2, 17), which appeared to be the case on the ryegrass. However, the very low N values and low N/S ratio where S was applied indicate a possible N deficiency.

The object of the field S treatments was to produce sufficient forage, with S concentrations ranging from deficient to sufficient, which could be fed to lambs on which gains could be measured. These plots had to be large enough to operate haymaking equipment and replication was not feasible, thus no statistical comparisons of the 10 treatments are made. Separate replicated S fertilizer trials have been run at four locations over a 20 year period in the same field. Each has shown a significant response to S (M. B. Jones, unpublished data).

Lamb gains generally increased with increasing S levels in both pelleted hays for both years (Table 2). The gains by lambs fed the clover-grass hay were greater than gains on the ryegrass. The smaller gains for ryegrass were probably the result of insufficient protein concentrations in the forage which varied from 6.4 to 8.0%. The National Research Council (11) indicates a minimum concentration of 10% protein in the diet for 30 kg lambs. In spite of low protein levels, lamb growth responses to S occurred for ryegrass both years, up the to highest level of applied S. Lamb gains were less on both hay types the 2nd year. This may have been due to the smaller pens used to house each pair of lambs for the 2nd year experiment.

Feed consumption did not vary consistently with S treatments in 1978 and increased significantly with increasing S only for the ryegrass fed in 1979. However, feed efficiency was increased by S fertilization. An average of 11 kg feed/kg gain was required where no S was applied on clover-grass, but only 9 kg feed/kg gain was required where S fertilizer was used. On ryegrass the average ratios were 48 and 18 for no S and S, respectively.

Sulfate S concentrations of the blood serum correlated well ($r^2 = 0.90$) with the mean daily intake of sulfate S (Fig. 1). A much lower correlation ($r^2 = 0.41$) was found if total S intake rather than sulfate S was used for comparison. For comparable levels of

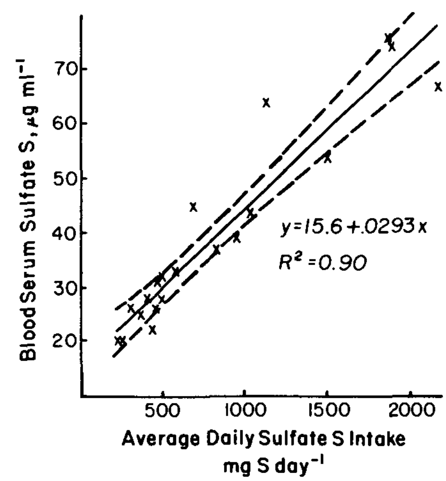

Fig. 1. The relationship of average daily sulfate intake to blood serum sulfate-S in lambs.

S intake, these serum sulfate S values are higher than those obtained with sheep fed alfalfa hay in a previous study (19), where serum sulfate concentrations of less than 20 μg/ml were found with S intakes of 1.3 to 1.5 g/day. Besides animal and plant species differences, there were different analytical methods used in the two studies. In the present study the serum was subjected directly to the reducing medium as prescribed in the Johnson-Nishita method (5), while previously a uranyl acetate precipitation preceded formation of a sulfate-containing colored complex.

The in vitro digestibility of clover-grass pellets was influenced very little by S fertilization, with values ranging from 69 to 71%, but for the ryegrass the values ranged from 58 to 68%, increasing as the rate of applied S was increased from zero to 90 kg/ha. Effects of S applications on in vivo digestibility values had been noted by Rendig and Weir (14) in their studies using alfalfa hay, as described earlier.

In order to identify and if possible quantify those parameters of chemical composition most closely related to animal responses, various correlations between the data were determined. Since the differences between species was quite marked, the data for clover-

Table 3. Simple correlation coefficients (r) showing the relationship between measured variables in S fertilized subclover-grass forage, 2-years data combined.

	Total S	SO_4-S	SO_4-S/Total S	N	N/S	Serum SO_4-S	Digestibility	Feed cons.
SO_4-S	0.80*	1.00						
SO_4-S/Total S	0.65*	0.97**	1.00					
N	0.77**	0.48	0.39	1.00				
N/S	−0.91**	−0.77**	−0.62	−0.48	1.00			
Serum SO_4-S	0.87**	0.95**	0.88**	0.87**	−0.89**	1.00		
Digestibility	0.34	0.51	0.72*	0.39	−0.23	0.53	1.00	
Feed consumption	−0.08	−0.48	−0.52	0.24	0.13	−0.38	−0.28	1.00
Relative ADG	0.60	0.71*	0.77**	0.55	−0.53	0.69*	0.80**	0.05

* $P \leq 0.05$.
** $P \leq 0.01$.

grass and ryegrass hay pellets will be presented and discussed separately.

Clover-grass

The correlation coefficients between the variables are shown in Table 3. Highly significant (1% level) r values were obtained for the linear relationship of total forage S, to sulfate-S, N, and N/S in the forage, and to the serum sulfate values. Total forage S was less closely ($r^2 = 0.36$; 10% level) related to the relative average daily gain (RADG). There was no significant correlation between total forage S concentrations and feed consumption, or to feed digestibility. The relationship between total forage S and RADG could be described slightly better ($R^2 = 0.40$) with a negative exponential model (Fig. 2).

When data for the 2 years were analyzed separately, forage S and RADG showed a linear relationship ($r^2 = 0.72$) in 1978, but the relationship between these two parameters in 1979 were best described by a negative exponential ($R^2 = 0.70$). The latter saturation-type response could be attributed to the higher rates of appliction in the 2nd year. From the data for the 1979 study, the S requirement (critical value) needed to obtain 90% of maximum ADG was estimated as 0.15% S. In 1978 the greatest gain occurred at the highest level of S or 0.20%. When data was combined for both years, the critical value was 0.19% S (Fig. 2). From their studies involving the feeding of alfalfa hay grown on soils of varied S contents to yearling wethers, Rendig and Weir (14) concluded that 0.155% total dietary S resulted in good gains. The National

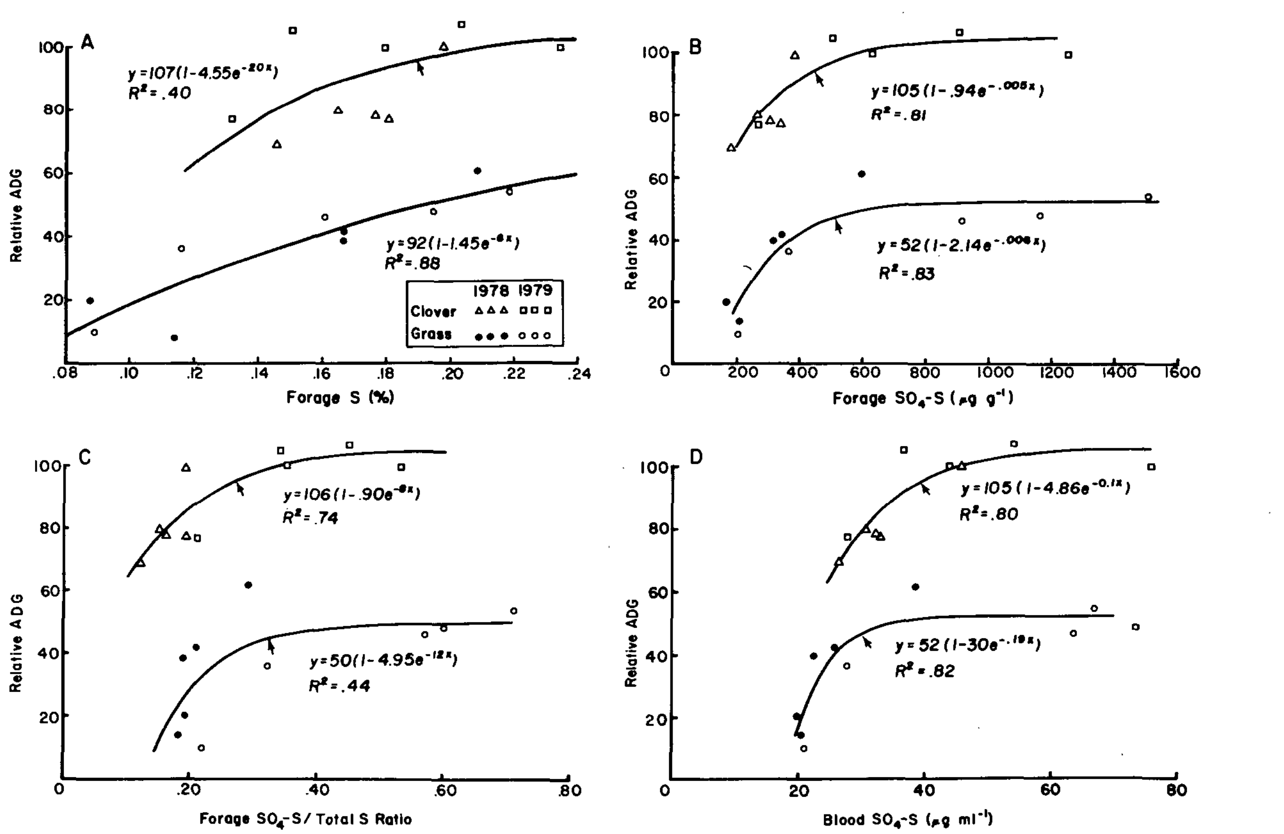

Fig. 2. The relationship of: a) % forage-S to relative average daily grain (relative ADG) of lambs, where 235 g/day = 100 in 1978 and 166 g/day = 100 in 1979; b) sulfate-S in forage to relative ADG; c) the ratio of sulfate-S/total S in forage to relative ADG; and d) blood serum sulfate-S to relative ADG.

Table 4. Correlation coefficients (r) showing the relationship between measured variables in S fertilized ryegrass; 2-years data combined.

	Total S	SO_4-S	SO_4-S / Total S	N	N/S	Serum SO_4-S	Digestibility	Feed cons.
SO_4-S	0.78**	1.00						
SO_4-S/Total S	0.64*	0.97**	1.00					
N	0.56	0.39	0.26	1.00				
N/S	−0.94**	−0.69*	−0.57	−0.31	1.00			
Serum SO_4-S	0.71*	0.64*	0.96**	0.30	−0.66	1.00		
Digestibility	0.88**	0.61	0.44	0.75*	−0.77**	0.55	1.00	
Feed consumption	0.54	0.39	0.27	0.37	−0.44	−0.39	0.51	1.00
Relative ADG	0.93**	0.71*	0.60	0.52	−0.90**	0.68*	0.82**	0.63

* $P \leq 0.05$.
** $P \leq 0.01$.

Research Council (11) indicates the S requirement to be in the 0.14 to 0.25% range. Using their protein requirement of 10.0% for 30 kg replacement lambs and a N/S ratio of 10:1, a requirement of 0.16% S is indicated.

The relationship between sulfate-S in clover-grass forage, N/S ratio, and blood serum sulfate was highly significant. The correlation between forage sulfate-S and feed consumption was not significant. The linear relationship of sulfate-S to RADG was significant, but the goodness of fit as indicted by $r^2 = 0.50$, was less than for the curvilinear relationship using the negative exponential ($R^2 = 0.81$) (Fig. 2). The critical value of 450 ppm sulfate-S for 90% of maximum RADG seems rather high compared to the critical value for subclover forage production which previous studies had indicated as being less than 200 ppm (8). Harvesting the hay in late May rather than in April may account for the higher sulfate values and thus the greater estimated critical values. Previous studies (6) had shown sulfate values increase as the subclover plants dry at the end of the growing season. This might explain the high critical sulfate values and the high critical sulfate-S/total S ratios.

The r values for the linear relationships of the sulfate-S/total S ratio to blood serum sulfate and to RADG were highly significant ($P < 0.01$). The relationship of sulfate-S to RADG was defined better by a negative exponential ($R^2 = 0.74$) than by the linear relationship ($r^2 = 0.59$) (Fig. 2). The critical sulfate-S/S ratio was 0.28, much higher than the 0.10 reported for plants (16).

Nitrogen concentrations in the forage were linearly related ($P < 0.01$) to blood serum sulfate and to RADG ($P < 0.10$). Clover-grass-N was not significantly related to feed consumption.

The N/S ratios in clover-grass were linearly related ($P < 0.01$) to blood serum sulfate level, but the N/S relationship to RADG was not significant. Values obtained in the present study ranged from those somewhat in excess to well below the value of 10 that Moir et al. (10) specified as being optimal for sheep. Bird et al. (1) in their review of the subject cite generally higher values, and discuss a number of plant and animal factors that lessen the likelihood of defining a generally applicable optimal N/S ratio.

Blood serum sulfate S was linearly related ($r^2 = 0.48$; $P < 0.05$) to RADG, but the negative exponential function gave a much better fit ($R^2 = 0.80$) (Fig. 2). Ninety percent of the maximum gain had occurred where the sulfate-S level in the blood reached 39 μg/ml.

In vitro digestibility was significantly related only to the sulfate-S/total S ratio of the chemical constituents of the forage ($r^2 = 0.52$, $P < 0.05$) and to RADG ($r^2 = 0.64$; $P < 0.01$). Feed consumption was not significantly related to forage composition, digestibility, nor RADG.

Ryegrass

Total S values for the ryegrass hay were more closely related (Table 4) to forage sulfate-S, N/S ratio, digestibility, and RADG ($P < 0.01$) than to sulfate-S/S ratio and blood serum sulfate-S ($P < 0.05$), or to forage N concentrations ($P < 0.10$).

The relationship between total S and RADG was described better by a negative exponential function ($R^2 = 0.88$) than by a linear function ($r^2 = 0.86$) (Fig. 2). The increasing ADG of lambs with increasing S is of particular interest because the gains came in spite of the low protein levels in the grass forage which ranged from 6.8 to 8.0%. The protein requirements for 30 kg replacement lambs is listed at 10% (11). In light of this fact, it is perhaps not surprising that the calculated best fit lamb response curve showed a continued increase over the entire treatment range used in the studies. Thus, no attempt was made to estimate the critical S value.

The sulfate-S levels in the ryegrass were related to N/S, blood serum sulfate-S, and to RADG ($P < 0.05$). An r^2 value of 0.50 for the linear relationship of sulfate-S vs. RADG, and R^2 of 0.83 for the best fit negative exponential indicates a curvilinear relationship with an estimated critical value of 510 ppm S (Fig. 2). This value seems high and may be due in part to N deficiency in the grass and some conversion of organic S to sulfate-S as the crop matured, as had been indicated in earlier studies with subclover (6).

The sulfate-S/S ratios were highly related to blood serum sulfate-S ($r^2 = 0.92$), but other variables were not significantly related to this ration. Sulfate-S/S and RADG were poorly correlated as indicated by values of r^2 (linear) an R (negative exponential function) of 0.36 and 0.44, respectively (Fig. 2).

Digestibility values were linearly related ($r^2 = 0.56$; $P < 0.05$) to N concentrations of the ryegrass, but the other indicated variables were not significantly related to the latter.

The N/S ratios in ryegrass were linearly related to blood serum sulfate-S ($P < 0.05$) and to digestibility and RADG ($P < 0.01$).

The relationship of blood serum sulfate-S to RADG was better described by a negative exponential function ($R^2 = 0.82$) than linearly ($r^2 = 0.46$). A critical value of 30 μg/ml was estimated from the negative exponential equation (Fig. 2).

In vitro digestibility values were linearly related to RADG ($r^2 = 0.67$; $P < 0.01$). Quantities of feed consumed were linearly related ($r^2 = 0.40$; $P < 0.10$) to RADG.

ACKNOWLEDGMENT

The authors thank John Hays and Gil Dow for handling the sheep and to Mike Center and W. A. Williams for statistical assistance.

LITERATURE CITED

1. Bird, P. R., M. J. Watson, and J. W. D. Cayley. 1978. The sulphur requirements of ruminants and the S and N status of perennial pastures in southern Australia. *In* J. C. Brogan (ed.) Sulphur in forages. Proc. Symp. Johnstown Castle Research Centre, Wexford, Ireland. An Foras Talluntais, Dublin.
2. Bolton, J., T. Z. Nowakowski, and W. Lazarus. 1976. Sulphur-nitrogen interactions effects on the yield and composition of the protein-N, non-protein N, and solule carbohydrates in perennial ryegrass. J. Sci. Food Agric. 27:553–560.
3. Chapman, H. D., and P. F. Pratt. 1961. Methods of analysis for soils, plants and waters. Univ. of California, Division of Agric. Sci.
4. Cowling, D. W., and L. H. P. Jones. 1970. A deficiency in soil sulfur supplies for perennial ryegrass in England. Soil Sci. 110:346–354.
5. Johnson, C. M., and H. Nishita. 1952. Microestimation of sulfur in plant materials, soils, and irrigation waters. Anal. Chem. 24:736–742.
6. Jones, M. B., and W. E. Martin. 1964. Sulfate sulfur concentration as an indicator of sulfur status in various California dryland species. Soil Sci. Am. Proc. 28:539–541.
7. ———, J. H. Oh, and J. E. Ruckman. 1970. Effect of P and S fertilization on the nutritive value of subclover (*Trifolium subterraneum*). Proc. N. Z. Grassl. Assoc. 32:69–75.
8. ———, J. E. Ruckman, W. A. Williams, and R. L. Koenigs. 1980. Sulfur diagnostic criteria as affected by age and defoliation of subclover. Agron. J. 72:1043–1046.
9. Martin, W. E., and J. E. Matocha. 1973. Plant analysis as an aid in the fertilization of forage crops. p. 393–426. *In* L. M. Walsh and J. D. Beaton (ed.) Soil testing and plant analysis, Rev. Ed., Soil Sci. Soc. Am., Madison, Wis.
10. Moir, R. J., M. Somers, and A. C. Bray. 1967–68. Utilization of dietary sulphur and nitrogen by ruminants. Sulphur Inst. J. 3:15–18.
11. National Research Council. 1975. Nutrient requirements of domestic animals, Number 5, Nutrient requirements of sheep. National Academy of Science, Washington, DC.
12. Rees, M. C., and D. J. Minson. 1977. The influence of supplements on the voluntary intake and digestibility of feed—an alternative of fertilizers? *In* G. J. Blair (ed.) Review in Rural Sci. III. Univ. of New England Press, Armidale, N.S.W. Australia.
13. Rendig, V. V. 1956. Sulfur and nitrogen composition of fertilized and unfertilized alfalfa grown on a sulfur-deficient soil. Soil Sci. Am. Proc. 20:237–240.
14. ———, and W. C. Weir. 1957. Evaluation by lamb feeding tests of alfalfa hay grown on low-sulphur soil. J. Anim. Sci. 16(2):451–461.
15. ———, and E. A. McComb. 1959. Effect of nutritional stress on plant composition: I. The interaction of added nitrogen with varying sulfur supply. Soil Sci. Soc. Am. Proc. 23:377–380.
16. Spencer, K., J. R. Freney, and M. B. Jones. 1978. Diagnosis of sulfur deficiency in plants. From "Plant Nutrition 1978" Proc. 8th Int. Colloquim of Plant Analysis and Fertilizer Problems. Auckland, N.Z., August 28-September 1, 1978. A. R. Ferguson and R. L. Bieleski (ed.) N.Z. DSIR Information Series #134 Wellington, Government Printer, 1978.
17. Stewart, B. A., and L. K. Porter. 1969. Nitrogen-sulfur relationships in wheat (*Triticum aestivum* L.) corn (*Zea mays* L.), and beans (*Phaseolus vulgaris*). Agron J. 61:256–271.
18. Tilley, J. A. A., and R. A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18:104–111.
19. Weir, W. C., and V. V. Rendig. 1954. Serum inorganic sulfate sulfur as a measure of the sulfur intake of sheep. J. Nutri. 54:87–96.