

EFFECT OF PHOSPHORUS AND SULPHUR FERTILIZATION ON THE NUTRITIVE VALUE OF SUBTERRANEAN CLOVER (*TRIFOLIUM SUBTERRANEUM*) *

M. B. JONES, J. H. OH, AND J. E. RUCKMAN†

Summary

Phosphorus or sulphur applied alone to a soil deficient in P and S did not increase subterranean clover (*Trifolium subterraneum*) yields; but when the two elements were applied together, a five-fold increase was obtained. The nutritive value of the clover was evaluated by measuring sheep rumen microbial activity using an anaerobic manometric technique. Subterranean clover fertilized with P alone increased the microbial activity about 6%. In contrast, the clover fertilized with S alone decreased activity about 5%. When P and S were applied together, microbial activity was increased 20 to 30%, depending on the rates applied. There was a positive correlation between the level of reducing sugars in the clover and the degree of rumen microbial activity.

APPLICATIONS of phosphorus and sulphur to deficient soils of California annual grasslands have generally resulted in striking increases in yields and pronounced changes in botanical and chemical composition of the forage. From work reported with other forage crops (Rendig and Weir, 1957; Weir, et al., 1958; Reid and Jung, 1965) it has been assumed that these changes improved the quality of the crop, but no direct measurements of quality have been made.

On annual grasslands it is difficult to obtain sufficient amounts of a single plant species to run digestion studies, especially at variable levels of soil fertility. Rumen microbial gas production, measured *in vitro*, has been shown to be highly correlated with dry matter intake and dry matter digestibility (Oh, *et al.*, 1969). Such techniques make it easier to study the changes in nutritive value which accompany changes in soil fertility, and it is possible to include in a study a greater number of fertility levels than would be practical under field conditions.

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† Agronomist, Assistant Research Bacteriologist, and Associate Specialist, respectively.

The purpose of this study was to determine relative nutritive values of subterranean clover grown at various levels of soil P and S.

PROCEDURE

In a glasshouse, 2 kg of Josephine soil, known from previous experimentation to be very deficient in P and S, and not responsive to applications of micronutrients, were placed in each of 64 plastic pots. Four levels of P (0, 25, 50, and 75 ppm) were applied as H_3PO_4 in all combinations with four levels of S (0, 5, 10, and 15 ppm) applied as H_2SO_4 . Each pot also received 50 ppm K as KCl. All fertilizers were applied in solution and, after drying, were mixed throughout the soil. Then, the soil was tested for P by the sodium bicarbonate method (Olsen et al., 1954). On February 24, 1969, 15 inoculated seeds of subterranean clover (*Trifolium subterraneum* 'Clare') were planted in each pot. Treatments were replicated four times.

The pots were weighed periodically throughout the growing period, and were watered as needed to maintain the soil moisture near field capacity. On May 2, when the clover was in flower, all forage was harvested, oven-dried at 68° C, weighed, and ground to pass a 40-mesh screen.

With 0.1 g subterranean clover as substrate, rumen microbial activity was measured by the Warburg manometric technique of Hungate et al. (1955) as modified by Oh et al. (1967). The ruminal inoculum was obtained, as follows, from sheep maintained on good quality lucerne hay. Ruminal fluid was strained through two layers of cheesecloth, and then centrifuged at 300 rpm for 45 seconds to remove large feed particles. The supernatant fluid was retained and centrifuged at 20,000 rpm for 15 minutes. This supernatant fluid was discarded, and the sedimented ruminal microbes were suspended in a buffer of 1.2% $NaHCO_3$ and 0.6% NaCl, preheated to 39°C. Seven ml of the microbial solution was used to ferment the substrate for 24 hours. Microbial activity was expressed as mmoles gas/0.1 g substrate.

Total N, P, and S in the forage were estimated, respectively, by the methods of AOAC (1965); Bridger et al. (1953) and Johnson and Nishita (1952). Carbohydrates were determined by the AOAC (1965) method as modified by Hassid (1936), and McCready et al. (1950).

RESULTS

The results are indicated in Table 1. Neither P nor S applied without the other produced a significant effect on

yield. Application of 25 ppm or more P plus 5 ppm or more S caused about a five-fold increase in yield. Soil tested by the sodium bicarbonate method for available P gave values of 2, 8, 15, and 23 ppm P for the 0, 25, 50, and 75 ppm treatments, respectively.

The concentration of P in the dried clover was about 0.09% when no P or S was applied. Adding 25 to 75 ppm, P but no S, nearly doubled the level. However, when S was applied, only a small significant increase in plant P was observed with 25 ppm P. An additional increase occurred with each increment of P added. Percentage S in the clover increased as the amount of S applied increased, but application of 25 ppm P reduced the S percentage. Additional increments of P had little effect on S percentages except at the 15 ppm S level, 50 ppm P increments reduced S. The concentration of N in the clover was decreased by the first increment of P and increased by the first two increments of S, with little difference at higher P and S levels.

The percentage of reducing sugars in the plants was not significantly changed by the addition of either P or S alone; but with the addition of at least 25 ppm P the first two increments of S increased reducing sugar. With the addition of S, only the first increment of P increased reducing sugar.

Application of 15 ppm S alone reduced sucrose percentage in the clover, but applying S with 25 ppm or more P increased sucrose. Phosphorus applications increased sucrose only where S had been applied.

Percentage starch in the clover was increased by the first increment of P only, and was decreased by the first two increments of S.

Microbial gas production was increased by the application of P only, but was reduced when S only was applied. There was an increase in gas production with the first 5 ppm increment of S where 25 ppm P had been applied, but, where 50 and 75 ppm had been applied, an increase in gas production resulted from applications of 5 and 10 ppm S.

DISCUSSION AND CONCLUSIONS

There are two factors involved in changing the rates of rumen microbial activity induced by P and S fertilization: (1) the P and S requirements for growth of the microbes; and (2) other chemical changes in the plants, such as concentration of reducing sugars, resulting from fertilization,

There are apparent critical levels of P and S in plant tissue, below which rumen microbial activity decreases

TABLE 1: EFFECT OF P AND S FERTILIZATION ON YIELD, CHEMICAL COMPOSITION, AND NUTRITIVE VALUE OF SUBTERRANEAN CLOVER AS INDICATED BY RUMEN MICROBIAL GAS PRODUCTION

Fertilizer		Yield (g/pot)	P (%)	S (%)	N (%)	N/S	Reducing Sugar as Glucose (%)	sucrose (%)	Starch (%)	Rumen Microbial Gas Production (moles/0.1 g)
P (ppm)	S (ppm)									
0	0	2.0	0.08	0.15	1.61	27	1.1	7.7	10.2	0.72
0	10	2.3	0.085	0.168	2.02	12	1.1	7.7	10.2	0.66
0	15	2.3	0.083	0.188	2.00	11	1.3	5.1	10.5	0.65
25	0	2.9	0.161	0.035	1.39	40	1.3	6.2	20.2	0.67
25	5	10.7	0.109	0.053	1.47	28	4.4	9.2	17.1	0.78
25	10	11.8	0.101	0.094	1.58	17	5.1	9.0	14.6	0.92
25	15	10.5	0.108	0.132	1.54	12	4.5	8.2	14.5	0.96
50	0	3.4	0.161	0.037	1.37	37	1.2	6.2	20.8	0.94
50	5	12.4	0.158	0.052	1.49	29	3.5	9.8	15.1	0.81
50	10	14.8	0.142	0.084	1.56	19	5.3	8.3	13.3	0.96
50	15	12.9	0.161	0.108	1.62	15	5.4	7.6	14.4	1.01
75	0	3.2	0.176	0.036	1.36	38	1.3	5.8	20.8	0.77
75	5	11.6	0.200	0.058	1.49	26	3.7	9.0	15.4	0.93
75	10	13.6	0.196	0.090	1.69	19	4.9	7.9	13.0	1.03
75	15	15.9	0.192	0.107	1.55	14	5.5	8.6	14.5	1.04

Values within a column followed by the same letter are not significantly different at the 5% level.

sharply when such plants are used as microbial substrate. Under the conditions of this experiment, these critical levels were 0.10% for P and 0.05% for S. Below these levels it appears that P or S were primary limiting factors. However, levels of reducing sugars were also lowest where P and S values were lowest. Thus, critical levels cannot be taken at face value. There was a small increase in microbial activity where P level went from 0.10 to about 0.14% at the two higher levels of S. Above this range there was no further increase. Likewise, small increases occurred when S level went from 0.05 to about 0.08% at the two higher levels of P. The care used in eliminating all P and S from the buffer solution and as much as possible from the inoculum, so that the only source of the two elements was the clover substrate, had an important influence on the results.

There was a positive correlation between microbial activity and the percentage of reducing sugars, except when P and S were most limiting. The relation of rumen microbial activity to concentration of sucrose was less intimate than to reducing sugars, although correlation coefficient values for sucrose were significant at some levels of P and S.

Above the P and S critical levels there was a negative correlation between microbial activity and starch percentage. The diet of the sheep providing ruminal inoculum has an influence on the characteristics of fermentation (Baumann and Foster, 1956). Had the sheep been on a grain diet rather than lucerne, this correlation may have been positive.

It is not surprising that percentage N in the clover was not well correlated with ruminal activity, since applied P decreased the N level and increased activity, while applied S increased both N and ruminal activity. However, it is surprising that the level of N in the clover was so low. It is not probable that additional increments of S would have increased the level of N, since the last increment of S applied did not increase N level in the plant. Subsequent tests have indicated that the strains of rhizobia used in this study were less effective N fixers than strains now available. Possibly this is the reason for low N values.

The information from this study supports the findings of Weir et al. (1958) who reported that lucerne hay which contained 0.10% P produced poor animal gains compared with hay containing 0.15% P. Values reported by Reid and Jung (1965) were far higher, 0.38% P for superphosphate fertilized fescue compared with 0.34% P for unfertilized grass. Sheep preferred the fertilized grass.

The S percentages in this study were generally lower than the hay studied by Rendig and Weir (1957), who indicated that animals performed better when fed hay containing 0.18% S rather than hay with 0.14% S.

The very low P and S levels reported in the forage of this study have been found under field conditions on the annual grasslands of California. Therefore, the low concentrations of P and S probably adversely affect the quality of feed as well as total production under field conditions. The addition of P and S fertilizers to these very deficient grasslands not only increases forage production when legumes are present, but evidence indicates that the nutritive value of that forage also is increased by both elements.

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DISCUSSION

Howard asked if gas production had been correlated with reducing sugars and starch together, to which Jones replied that., when reducing sugars were added to sucrose, a similar pattern was obtained.

Bailey suggested that the starch levels were high by New Zealand standards and asked how they were obtained. Jones said the old classical chemical methods had been used. He had extracted sugars, reducing sugars and then sucrose. The remaining starch was hydrolysed to sugar and measured in this way. The level was higher than expected but other methods had verified the results. It could be that the level was affected by low N content and the fact that the material was grown indoors.

Smith (U.S.A.) thought it might be possible that photosynthesis had been restricted in the plants because they were grown at too low temperatures. Jones replied that the plants grew over the period February-May, with day temperatures 80 to 90°F and night 50°F. Smith further commented that any factor slowing down growth could cause accumulations so temperatures could have an effect. Jones agreed that the same situation could occur in the field where drought conditions would cause accumulations.

Ludecke considered that even at the high levels sulphur could still be deficient and asked if a greater growth response would be achieved at higher rates of sulphur. Jones said he would certainly use higher levels of S in further trials. In the present trial they had wanted to find the critical level.

Asked by Katznelson (Israel) if fertilizers had affected the phenology of the plant, Jones replied that flowering had been earlier with phosphorus but no such effect had been noticed from sulphur.