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Pastures consisting of mixtures of subclover (Trifolium subterraneum) and grasses have responded to sulfur fertilization on many sites in Douglas County, Oregon. The objectives of this study were to examine specific changes in forage quality which occur as sulfur is applied in excess of the amount required for maximum yield of dry matter.

Plant samples and yield data were obtained from field plots treated with 0, 10, 20, 40, 80 and 160 pounds of sulfur per acre in the form of gypsum. Samples were then examined for species composition, total nitrogen, total sulfur and in vitro digestibility.

Dry matter yields were not significantly increased by sulfur application. However, the percentage of clover in the forage changed significantly. The amount of clover increased from 42% in the check plot to 81% when 20 pounds of sulfur per acre was applied. As the sulfur rate increased up to 160 pounds per acre, the percen age of

subclover declined to 65%. Subclover has a higher requirement for sulfur than the grasses. This is reflected by the sharp increase in clover with the application of 20 pounds of sulfur per acre. At higher rates of sulfur application, the companion grasses became competitive with the clover, apparently due to the addition of nitrogen to the plant community through biological fixation.

The increase in nitrogen and sulfur content with increasing sulfur fertilization was highly significant for both the grass and clover.

The increase in the nitrogen content of the grass from 1.2% in the check plot to 1.8% at the rate of 160 pounds of sulfur is attributed to underground transfer of nitrogen from the clover to the grass. The sulfur content increased at a more rapid rate than did the nitrogen content which resulted in a narrowing of the nitrogen to sulfur ratio. The nitrogen to sulfur ratio narrowed from 14:1 to 9:1 in the grass, from 22:1 to 13:1 in the clover and from 18:1 to 12:1 in the forage as sulfur application was increased from 0 to 160 pounds per acre.

Average digestibility as measured with the <u>in vitro</u> technique was 36 and 49% respectively for grass and clover. Digestibility of the forage increased significantly with sulfur applications, while the digestibility of the grass or clover measured separately was not changed.

In summary, sulfur fertilization influences the quality of subclover-grass forage largely through changes in species composition, nitrogen content, and by narrowing the nitrogen to sulfur ratio. The increase in forage digestibility is due primarily to the change in species composition and nitrogen content.

Some Effects of Sulfur Fertilization on the Nutrient Value of Subclover-Grass Forage

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SOME EFFECTS OF SULFUR FERTILIZATION ON THE NUTRIENT VALUE OF SUBCLOVER-GRASS FORAGE

INTRODUCTION

The hill lands of Western Oregon are well suited for the production of subclover (Trifolium subterraneum). Subclover is a winter annual which is quite productive when supplied with adequate nutrients. Sulfur is an important plant nutrient which often limits subclover growth, since many Western Oregon soils are low in this element.

This study was conducted to evaluate the influences of sulfur fertilization upon the yields of subclover-grass pastures and the quality of the forage produced. Sulfur is required for plant and animal growth. It is therefore important to know if the amount of sulfur required for maximum plant growth is the optimum level needed by the ruminant animal consuming that forage. Since both nitrogen and sulfur are required for the synthesis of protein, an inadequate amount of sulfur may limit this process in the animal even though that level of sulfur produced maximum forage yields. Sulfur is also needed for symbiotic fixation of nitrogen by the subclover plant. If applications of sulfur fertilizer beyond that quantity needed for the maximum yield of dry matter change the quality of the forage to the ruminant animal, then it is of considerable agronomic importance to be aware of these changes.

In evaluating the effects that sulfur fertilization has upon the nutritional quality of subclover-grass forage, consideration was given to the following facets in this study:

(1) The rate of sulfur required for maximum forage production.

(2) Changes in species composition with the application of sulfur.

(3) Increases in suflur content of the plant species.

(4) The effect that sulfur has upon nitrogen fixation and resultant nitrogen content of the forage.

(5) Changes in the nitrogen to sulfur ratio with increased levels of sulfur fertilizer.

(6) The influence that sulfur has upon the digestibility of the forage as measured by the in vitro technique.

REVIEW OF LITERATURE

Subclover-Grass Pastures in Oregon

Much of the agricultural land in Western Oregon is suited only to restricted types of production. Land capability classifications describe the capabilities and limitations of the hill lands found in Western Oregon (Hackensmith and Steele, 1949). Much of this type of land is not suited for cultivated crops, but is quite well suited to varying degrees of grazing by livestock. There are many acres which, under sound management, can produce a substantial quantity of forage when planted to improved grass-clover pasture (Dawson and McGuire, 1972).

Subterranean clover (Trifolium subterraneum), commonly called subclover, originated in the Mediterranean area and the Near East. Morley (1961) estimated that subclover had been established on 10 million acres in Australia at that time, with a future potential of satisfactory production on 40 million acres. Subclover is a winter annual which is well adapted to dry summers and relatively warm moist winters. The area west of the Cascade mountains in Oregon and California has these climatic conditions (Rampton, 1952; Williams, Love and Berry, 1957). The subclover plant produces a small inconspicuous inflorescence which contains from three to seven florets. Once flowering and fertilization has taken place, a

bur containing the seed forms. The peduncle then elongates toward the ground. This process in conjunction with the bristles of the bur give subclover a high tolerance to heavy grazing in a permanent pasture situation. Subclover also has the distinct advantage of being especially tolerant to acid soils (Heath et al., 1973).

Growth of a productive subclover-grass pasture requires a good supply of nitrogen, phosphorus, and sulfur (Dawson and McGuire, 1972). The supply of these nutrients may be derived from the soil, fertilizers and biological fixation of nitrogen. In a pasture situation, all these sources may be utilized.

The productivity of a permanent pasture involves the inter-relationship of soil, plant and animal factors. This may have a distinct advantage over a crop which is removed from the field at harvest time. In the pasture situation, the natural nitrogen and sulfur cycles may be taken advantage of to conserve nutrients and minimize fertilizer inputs (Dawson and McGuire, 1972).

The productivity of a grass-clover pasture can easily be limited if adequate amounts of essential nutrients are not supplied. A plant's requirement for a nutrient can be defined as the amount of that nutrient which is needed for the maximum yield of dry matter (Thompson, Smith and Moore, 1970). This amount may vary considerably among plant species.

Nutrient requirements are influenced by two relationships.

There is the relationship between plant growth and the nutrient supplying power of the soil. A relationship also exists between plant growth and the amount of nutrient taken up and distributed within the plant. McLachlan (1975) has shown that the amount of an element which fulfills these requirements may vary among species.

Role of Sulfur in Plant Growth

Sulfur Sources

Sulfur deficiencies are occurring on agricultural lands with greater frequencies in many parts of the world (Burns, 1968). There are several reasons for this greater occurrence of sulfur deficiencies in agricultural production. First, modern trends in the fertilizer industry have been towards the use of high analysis fertilizers. These fertilizers are either devoid or of very low in sulphur content. Secondly, with the increased use of natural gas and a decrease in the use of high sulfur containing coals, less sulfur is being released into the atmosphere (Coleman, 1966). Since some of this atmospheric sulfur would eventually be added to the soil by rain, another substantial source of sulfur for plant growth has been reduced. Lastly, with greater yields resulting from modern technological advancement, more sulfur per acre is in demand to meet plant growth requirements (Burns, 1968).

The total amount of sulfur in the earth is relatively small, comprising only 0.06% of the earth's crust. The ultimate source of soil sulfur is from metallic sulfides of plutonic rocks (Tisdale and Nelson, 1975). In so far as plants are concerned, the "soil sink" for sulfur is the organic matter. This supply of organic sulfur must be broken down microbially before it can be taken up by plants (Burns, 1968). Almost all suflur is taken up by plants in the sulfate form except for plants grown on flooded soil conditions such as rice. These plants may take up sulfur in more reduced forms (McLachlan, 1975).

The sulfur content of soil organic matter is relatively uniform as indicated by a fairly constant nitrogen to sulfur ratio of 10:1.2 to 10:1.5. This steady state of equilibrium has implications relevant to the importance of sulfur in the formation and decomposition of soil organic matter (Tisdale and Nelson, 1975). Often less than 10% of the total soil sulfur is present as available or adsorbed sulfate (McLachlan, 1975) and, even though sulfur may be present, plant deficiencies may be apparent due to an inability to utilize the sulfur present.

Role of Sulfur in Plants

Sulfur is required in a number of plant compounds occurring in both organic and inorganic forms (McLachlan, 1975). Sulfur is

required for the production of the amino acids: cystine, methionine and cysteine (Tisdale and Nelson, 1975) and these three sulfur containing amino acids make up 90% of the total sulfur content in the plant (Allaway and Thompson, 1966). These sulfur amino acids are used to synthesize essential proteins (Tisdale and Nelson, 1975), each of which is composed of a very specific amount, type and arrangement of amino acids (Salisbury and Ross, 1969). Proteins are required for two vital functions by living organisms. First, proteins make up catalytic proteins (enzymes) which catalyze biosynthetic and catabolic reactions for the growth, maintenance and development of cells. Second, certain proteins make up the structure of various cellular membranes (McLachlan, 1975). Because sulfur is a constituent of many different plant compounds, it is an essential element for plant growth. Some of the biochemical systems in which sulfur becomes involved include (Allaway and Thompson, 1966; Jones, Oh and Ruckman, 1972; Tisdale and Nelson, 1975):

1. Synthesis of sulfur containing amino acids (cystine, methionine, cysteine) and the proteins formed with these.
2. Enzyme systems and catalysts (papainases).
3. Constituents of certain vitamins (thiamine, biotin), coenzyme A and glutathione.
4. Electron carriers (cytochromes).
5. Sulfur is present in certain plant oils such as mustard, onion

and flax.

6. Disulfide (S-S) bonds which have recently been associated with the structure of protoplasm.
7. Sulfhydryl (S-H) bonds.
8. Sulfur also plays an important part in the nitrogenase enzyme, associated with the fixation of nitrogen by rhizobia.
9. A part of the biocompound ferredoxin, a constituent in the photosynthetic light reaction.

Of the many compounds which contain sulfur, there are a few which are required for normal functioning of the cell (Thompson et al., 1970). These include: the sulfur amino acids, (cystine, methionine, cystiene), glutathione, S-adenosyl methionine and the cofactors thiamine-pyrophosphate, biotin, lipoic acid and coenzyme A. There are also some secondary sulfur-containing plant compounds which presently are not thought to play important metabolic roles (McLachlan, 1975). However, these compounds may have an agronomic importance. They may directly or indirectly impart vile odors, toxic properties, pleasant flavors and tantalizing odors to animal and human foods. Nearly all of the methionine, cystine and cysteine are contained in proteins (Allaway and Thompson, 1966). These proteins have a significant effect upon the biological value of the plant produced. In the evaluation of protein quality, both the supply of amino acids and their relative amounts are of importance (Maynord

and Loosli, 1969). Since much of the world is consuming a diet deficient in methionine, it is of value to know that protein quality may be limited by the lack of sulfur amino acids (Tisdale and Nelson, 1975). Methionine has some unique properties which make it a very important biochemical compound. Methionine, usually in the form of S-adenosyl methionine (McLachlan, 1975), is used as a methyl donor directly or indirectly in many reactions. The methyl group of methionine is involved in the biosynthesis of plant lignin, pectin, chlorophyll and flavoids. Methionine is also a needed substrate for the synthesis of thiamine and ethylene. The other sulfur containing amino acid present in a relatively large abundance is cystine. Cystine is an essential metabolic intermediate in the synthesis of glutathione and Coenzyme A. Coenzyme A is a cofactor in virtually all the reactions involving fatty acids.

A deficiency of sulfur results in plant symptoms which include retarded growth, chlorosis and stunted thin stems. These symptoms are much like those of nitrogen deficiency (McLachlan, 1975; Tisdale and Nelson, 1975). Sulfur deficiency symptoms as observed in subclover (McLachlan, 1975) seem to start with chlorosis of the younger leaves then spread to the older leaves. Apparently suflur is translocated only to a very limited extent from older to younger plant parts as a deficiency occurs. Sulfur movement is much more restricted than nitrogen or phosphorus, thus making the younger

leaves the best indicator of plant sulfur status (McLachlan, 1975).

Plant Response to Sulfur Fertilization

Methods commonly used to determine the need for sulfur fertilizer application include soil testing and chemical analysis of the plant tissue. Soil tests have been used in other areas of the country to determine the sulfur status of the soil (Fox et al., 1964; Mays, 1974). At the present time, the use of a soil test is not considered to be the most reliable means of assessing the amount of plant available sulfur in western Oregon soils.

Sulfur is a cyclic element and acts much like nitrogen. The amounts available to plants can vary greatly from time to time under specific conditions. The bulk of soil sulfur is contained in the organic matter and must undergo mineralization by soil microorganisms before it can become available for plant growth. The rate of sulfur mineralization depends upon environmental conditions just as nitrogen mineralization does. Sulfur availability may fluctuate widely with changes in moisture, aeration, temperature, and soil pH (Buckman and Brady, 1969). Because of the fluctuation of sulfate levels in the soil, often a more reliable evaluation of plant sulfur needs may be made from a plant chemical analysis.

To predict the need for sulfur fertilization, the level of sulfur in the plant which constitutes a sufficient supply must be known.

This critical level varies widely with plant species. Fox et al. (1974) lndicates alfalfa grown under adequate sulfur fertilization will contain from 0. 22 to 0. 25% S on a dry weight basis at 1/10 bloom. The critical level used in predicting the need for sulfur was 0. 22% for alfalfa at the 1/10 bloom stage of growth. Pumphrey and Moore (1965a) have shown that plants with a sulfur level less than 0. 22% responded to sulfur application. The critical level reported for maximum growth of sugar cane is approximately 0. 05% (Stanford and Jordan, 1966) while the critical amount for white clover is 0. 26% (McNaught and Chrisstoffels, 1961); the need for Italion rye grass was best defined by 100 ppm sulfur on a dry weight basis in leaf blade tissue (Ulrich and Hylton, 1968).

Under field conditions the benefits from sulfur fertilization are often reflected in yield increases as well as a change in the chemical constituents of the plants grown. Harward, Chao and Fang (1962) indicate that on soils where sulfur application to alfalfa increased yields, the sulfur concentration in the plants also increased. The form of sulfur applied will also have a bearing upon availability to the plant as well as the residual effects. Two common forms of sulfur used in fertilizers are sulfates and elemental sulfur. The sulfate fertilizers are immediately available to the plant (Buckman and Brady, 1969) while elemental sulfur must undergo microbial oxidation in order to become available to the plant (Starkey, 1966).

Gypsum and elemental sulfur applied to clover grass pasture at the rate of 40 lbs of sulfur per acre increased yields 100% the first season (Jones and Ruckman, 1966). The second season the elemental sulfur application gave the better yield. This experiment showed that 40 pounds of sulfur per acre, applied as gypsum, supplied adequate sulfur for subterranean clover and grass pasture production during the first season only. The same rate of sulfur applied as elemental sulfur as sufficient for 2 years. Jones (1964) reported that, when applying sulfur in the gypsum form, the maximum yield of subclover, rose clover and Harding grass was obtained with 40 pounds of sulfur per acre the first year. The 20 pound application of gypsum gave approximately the same yield the first year as the 80 pound rate did the second year.

In addition to yield increases which have been observed with sulfur fertilization, the chemical composition also changes. One of the most prominent changes in chemical composition is the change in plant sulfur and nitrogen content (Harward et al., 1962). These two elements are closely associated due to their requirements for protein synthesis (Thompson et al., 1970). Environmental factors may influence the sulfur amino acid concentration in the plant by influencing the kind of protein being synthesised (Allaway and Thompson, 1966). The external supply of sulfur and the genetics of the plant also influence the sulfur amino acid concentration. When

When sulfur is applied to deficient plants, the change in sulfur concentration is largely due to the increased methionine and cystine in the plant (Tisdale et al., 1950; Saalbach, 1966). It is also interesting to note that plants differ genetically in their ability to produce these sulfur amino acids. Tisdale et al. (1950) have shown that two strains of alfalfa vary considerably in their ability to synthesize methionine and cystine under the same external sulfate concentrations. On sites where alfalfa responded to sulfur application in terms of yield, there was a corresponding increase in plant nitrogen and sulfur. Harward et al. (1962) found that applying gypsum to alfalfa increased the yield and the nitrogen and sulfur concentrations in the plants. In the same experiment it was noted that plant nitrogen and sulfur were greater at the early bloom stage compared to more mature plants. In order to evaluate the sulfur status of the plant, using plant analysis, a specific stage of maturity must be stipulated.

An important change that takes place with the application of sulfur fertilizer is that of plant species composition. The first year after applying 40 pounds of sulfur per acre to subclover-grass pasture, the yield was increased from 3675 to 6976 pounds of dry matter per acre; at the same time the clover content of the stand rose from 30 to 70% (Adams, 1973). Competition from the grass is one reason for a low clover stand at lower soil sulfur levels. If sulfur is inadequately supplied to a grass-clover pasture, the amount of clover will

decline (Dawson and McGuire, 1972; Tisdale et al., 1975). In order for clover to be competitive with grasses, higher levels of sulfur are required (Mays, 1974). Walker and Adams (1958) report a luxury consumption of sulfur by grasses. In the absence of applied sulfur 98% of the total sulfur uptake was by the grass. Jones (1964) reports that where maximum yields were obtained from 40 pounds of sulfur per acre applied as gypsum to subclover-grass pasture, the ratio of clover to grass increased up to the 80 pounds of sulfur per acre rate. In this case there was a small increase in plant sulfur concentration while there was a considerable increase in sulfur uptake per acre due to the increased yield of the subclover. Generally, in a grass-clover pasture, sulfur is applied to meet the needs of the legume. Under these conditions, the sulfur supply for the grass appears to be adequate (McLachlan, 1975).

A very important result of sulfur fertilization to a grass-legume pasture is the resultant increase in nitrogen fixed by the legume. Adams (1973) showed that in a four year trial, where 88 kilograms of sulfur per hectare was applied as gypsum, the average nitrogen yield increased 97% and the average dry matter production increased 44% over the control. When insufficient sulfur is available to the clover, this has the indirect effect of depressing nitrogen fixation (Walker and Adams, 1958). Sulfur not only affects nitrogen fixation through the health and well being of the legume, but is also required

directly by rhizobia (Tisdale and Nelson, 1975). Sulfur is a constituent of the nitrogenase enzyme system (Eady and Postgate, 1974; Israel et al., 1974; Tisdale and Nelson, 1975) as well as a constituent of other proteins required for biochemical reactions by the nitrogen fixing bacteria.

White clover yields were increased with the application of sulfur whereas yields of the companion grass declined (Jones and Ruckman, 1966). Under these conditions both nitrogen and sulfur were limiting. The legume however, was able to overcome these deficiencies by the application of sulfur. The clover portion of the stand increased from 12% in the control to 59% and 62% for the gypsum and elemental sulfur treated plots the first year. Dawson and McGuire (1972) estimated that subterranean clover could produce 6000 pounds of dry matter per acre. This would reauire 180 pounds of nitrogen. If the soil contained 0.2% nitrogen, mineralization could provide 50 pounds of plant available nitrogen. Effectively nodulated subclover would thus symbiotically fix 150 lbs of nitrogen per acre. The combination of these two sources of nitrogen would more than provide the needs for subclover growth. In fact, nitrogen will become available to the companion grass through underground transference from the clover roots (Jones and Ruckman, 1966; Adams, 1973). When sulfur was applied to a grass-clover pasture, Jones and Ruckman (1966) observed that total yield, nitrogen content and amount of clover in the stand all

increased the first year. The second year there was a significant increase in the grass yield, suggesting that additional nitrogen became available to the grass as a result of fixation by the legume. The grass usually will not respond to added sulfur until excess nitrogen is fixed by the clover (Jones, 1964). However, once nitrogen becomes available to the grass, these species then become more vigorous competitors for the available sulfur (Dawson and McGuire, 1972). If sulfur application is not continued at a rate needed for the legume, the grass may again force the clover out of the stand (Dawson and McGuire, 1972). Subclover appears to have a much higher requirement for sulfur than the grass species (Jones, 1964).

Nitrogen and Sulfur Relationships in Plants

When sulfur is applied to alfalfa there are changes that take place in plant constituents which are apparent in the chemical analysis of the tissues. Pumphrey and Moore (1965b) pointed out that among the changes that take place with sulfur application are yield, nitrogen content, and sulfur content. Harward et al. (1962) noted that the nitrogen and sulfur contents changed with stage of maturity making it difficult to determine the plant sulfur status based on the total sulfur content. The sulfur content of plants may be affected by plant species, stage of maturity, sulfur status of the soil and season (Mays, 1974). The ratio of nitrogen to sulfur is more consistent

than either total nitrogen or total sulfur. Pumphrey and Moore (1965b) observed that the nitrogen to sulfur ratio remained relatively constant during the first cutting with this ratio being narrower in the sulfur fertilized plots. They concluded that unfertilized alfalfa had a nitrogen to sulfur ratio of 17:1 for all stages of growth. This close relationship of nitrogen to sulfur in living tissue can be attributed to proteinaceous material and its content of sulfur amino acids. The amount and sequence of amino acids that are required for the synthesis of any particular protein molecule is determined by the genetics of the plant (Thompson et al., 1970). Therefore the amount of sulfur containing amino acids that are found in the plant should vary only with a change in the amounts of specific proteins. When sulfur is deficient for plant growth, practically all of the plant sulfur is in the form of proteins (Williams et al., 1957). When sulfur is limiting protein formation, nonprotein nitrogen will accumulate, resulting in a widening of the nitrogen to sulfur ratio (Thompson et al., 1970). High nitrate levels in plants have been found to be associated with a wide nitrogen to sulfur ratio by Tisdale and Nelson (1975). When sulfur is more than adequate or nitrogen is limiting, then nonprotein sulfur accumulates narrowing the nitrogen to sulfur ratio. Much of the nonprotein sulfur in plants is in the form of sulfate (Dijkshoorn, Lampe and Van Burg, 1960; Thompson et al., 1970). Fertilization of wheat with sulfur has resulted in increased levels

of both nitrogen and sulfur in the wheat plants (Steward, Porter and Viets, 1966). Spencer (1959) reported that sulfur application to white clover increased sulfur content as well as the amount of protein nitrogen. In nonfertilized plots, protein sulfur accounted for the bulk of the plant sulfur, while in fertilized plots increasing amounts of organic nonprotein sulfur and sulfate were present (Spencer, 1959). With such a relationship existing between nitrogen and sulfur in plant tissues, the N:S ratio has been used as a tool to evaluate the sulfur status of the plant (Dijkshoorn et al., 1960). The critical N:S ratio will differ for plant species. Pumphrey and Moore (1965a) using critical N:S ratio of 11:1 for alfalfa, found that above this ratio plants would respond to sulfur fertilization while plants with N:S ratios below this did not respond to sulfur application. The N:S ratio of wheat was 14:1 in situations where sulfur was not limiting (Stewart et al., 1966). Dijkshoorn and Van Wijk (1967) reported that legumes have a N:S ratio of about 17:1 while the ratio in grasses is about 14:1.

Sulfur in Ruminant Nutrition

When evaluating sulfur in ruminant nutrition, there are two distinct areas which must be studied. First, the animal needs a supply of sulfur for its life functions. If inadequate amounts are available in the diet, there are definite effects on animal performance (McLachlan, 1975). Secondly, sulfur affects the plant quality

which in turn affects the animal (Tisdale and Nelson, 1975). The ruminant has different dietary requirements than a monogastric animal due to the population of microorganisms contained in the rumen. The ruminant can derive all of its essential organic nutrients from a diet which would be inadequate to a monogastric animal. A wide and diverse genetic potential of the rumen microorganisms widens the range of materials digested. This adds to the synthetic capacity of the ruminant system as a whole (Moir, Somers and Bray, 1967).

Sulfur plays an important metabolic role in ruminant nutrition because it is a constituent of cystine and methionine which are needed for protein production as well as being needed for the synthesis of the vitamins, biotin and thiamine, along with many important enzymes (Elam, 1975). Many important biochemical processes which occur in the animal body involve sulfur containing compounds. Sulfur is part of structural entities, such as collogen, which is the basis of muscle structure and the most widely distributed protein in the animal body (Ziegler, 1968). Sulfur is also a constituent of oxygen carriers (cytochromes) and hormones, such as insulin (Allaway and Thompson, 1966).

Sulfur Deficiency

A deficiency of sulfur in the animal's diet will have immediate

effects upon the rumen microbes with further resultant effects upon the animal itself (McLachlan, 1975). Sulfur deficiency has been found to decrease rumen activity, having the following consequences:

1. Microbial protein synthesis is reduced. As a result, less dietary nitrogen, especially nonprotein nitrogen, is utilized. Blood urea levels increase and more urea is excreted in the urine. The nitrogen retention declines.
2. Less organic matter is digested in the rumen, decreasing the energy retention.
3. Feed intake also declines.
4. Inhibition of the acrylate pathway preventing conversion of lactate to propionate occur when there is a high concentration of carbohydrates in the diet.

Sulfur Utilization

Sulfur amino acids are the primary source of sulfur in animal nutrition (Allaway and Thompson, 1966). However, in the case of ruminants this need not be the only source of sulfur. The rumen microbes are capable of utilizing other forms of sulfur to fulfill the requirements of the animal (Albert et al., 1956), but animals are considered to be unable to reduce sulfate (McLachlan, 1975). In contrast, plants and many bacteria, including some of which are found in the rumen, are able to reduce sulfate to sulfide. This has

been proven by their ability to grow when sulfate is supplied as their only source of sulfur (Peck, 1970). Sulfur in many chemical forms is reduced to sulfide by the microorganisms in the rumen (McLachlan, 1975), and once reduced to sulfide, various processes may occur. The sulfide may be used to synthesize microbial protein or absorption of sulfide may occur through the rumen wall or the small intestine and enter the blood. This sulfide is oxidized by the liver to sulfate which is then excreted. This reaction is important in that sulfate is much less toxic than sulfide (L'Estrange, Upton and McAleese, 1970). The ruminant animal receives a large part of its sulfur amino acids from microbial protein rather than dietary sources as in the case of the monogastric animal (McLachlan, 1975).

Sulfur deficiency symptoms have been most noticeable when a low quality roughage has been fed (Rural Research, 1972). In a study with spear grass (Heteropogon contortus), a nutritional deficiency of some type was apparent during winter months when the crude protein content of the feed dropped to a low of 3%. Addition of urea to the diet of sheep and cattle did not increase the utilization of spear grass. The energy intake still remained below the maintenance level for these animals. When sulfur was added with urea, the utilization of spear grass increased to the point where energy intake was above the maintenance level. It appears that both nitrogen and sulfur were needed to overcome the deficiency. This

supplementation increased the feed intake of both sheep and cattle, but the digestibility of the spear grass increased only with the sheep (Kennedy and Siebert, 1971). This is explained by the fact that cattle are more efficient in recycling sulfur back to the rumen than are sheep (Bird, 1974). Also, sheep have a relatively greater need for sulfur in the diet due to the needs of wool production. Kennedy (1974) reported that sulfate supplementation to rations of nonlactating cattle fed tropical grasses would be of little benefit unless a nonprotein nitrogen source of nitrogen was provided in addition. Bird (1974) has shown that the supplementation of wheat straw with urea and sulfur as Na_2SO_4 increased intake of energy, efficiency of digestion and live weight gains of sheep. The fiber digestion of oat hulls was increased by supplementation with sulfate and urea (Bray and Hemsley, 1968) and there is also a greater retention of both nitrogen and sulfur by the animal. Sulfur recycling seems to be important for those animals consuming poor quality roughage (Hume and Bird, 1969). Increased sulfate and decreased nitrogen in the saliva have been apparent with sulfur supplementation (Bray and Hemsley, 1968). Sulfate content of the saliva as well as the level of sulfide in the rumen will decrease when the sulfur level of the diet is reduced (McLachlan, 1975). Sulfur supplementation to the ruminant diet supplies sulfur needed by the microbes to synthesize protein.

The exact amount of sulfur which is required for the ruminant

is not known (Elam, 1975) because different species will have different requirements, and these requirements change with physiological age. The ability to measure requirements is also complicated by the methods of evaluation. These may include:

1. In vitro versus in vivo techniques.
2. Natural versus purified diets.
3. The source of sulfur, along with the nitrogen content affecting the nitrogen to sulfur ratios.
4. Other plant factors which are affected by sulfur content.

McLachlan (1975) indicates that a response to sulfur supplementation is not expected if the ruminant diet contains more than 0.1% sulfur. Estimations of the sulfur requirements of dairy cows are 0.15 to 0.20 percent of the dry matter intake and 0.25% of dry matter is suggested as a recommended rate of supplementation (Bull, 1971). The sulfur needs of calves fed a purified diet containing urea were met with 0.3% elemental sulfur were fed with no deleterious effects (Chalupa et al., 1971).

The amount of sulfur supplementation may vary with the source of sulfur. Fattening lambs on a purified diet containing 4% urea were supplemented with methionine, Na_2SO_4 and elemental sulfur (Albert et al., 1956). Seventy percent less sulfur was required as methionine than as elemental sulfur and 50% less Na_2SO_4 as compared to elemental sulfur. Protein production by the rumen microbes was

not influenced by the form of sulfur supplementation (Hume and Bird, 1969). The sulfur amino acids are by far the primary source of sulfur in animal nutrition (Allaway and Thompson, 1966).

There is little evidence that methionine is required as an essential nutrient by rumen microorganisms, though it appears that cystine is obligatory for many rumen microbes (Moir et al., 1967). Microorganisms must be supplied with a sulfur source but cystine may be synthesized. The microbes are able to derive methionine from cystine, however, the reverse does not occur. Therefore, it is believed that a supplementation of methionine as the only source of sulfur would create a deficiency of cystine (Moir et al., 1967).

Influence of Sulfur upon Forage Quality

Since sulfur is present in plant protein in a fairly definite amount, determined genetically, the animal is somewhat protected from extreme sulfur deficiency. The sulfur content of the plant being consumed may not be optimal for animal growth, but sulfur deficiencies are not as dramatic as for other elements (Allaway, 1970). On the other end of the scale, rumen microorganisms have a very high tolerance to sulfur toxicity (Kennedy, 1974).

The sulfur status of the plant is quite an important link in supplying the animals' needs for this element. When fertilized with sulfur, the total sulfur content of the plant increases somewhat more

than does the sulfur amino acid content (Allaway and Thompson, 1966). The ruminant animal is fortunate in being able to use the total sulfur content of the plant. Sulfur fertility levels also influence other important growth facets of the plant. Forages low in sulfur are generally high in lignin and fiber, and low in protein and soluble carbohydrates. These forages are low in digestibility and it is unlikely that the energy intake would be sufficient even if sulfur was supplemented (McLachlan, 1975). Experimentation with Pangola grass showed that sulfur fertilization increased sulfur content, dry matter intake and digestibility. Conclusions suggest that the improvement in nutritional quality through sulfur fertilization may be more beneficial than simple sulfur supplementation of the ration (Rees, Minson and Smith, 1974).

The value of forage is often greatly influenced by the protein content. The crude protein content has traditionally been calculated by multiplying the total plant nitrogen by 6. 25 (Crampton and Harris, 1969). This value may be a good indication of forage quality to the ruminant because of the microbial conversions of all nitrogenous compounds. Tisdale et al. (1950) presented data which show that, with alfalfa grown under non-sulfur fertilized conditions, the sulfur content, methionine and cystine levels were the lowest at the highest nitrogen content. The importance of the nitrogen to sulfur relationship should be considered, in the evaluation of forage quality.

Allaway and Thompson (1966) suggest that at maximum plant growth rates, there may be suboptimal levels of sulfur for the ruminant. In this respect, sulfur fertilization beyond the needs of the plant would possibly increase the value of the forage to the animal in addition to the benefits of increased yields.

Under sulfur deficiency situations, nonprotein nitrogen will accumulate in some forages (Odelien, 1963). Non-legumes which are supplied with abundant nitrogen will accumulate nitrites, amides and nitrates in large quantities (Tisdale and Nelson, 1975). Large amounts of nitrates are toxic to ruminant animals (Crampton and Harris, 1969).

Nitrogen to Sulfur Ratios

High nitrate concentrations in plants are associated with wide nitrogen to sulfur ratios (Tisdale et al., 1950). Allaway and Thompson (1966) indicate that the optimum N:S ratio for plant growth is above the 10:1 to 15:1 N:S ratio which is thought to be optimum for the ruminant. The N:S ratio in the body tissue is 15:1 (Pund, 1969). Rumen bacteria also have a nitrogen to sulfur ratio of 15:1 (Moir et al., 1967). Davis, Williams and Loosi (1954) suggested that a N:S ratio of approximately 15:1 is adequate for the ruminant. Also, unless a forage is grown on a soil deficient in sulfur, there is little chance of a sulfur deficiency when nonprotein nitrogen is added in

amounts up to 3% of the concentrate in the ration. In evaluating the amount of sulfur needed in a dairy ration, the requirements for milk production must also be considered. Milk contains 0. 20% sulfur mostly in the form of methionine and cystine. In order to meet these demands, dietary sulfur should not be less than 0. 13% with a N:S ratio of 12:1 (Conrad and Bouchard, 1973). If an animal consumes a diet with a N:S ratio which is too wide, the animal compensates for this by wasting nitrogen (Allaway, 1970). This is a primary concern with sulfur deficient feeds. Rations containing urea should have a N:S ratio of slightly narrower than 10:1 (Pund, 1969). This is necessary for optimum use of the nitrogen (Moir et al., 1967). The use of urea or some other nonprotein nitrogen may widen the N:S ratio of the diet furthering the deficiency of sulfur. The results are suboptimal growth and productivity by the ruminant consuming this ration (Allaway, 1970). Even though ruminal protein has a N:S ratio of 15:1, for efficient use of urea at 40% of the dietary nitrogen, the N:S ratio of the ration should be slightly narrower than 10:1 (Moir et al., 1967).

The return of nitrogen and sulfur through the rumen wall and saliva is another factor which could influence the dietary requirements for nitrogen or sulfur. One reason that a narrower N:S ratio is required in the feed than in the rumen protein may be due to the difference in the amount of sulfur recycled as compared to nitrogen.

The ratio of recycled nitrogen to sulfur is from 70:1 to 80:1 Moir et al., 1967). Cattle require less dietary sulfur and can do better on a wider N:S ratio in the diet than sheep. This may be due not only to the greater demand for sulfur by sheep but also to an apparent more efficient recycling of the sulfur by cattle (Bird, 1974).

Forage Quality and In Vitro Digest Evaluation

Jones et al. (1972) have evaluated the effects that sulfur and phosphorus have on the digestibility of subclover. They found that 0.1% phosphorus and 0.05% sulfur were the critical levels required by the rumen microorganisms. The application of both elements increased the amount of reducing sugars and glucose in the plant. Application of either element singularly did not have this effect. Using in vitro fermentation procedures, there was an increase in digestibility with an increase in reducing sugars resulting from sulfur and phosporus application (Jones, Oh and Ruckman, 1970; Jones et al., 1972).

Nitrogen is quite often the most limiting factor in the utilization of feedstuffs (Oh, Longhurst and Jones, 1969). Both protein and nonprotein nitrogen are largely degraded to ammonia in the rumen. This ammonia is then used for microbial protein synthesis, which in turn serves as a source of amino acids for the animal (Moir et al., 1967). The microbial protein is of a relatively high biological value

(Church, 1975). A significant difference in dry matter and cellulose digestion has been observed with nitrogen fertilization (Reid and Jung, 1965). In this case the intake of feed did not significantly increase with nitrogen application. An increase in cellulose digestion has been observed by the addition of sulfur with in vitro methods (Bull and Vendersall, 1973), but no differences in digestibility due to the source of sulfur were observed. The in vitro digestion of starch was increased 32.5, 29.4 and 22 percent, respectively, with addition of inorganic sulfur, cystine and methionine to a basal sulfur-free ration (Kennedy, Mitchell and Little, 1968).

The digestibility of various plant components may differ among species. Rendig and Weir (1957) have shown that the digestibility of the crude protein in alfalfa is significantly higher than that of orchard grass. Also, the crude fiber of orchard grass was more digestible than that of alfalfa. These examples show that factors affecting changes in plant species and/or plant chemical constituents may cause quality changes in the forage to the ruminant.

The in vitro digestion procedure used in this research was highly correlated with actual in vivo digestion (Quicke et al., 1959; Oh, Baumgardt and School, 1966; Barnes, 1967; Mellenberger et al., 1970). In order to evaluate the digestibility as accurately as possible, it helps to obtain rumen fluid from an animal consuming the same

forage as will be digested in the in vitro procedure (Hinders and Ward, 1961).

MATERIALS AND METHODS

Field Experiment

A sulfur fertility experiment[1] was established in the fall of 1974 to evaluate the effects of various rates and forms of sulfur fertilizer upon subclover-grass pasture. The site of this experiment is identified as the SW 1/4, Sec 28, T 26 S, R 4 W, which is located approximately ten miles east of Roseburg in Douglas County, Oregon.

The soils at this site are a complex of the Nonpareil, Sutherlin and Oakland series. The Nonpareil series is a member of the loamy, mixed, mesic shallow family of Dystric Xerochrepts. This series typically has brown loamy A horizons and dark yellowish brown B horizons formed in colluvium and residuum weathered from tuffaceous sandstone, siltstone or shale. The Sutherlin series belongs to the fine-loamy over clayey mixed mesic family of Ultic Haploxeralfs. Typically this series has dark brown silty clay loam A horizons and brown silty clay loam Bt horizons abruptly overlying yellowish brown clay IIC horizons. The Oakland series is a member of the fine mixed mesic family of Ultic Haploxeralfs. It has dark brown loam A horizons and brown silty clay loam B2t horizons overlying sandstone

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[1] Experiment established by T. L. Jackson, Department of Soil Science and Wayne D. Mosher, Douglas County Extension Agent, Oregon State University.

and saprolite. The typifying pedons for these three series have been described (Appendix 1) by the USDA, Soil Conservation Service (1973).

These soils are present to a moderate extent in Douglas County, occurring on sloping uplands at elevations of 300 to 2, 500 feet. These areas have warm dry summers and cool moist winters with 30 to 50 inches of annual precipitation.

A soil sample was taken from the surface six inches of the experimental area in mid September, 1974. Table 1 shows the chemical analysis (Roberts et al., 1971) of this soil sample. The pH and SMP tests show that this soil is moderately acid, however, it is quite suitable for subclover. There are adequate amounts of Ca, Mg and K, but the level of P is low.

Table 1. Results of soil analysis

pH	SMP[1]	CEC	Ca	Mg	K	P
		---meq/100 g---			---ppm---	
5.8	6.3	14.1	7.9	2.2	250	16

[1] Lime requirement test

The site of this experiment had previously been planted to subclover and native grasses. Single super phosphate had been applied at the rate of 200 lb per acre every other year for the past ten years. During the past several years this subclover-grass

pasture had been grazed by sheep.

The field experiment was of the randomized block design, containing 24 treatments with five replications. Each plot consisted of a 6 x 20 ft area. The sulfur treatments along with a blanket application of 60 lbs of P_2O_5 per acre were applied October 10, 1974. Only the gypsum treatments and the check plots were selected to evaluate the effects of sulfur upon forage quality. The sulfate form of sulfur was chosen because a plant response could be expected the first year (Buckman and Brady, 1969).

The plots were harvested on June 4, 1975. At that time, the clover had formed burs and the grass seed heads were fairly mature. Plant samples were randomly taken by hand from each plot. Yield information was collected by mowing a 3 x 17 ft section from the center of each plot. The plant material was weighed and the dry matter (DM) yields were calculated using plant samples which were taken at harvest.

Plant samples were hand separated to obtain pure samples of subclover and grass. These samples, as well as a forage sample as taken in the field were then oven dried at 75° C, ground with a Wiley Mill to pass a 20 mesh screen and stored in manila envelopes for analysis.

Plant Sulfur Analysis

Plant sulfur was determined according to the procedure modified by D. T. Westerman (Appendix 2). One half gram samples were dry ashed after adding 3 ml of a saturated magnesium nitrate solution. The ash was dissolved in hydrochloric acid and the sulfur measured turbidimetrically as barium sulfate following the addition of barijm chloride.

Plant Nitrogen Analysis

Plant nitrogen was determined by the micro-kjeldahl method, similar to the method used by M. L. Jackson (Appendix 3). Three tenths of a gram of sample was digested with sulfuric acid and catalyst, distilled with the ammonia being captured in Boric Acid. Nitrogen content was then determined by titrating with hydrochloric acid.

Species Composition

The determination of the relative amounts of grass and clover in the total forage was calculated using hand separations and total plant nitrogen information. The relative amounts of each species can be calculated algebraically using the nitrogen contents of the grass, clover and forage since the grass and clover are the only two constituents of the forage.

In Vitro Digestibility

In vitro digestibility of the forage samples was done using a method similar to Mellenberger et al. (1970).

1) Inoculum:

Rumen fluid was obtained from a fistulated steer fed a diet of subclover-grass forage. The fluid was strained through 4 layers of cheese cloth and gassed with CO_2. It was then placed into a separatory funnel held at 39° C for one hour while it separated into three distinct layers. The middle layer was then used as the source of inoculum.

2) Buffer solution:

McDougall's nutrient buffer solution was used with the substitutuion of MgO for $MgSO_4$ as the Mg source (Appendix 4).

3) Plant samples:

0.5 g of plant sample was weighed into 50 ml screw cap fermentation tubes.

4) Inoculation and Digestion:

Fermentation tubes were inoculated with 35 ml of a 1:1 mixtuer of rumen fluid and buffer solution. The tubes were then gassed with CO_2, capped, shaken and placed horizontally into a 39° C water bath and incubated for 24 hours. Twice during the digestion period, the tubes were shaken and the excess gas released.

The digested plant material was filtered through a preweighed sintered glass Gooch type crucible. The crucible and its contents were then dried at 100° C and weighed. The dry matter contents of the inoculum were determined in a similar manner.

5) Calculations:

Percent Digestion =

$$\left[1 - \frac{\text{weight after digestion - D. M. in inoculum}}{\text{weight of plant sample}}\right] X\ 100$$

RESULTS AND DISCUSSION

The plant samples collected from the sulfur fertility plots were analyzed for total sulfur, total nitrogen and digestibility. Yield information was collected at harvest time and the species composition was determined using hand separations. The results of all analyses are listed (Appendix 5) in the order in which they are discussed below.

Yield

A significant response in dry matter yield response was not observed from the application of sulfur (Table 2). However, there was a significant change in species composition (Figure 1). The lack of a yield response with sulfur application indicates that this site was not deficient enough in this plant nutrient to limit forage yield. Past applications of sulfur fertilizer were probably responsible for the adequacy of sulfur on this site.

Species Composition

The significant species change that occurred (Figure 1) suggests that changes in forage quality may be made by applying amounts of sulfur beyond what is required for maximum dry matter production. This information strongly supports the hypothesis that the subclover and grasses differ in their requirements for sulfur (Jones, 1964).

Figure 1. Species composition

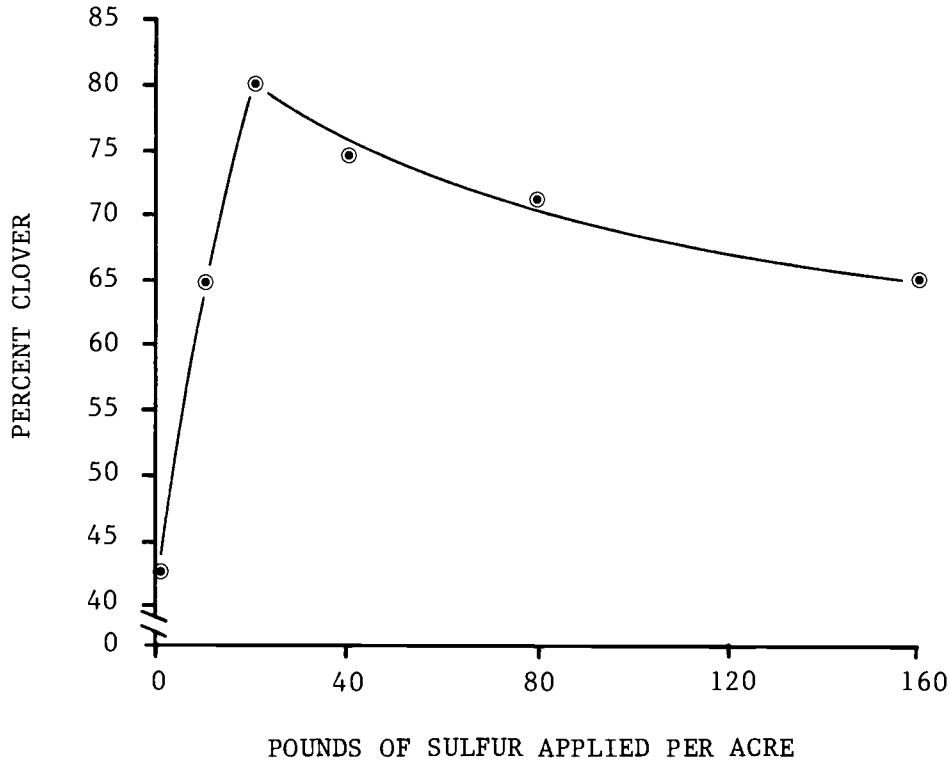

Table 2. Yield and species composition

Treatment	Mean Yield lbs. DM/Acre	Mean Percent Clover
0 S	4695	42.4
10 S	4679	64.4
20 S	4627	81.0
40 S	5233	73.8
80 S	5695	71.0
160 S	5006	65.0
ANOVA		
Error Mean Square	344062	216.867
F Value	2.5329	4.016*

Without the application of sulfur, the forage stand is dominated by the grass. In this experiment the clover made up 42 percent of the stand in the non-sulfur treated plots. The relative abundance of grass in the non-sulfur plots was probably due to the lower sulfur requirement of grass and also to a greater ability of grass to absorb sulfur compared to clover. In this situation, the clover was probably deficient in sulfur and less able to compete for other nutrients and therefore less productive.

When sulfur was applied at the rates of 10 and 20 pounds per acre the amount of clover in the stand increased to 64 and 81 percent respectively. Twenty pounds of sulfur applied per acre appears to fulfill the sulfur requirements of subclover at this location. As sulfur applications increased to 40, 80 and 160 pounds per acre, the percentage of subclover in the stand declined to 73, 71 and 65 percent respectively. This decrease in the amount of subclover was possibly due to the competitive effect exerted by the grass and could be explained on the basis that when 20 pounds of sulfur per acre or more were applied, the subclover biologically fixed nitrogen in excess of what was required for its own growth. The excess nitrogen became available to the companion grass which when supplied with additional nitrogen was more productive and more competitive with the subclover. This hypothesis was supported by a highly significant increase in the nitrogen content of the grass as the rate

of sulfur fertilization increased (Table 4).

Plant Sulfur Content

The application of sulfur fertilizer resulted in highly significant increases in plant sulfur content (Table 3). The sulfur content of the subclover was consistently higher than the grass at all levels of sulfur fertility (Figure 2). The subclover sulfur content also increased at a greater rate than did the sulfur content of the grass. When sulfur is applied to deficient plants the change in sulfur content of the plant is largely due to an increase in the sulfur amino acid concentration (Saalbach, 1966). Considering this fact, the differences in sulfur content between grass and clover may be explained by the differences in the ability of the plant species to synthesize sulfur amino acids (Tisdale et al., 1950).

Plant Nitrogen Content

The application of sulfur fertilizer resulted in a highly significant increase in the total nitrogen content of the grass and clover (Table 4). This increase in plant nitrogen can be attributed to biological fixation. Sulfur most likely stimulated an increase in nitrogen levels in two ways. First of all, when the sulfur requirements of the subclover are fulfilled, the plant is going to be more healthy and productive. Secondly, sulfur is required directly by the rhizobia

Figure 2. Plant sulfur

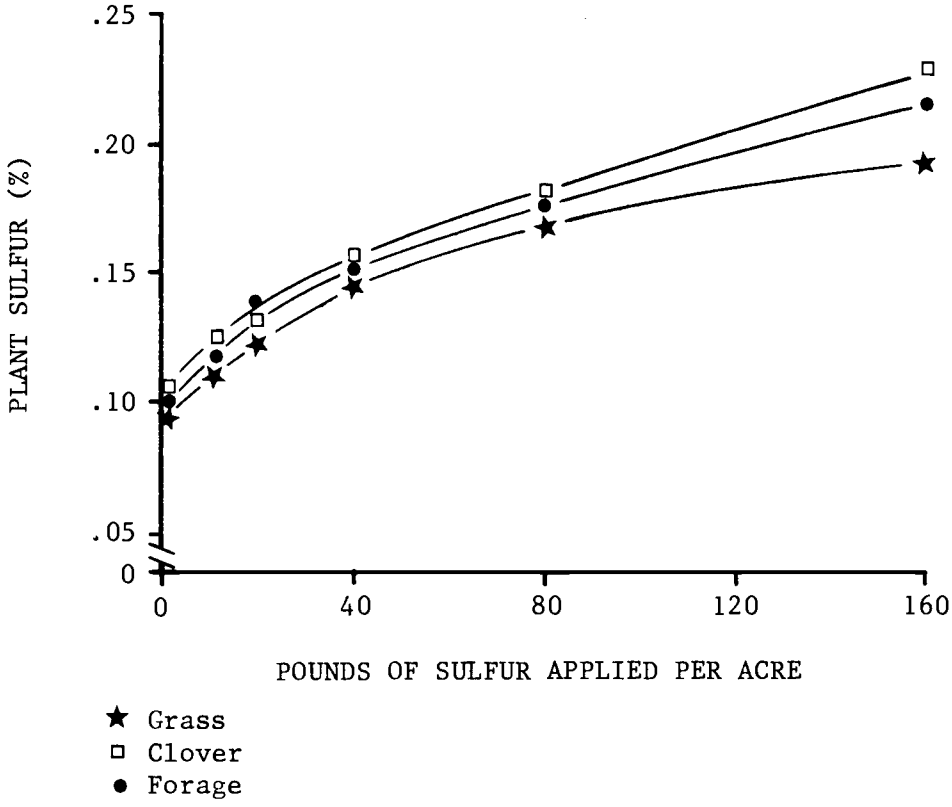

Table 3. Plant sulfur

Treatment	Mean Sulfur Content (%)		
	Grass	Clover	Forage
0 S	0.09	0.10	0.10
10 S	0.11	0.12	0.12
20 S	0.12	0.13	0.13
40 S	0.14	0.15	0.15
80 S	0.17	0.18	0.18
160 S	0.20	0.23	0.21
ANOVA			
Error Mean Square	0.0002756	0.0002796	0.0003007
F Value	31.2693**	40.7172**	30.7346**

Figure 3. Plant nitrogen

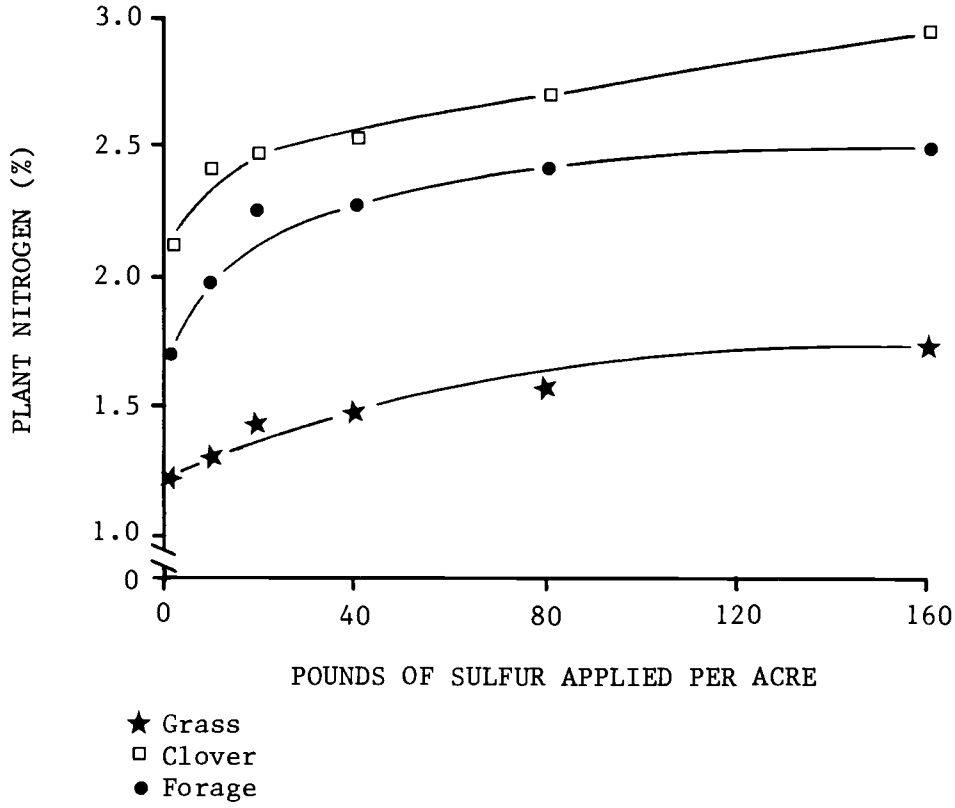

Table 4. Plant nitrogen

Treatment	Mean Nitrogen Content (%)		
	Grass	Clover	Forage
0 S	1.24	2.18	1.70
10 S	1.36	2.40	1.98
20 S	1.42	2.46	2.26
40 S	1.44	2.54	2.26
80 S	1.54	2.74	2.40
160 S	1.80	2.90	2.52
ANOVA			
Error Mean Square	0.03130	0.06180	0.03843
F Value	5.8190**	5.2611**	11.6288**

for the nitrogenase and other enzyme systems (Eady and Postgate, 1974; Israel et al., 1974). The subclover consistently had a higher nitrogen content than the grass at all levels of sulfur (Figure 3).

The nitrogen content in the clover increased much more with the first increment of sulfur than the grass. The slight lag in nitrogen content increase shown by the grass was probably due to the dependence of grass upon the nitrogen fixed by the clover. At lower sulfur levels, nitrogen was not fixed in amounts at which it would be available to the companion grass. Once adequate sulfur was available to the clover, more nitrogen was fixed biologically and the nitrogen content of the grass increased as an indirect effect of sulfur fertilization. The nitrogen content of the total forage (Figure 3) was a result of the amount of nitrogen in both the grass and clover as well as the relative abundance of each in the stand.

Plant Nitrogen to Sulfur Ratio

A highly significant decrease in the nitrogen to sulfur ratio took place when higher rates of sulfur were applied (Figure 4). The narrowing of this ratio could be attributed to the fact that the plant sulfur concentration increased at a faster rate than the plant nitrogen content. Without an application of sulfur, the nitrogen to sulfur ratio for clover and grass was quite wide (Table 5) and narrowed at a faster rate in clover relative to the grass with increased rates of sulfur

Figure 4. Nitrogen to sulfur ratio

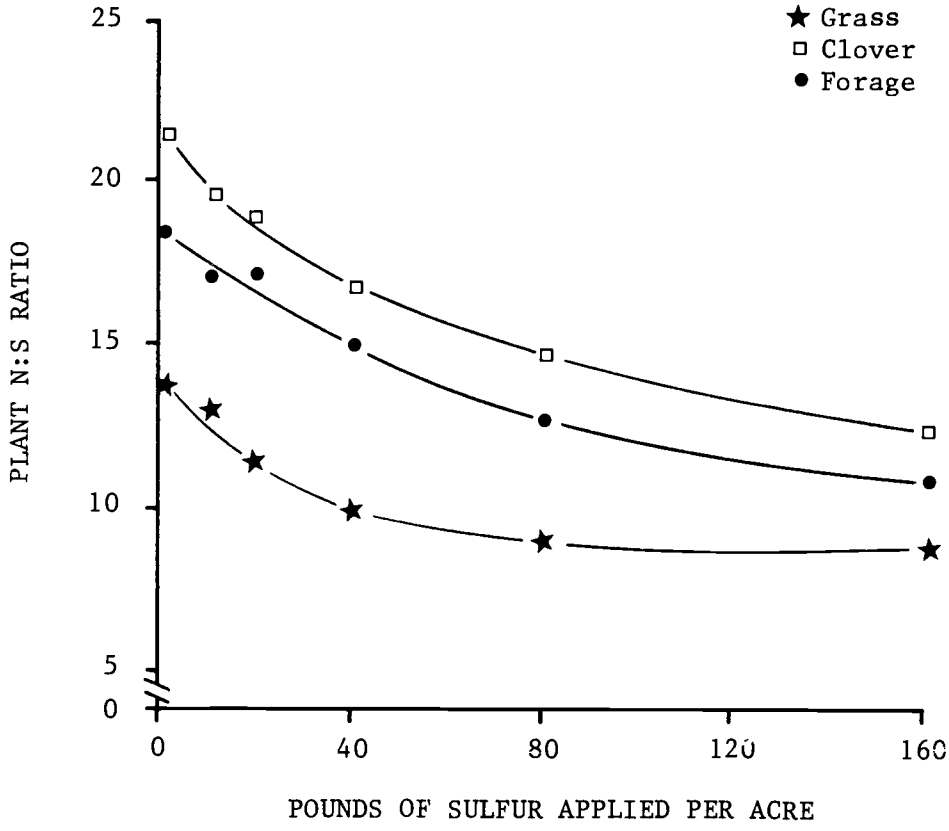

Table 5. Nitrogen to sulfur ratio

Treatment	Mean N:S Ratio		
	Grass	Clover	Forage
0 S	13.6	21.8	18.1
10 S	13.1	19.5	17.1
20 S	11.7	19.3	17.2
40 S	10.0	16.6	15.1
80 S	9.3	15.0	13.5
160 S	9.1	12.5	11.8
ANOVA			
Error Mean Square	2.97799	1.84293	1.64094
F Value	6.1446**	31.5187**	17.9751**

application.

At the 160 pound increment, the forage N:S ratio of 12:1 was within the range desirable for the ruminant animal (Davis et al., 1954; Pund, 1969). Supplying nitrogen and sulfur in an appropriate ratio should permit a more efficient utilization of the plant nitrogen.

In Vitro Digestibility

In this study subclover was consistently more digestible than the grass using the in vitro digestion procedure (Table 6). Although there was no significant difference in the digestibility of the grass or clover with the application of sulfur, the digestibility of the total forage did increase significantly (Table 5). It is quite likely that this trend was due to the increasing levels of nitrogen in the forage, along with a change in species composition. It is also interesting to note that the N:S ratio was narrower at the higher digestibility levels.

Figure 5. Forage digestibility

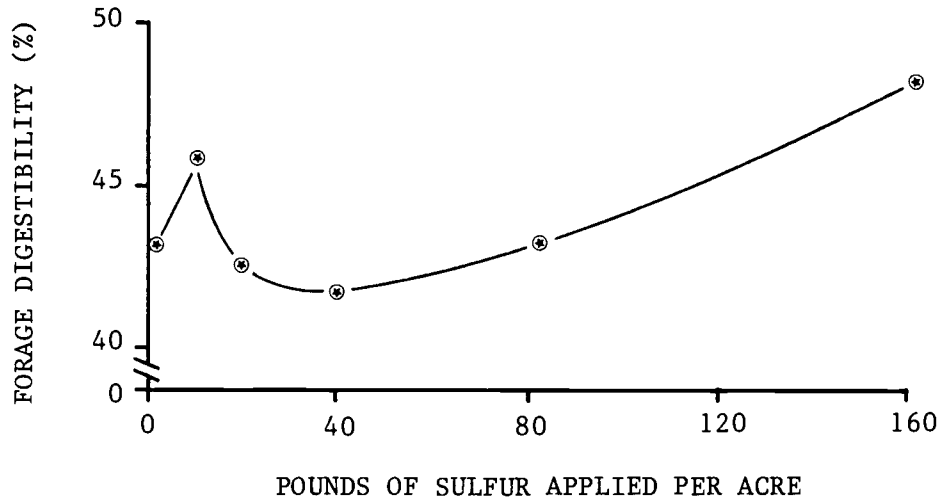

Table 6. In vitro digestibility

Treatment	Mean Digestibility (%)		
	Grass	Clover	Forage
0 S	37.7	50.5	43.6
10 S	37.8	51.3	45.9
20 S	34.6	48.9	42.6
40 S	33.6	46.9	41.9
80 S	34.8	47.9	43.4
160 S	34.1	50.4	48.1
ANOVA			
Error Mean Square	8.66583	13.8977	7.23617
F Value	1.9942	1.0786	3.8171*

CONCLUSIONS

This experiment has shown that the application of sulfur fertilizer to grass-clover pastures can have nutritional benefits to the ruminant animal. These benefits may be achieved by applications of sulfur in excess of that amount which shows a significant yield response. The nutritional quality of the forage was improved as a result of the following changes:

1. Although the total yield did not increase, the relative productivity of each species did change. Increasing the amount of subclover in the forage through sulfur fertilization was beneficial because this species is more digestible than the grass.
2. Sulfur application had a highly significant effect upon the nitrogen content of both the clover and the grass. The results indicate that the grass acquired additional nitrogen due to greater nitrogen fixation by the clover. When the grass obtained additional nitrogen, it then became more competitive with the clover.
3. The sulfur content of the forage increased resulting in a narrowing of the N:S ratio.
4. The application of sulfur significantly increased the digestibility of the forage as measured by the <u>in vitro</u> digestion procedure.

BIBLIOGRAPHY

Adams, A. F. R. 1973. Sulphur on New Zealand pastures - effect of rates and forms. Sulphur Inst. J. 9:14-16.

Albert, W. W., U. S. Garrigus, R. M. Forbes and H. W. Norton. 1956. The sulphur requirement of growing - fattening lambs in terms of methionine, sodium sulfate and elemental sulfur. J. Anim. Sci. 15:559-569.

Allaway, W. H. 1970. The scope of the symposium: Outline of current problems related to sulfur in nutrition In: O. H. Muth and J. E. Oldfield (ed.) Symposium: Sulfur in Nutrition. The AVI Publishing Co., West Port, Connecticut.

Allaway, W. H. and J. F. Thompson. 1966. Sulfur in the nutrition of plants and animals. Soil Sci. 101:240-247.

Barnes, R. F. 1967. Collaborative in vitro rumen fermentation studies on forage substrates. J. Anim. Sci. 16:1120-1130.

Bird, P. R. 1974. Sulphur metabolism and excretion studies in ruminants. XIII intake and utilization of wheat straw by sheep and cattle. Aust. Agric. Res. 25:631-642.

Bray, A. C. and J. A. Hemsley. 1968. Sulphur metabolism of sheep. IV. The effect of a varied dietary sulphur content on some body fluid sulphate levels and on the utilization of urea-supplemented roughage by sheep. Aust. J. Agric. Res. 20: 759-773.

Buckman, H. O. and N. C. Brady. 1969. The Nature and Properties of Soils. The Macmillan Company/Collier-Macmillan Limited, London.

Bull, L. S. 1971. Corn and corn silage rations may be low in sulphur. Sulphur Inst. J. 7:7-8.

Bull, L. S. and J. H. Vendersall. 1973. Sulfur source for in vitro cellulose digestion and in vivo ration utilization, nitrogen metabolism and sulfur balance. Dairy Sci. 56:106.

Burns, G. R. 1968. Oxidation of sulphur in soils. The Sulphur Institute. Technical Bulletin Number 13. Washington, D. C.

Chalupa, W., R. R. Oltjen, L. L. Slyter and D. A. Davis. 1971. Sulfur deficiency and tolerance in bull calves. J. Anim. Sci. 33:278.

Church, D. C. 1975. Digestive Physiology and Nutrition of Ruminants. O & B Books, Corvallis, Oregon.

Coleman, R. 1966. The importance of sulphur as a plant nutrient in world crop production. Soil Sci. 101:230-239.

Conrad, H. R. and R. Bouchard. 1973. Sulfur requirements of dairy cows. Ohio Report. 58:102-103.

Crampton, E. W. and L. E. Harris. 1969. Applied Animal Nutrition, W. H. Freeman and Company. San Francisco.

Dawson, M. D. and W. S. McGuire. 1972. Recycling nitrogen and sulfur in grass-clover pastures. Agricultural Experiment Station. Oregon State University, Corvallis, Station Bulletin 610.

Davis, R. E. Constance Williams and J. K. Loosi. 1954. Studies on sulphur to nitrogen ratios in feeds for dairy cows. J. Dairy Sci. 37:813-818.

Dijkshoorn, J. E. M., Lampe and P. F. J. Van Burg. 1960. A method of diagnosing the sulphur nutrition status of herbage. Plant and Soil. 13:227-241.

Dijkshoorn, W. and A. L. Van Wijk. 1967. The sulphur requirements of plants as evidenced by the sulphur-nitrogen ratio in the organic matter. A review of published data. Plant and Soil. 26:129-157.

Eady, R. R. and J. R. Postgate. 1974. Nitrogenase. Nature. 249: 805-810.

Elam, C. J. 1975. Sulfur requirement of ruminants. Feedstuffs. 47:23.

Fox, R. L., A. D. Flowerday, F. W. Hosterman, H. F. Rhoades and R. R. Olson. 1964. Sulfur fertilizers for alfalfa production in Nebraska. The Agricultural Experiment Station, Lincoln, Nebraska. Research Bulletin 214.

Hackensmith, R. D. and J. G. Steele. 1949. Recent trends in the use of the land-capability classification. Soil Sci. Soc. Am. Proc. 14:383-388.

Harward, M. E., T. T. Chao, and S. C. Fang. 1962. The sulfur status and sulfur supplying power of Oregon soils. Agron. J. 54:101-106.

Heath, M. E., D. S. Metcalfe and R. E. Barnes. 1973. Forages: The Science of Grassland Agriculture. Iowa State University Press. Ames, Iowa.

Hinders, R. G. and G. W. Ward. 1961. Influence of various feeds on in vivo and in vitro production of volatile fatty acids. J. Dairy Sci. 44:1119-1133.

Horwitz, W. (ed). 1975. Methods of Analysis of the Association of Official Analytical Chemists. Section 3.059. The Association of Official Analytical Chemists, Washington, D. C.

Hume, I. D. and P. R. Bird. 1969. Synthesis of microbial protein in the rumen. IV. The influence of the level and form of dietary sulphur. Aust. J. Agri. Res. 21:315-322.

Israel, D. W., R. L. Howard, H. J. Evans, S. A. Russell. 1974. Purification and characterization of the molybdenum - iron protein component of nitrogenase from soybean nodule bacteroids. J. Biol. Chem. 249:500-508.

Jackson, M. L. 1958. Soil Chemical Analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Jones, Milton B. 1964. Effect of applied sulfur on yield and sulfur uptake of various California dryland pasture species. Agron. J. 56:235-237.

Jones, M. B. and J. E. Ruckman. 1966. Gypsum and elemental sulfur as fertilizers on annual grassland. Agron. J. 58:409-412.

Jones, M. B., J. H. Oh, and J. E. Ruckman. 1970. Effect of phosphorus and sulphur fertilization on the nutritive value of subterranean clover (Trifolium subterraneum) Proceedings of the New Zealand Grassland Association. 32:69-75.

Jones, M. B., J. H. Oh, and J. E. Ruckman. 1972. Effect of S & P on nutritive value of clover. Sulphur Inst. J. 8:2-5.

Kennedy, L. G., G. E. Mitchell, Jr. and C. O. Little. 1968. Sulphur stimulates starch digestion. Sulphur Inst. J. 4:8-9.

Kennedy, P. M. 1974. The utilization and excretion of sulphur in cattle feed on tropical roughages. Aust. J. Agric. Res. 25: 1015-1022.

Kennedy, P. M. and B. D. Siebert. 1971. The utilization of spear grass (Heteropogon contortus). II. The influence of sulphur on energy intake and rumen and blood paramenters in cattle and sheep. Aust. J. Agric. Res. 23:45-56.

L'Estrange, J. L., P. K. Upton and D. M. McAleese. 1970. Sulphur metabolism in the ruminant. pp. 277-194. In: Sulphur in Agriculture, An Foras Taluntais, Dublin, Ireland.

McLachlan, K. D. 1975. Sulphur in Australasian Agriculture. Sydney University Press. Press Building, University of Sydney.

McNaught, K. J. and R. J. E. Chrisstoffels. 1961. Effect of sulfur deficiency on sulphur and nitrogen levels in pastures and lucerne. New Zeal. J. Agric. Res. 4:177-196.

Maynand, L. A. and J. K. Loosli. 1969. Animal Nutrition. McGraw-Hill Publications. New York.

Mays, D. A. (Ed.) 1974. Forage Fertilization. American Society of Agronomy, Crop Science Society of America. Soil Science Society of America. Madison, Wisconsin, U. S. A.

Mellenberger, R. W., L. D. Satter, M. A. Millett and A. J. Baker. 1970. An in vitro technique for estimating digestibility of treated and untreated wood. J. Anim. Sci. 30:1005-1011.

Moir, R. J., M. Somers and A. C. Bray. 1967. Utilization of dietary sulphur and nitrogen by ruminants. Sulphur Inst. J. 3:15-18.

Morely, F. H. W. 1961. Subterranean clover. In: A. G. Norman (ed.). Advances in Agronomy Soils. pp. 57-123. Academic Press Inc. New York.

Odelien, M. 1963. The effect of sulfur supply on the quality of plant products. Tidsskr. Norske. Land. 70:35.

Oh, H. K., B. R. Baumgardt and J. M. School. 1966. Evaluation of Forages in the Laboratory. V. Comparison of chemical analyses, solubility tests, and in vitro fermentation. J. Dairy Sci. 49:850-855.

Oh, H. K., W. M. Longhurst and M. B. Jones. 1969. Relation of nitrogen intake to rumen microbial activity and consumption of low-quality roughage by sheep. J. Anim. Sci. 28:272-278.

Peck, H. D., Jr. 1970. Sulfur requirements and metabolism of microorganisms In: O. H. Muth and J. E. Oldfield (ed.) Symposium: Sulfur in Nutrition. The AVI Publishing Co., Westport, Connecticut.

Pumphrey, F. V. and D. P. Moore. 1965a. Diagnosing sulfur deficiency of alfalfa (Medicago sativa L.) from plant analysis. Agron. J. 57:364-366.

Pumphrey, F. V. and D. P. Moore. 1965b. Sulfur and nitrogen content of alfalfa herbage during growth. Agron. J. 57:237-239.

Pund, W. A. 1969. Sulphur improves urea treated corn silage. Sulphur Inst. J. 5:7-9.

Quicke, G. V., O. G. Bentley, H. W. Scott and A. L. Moxon. 1959. Cellulose digestion in vitro as a measure of the digestibility of forage cellulose in ruminants. J. Anim. Sci. 18:275-286.

Rampton, H. H. 1952. Growing Subclover in Oregon. Oregon Agricultural Experiment Station, Corvallis Bulletin 432.

Rees, M. C., D. J. Minson and F. W. Smith. 1974. The effect of supplementary and fertilizer sulphur on voluntary intake, digestibility, retention time in the rumen and site of digestion of pangola grass in sheep. J. Agric. Sci., Camb. 82:419.

Reid, R. L. and G. A. Jung. 1965. Influence of fertilizer treatment on the intake, digestibility and palatibility of tall fescue hay. J. Anim. Sci. 24:615-625.

Rendig, V. V. and W. C. Weir. 1975. Evaluation by lamb feeding tests of alfalfa hay grown on a low-sulfur soil. J. Anim. Sci. 16:451-461.

Roberts, S., R. V. Vodraska, M. D. Kauffman, and E. H. Gardner. 1971. Methods of Soil Analysis Used in the Soil Testing Laboratory at Oregon State University. Agricultural Experiment Station, Oregon State University, Corvallis, OR. Special Report 321.

Rural Research. 1972. Utilization of dry feed. Rural Research, P. O. Box 225, Dickson A. C. T. 2602 Australia. 78:23-26.

Saalbach, E. 1966. Sulphur fertilization and protein quality. The Sulphur Inst. J. 2:2-5.

Salisbury, F. B. and C. Ross. 1969. Plant Physiology. Wadsworth Publishing Company, Inc. Belmont, California.

Silvernale, C. E. 1975. Personal communications. Douglas County Soil Scientist. 205 SE Jackson, Roseburg, Oregon.

Spencer, K. 1959. Growth and chemical composition of white clover as affected by sulphur supply. Aust. J. Agric. Res. 10:500-509.

Stanford, G. and H. V. Jordan. 1966. Sulfur requirements of sugar, fiber and oil crops. Soil Sci. 101:258-266.

Starkey, R. L. 1966. Oxidation and reduction of sulfur compounds in soils. Soil Sci. 101:297-306.

Steward, B. A., L. K. Porter and F. G. Viets, Jr. 1966. Effect of sulphur content of straws on rates of decomposition and plant growth. Soil Sci. Soc. Am. Proc. 30:355-358.

Tabatabai, M. A. and J. M. Bremmer. 1970. A simple turbidimetric method of determining total sulfur in plant materials. Agron. J. 62:805-806.

Thompson, J. F., I. K. Smith and D. P. Moore. 1970. Sulfur requirements and metabolism in plants. In: O. H. Muth and J. E. Oldfield (ed.) Symposium: Sulfur in Nutrition. The AVI Publishing Co. West Port, Connecticut.

Tisdale, S. L., R. L. Davis, A. F. Kingsley and E. T. Mertez. 1950. Methionine and cystine content of two strains of alfalfa as influenced by different concentrations of the sulfate ion. Agron. J. 42:221-225.

Tisdale, S. L. and Werner, L. Nelson. 1975. Soil Fertility and Fertilizers. The Macmillan Company. New York.

Ulrich, A. and L. O. Hylton, Jr. 1968. Sulphur nutrition of Italina ryegrass measured by growth and mineral content. Plant and Soil. 29:274-284.

United States Department of Agriculture, Soil Conservation Service, National Cooperative Soil Survey. 1973. Tentative description for the Nonpareil, Sutherlin and Oakland soils series.

Walker, T. W. and A. F. R. Adams. 1958. Competition for sulphur in a grass-clover association. Plant and Soil. 9:353-366.

Westermann, D. T. 1975. Personal communication, Snake River Conservation Research Center, Route 1, Box 186, Kimberly, Idaho.

Williams, W. A., R. M. Love, and L. F. Berry. 1957. Production of range clovers. California Agricultural Experiment Station Cir. 458.

Ziegler, T. P. 1968. The Meat We Eat. The Interstate Printers & Publishers Inc. Danville, Illinois.

APPENDICES

APPENDIX 1. SOIL SERIES DESCRIPTION

Nonpareil Series Profile Description

A1 0-4"--Brown (10YR 4/3) loam, pale brown (10YR 6/3) dry; moderate fine subangular blocky structure; hard, friable, slightly sticky, slightly plastic; many very fine roots; many very fine tubular pores; very strongly acid (pH 4. 8); clear smooth boundary (2 to 10 inches thick).

B21 4-14"--Brown (10YR 4/3) loam, pale brown (10YR 6/3) dry; weak medium prismatic parting to moderate fine subangular blocky structure; hard, friable, sticky, plastic; common very fine roots; many very fine tubular pores; 10 percent weathered pebbles; very strongly acid (pH 4. 8); clear wavy boundary (4 to 14 inches thick).

B22 14-17"--Dark yellowish brown (10YR 4/4) loam, light yellowish brown (10YR 6/4) dry; moderate fine subangular block structure hard, friable, sticky, plastic; common very fine roots; many very fine tubular pores; 15 percent weathered pebbles; very strongly acid (pH 4. 8); gradual wavy boundary (4 to 6 inches thick).

C 17-24"--Weathered sandstone; common black stains and reddish brown (5YR 4/5) clay films in fractures.

Sutherlin Series Profile Description

A11 0-3"--Dark brown (7.5YR 3/2) silty clay loam, yellowish brown (10YR 5/4) dry; moderate fine subangular blocky and moderate fine granular structure; hard, friable, sticky, plastic; many medium, fine and very fine roots; many fine tubular and irregular pores; medium acid (pH 6.0); abrupt smooth boundary (2 to 8 inches thick).

A12 3-11"--Dark brown (7.5YR 3/4) silty clay loam, light yellowish brown (10YR 6/4) dry; moderate fine subangular blocky structure; hard, friable, sticky, plastic; many medium and common fine and very fine roots; many fine tubular pores; medium acid (pH 5.6); clear smooth boundary (4 to 10 inches thick).

B1 11-15"--Dark brown (7.5YR 3/4) silty clay loam, light brown (7.5YR 6/4) dry; weak medium and fine subangular blocky structure; hard, friable, sticky, plastic; many medium and common fine and very fine roots; many fine tubular pores; strongly acid (pH 5.2); clear smooth boundary (4 to 12 inches thick).

B2t 15-25"--Brown (7.5YR 4/4) silty clay loam, reddish yellow (7.5YR 6/6) dry; moderate fine subangular blocky structure; hard, firm, sticky, very plastic; common medium,

fine and very fine roots; many fine tubular pores; common moderately thick clay films on faces of peds; strongly acid (pH 5. 4); abrupt smooth boundary (8 to 14 inches thick).

IIC1 25-45"--Yellowish brown (10YR 5/4) clay, brownish yellow (10YR 6/6) dry; common fine faint light brownish gray (10YR 6/2) mottles; weak coarse prismatic structure; very hard, very firm, very sticky, very plastic; few fine and very fine roots; few fine pores; common fine black stains; common fine black concretions; very strongly acid (pH 4. 8); gradual wavy boundary (15 to 50 inches thick).

IIC2 45-60"--Siltstone saprolite; common thick dark brown (7. 5YR 4/4) and reddish brown (5YR 4/4) clay films in rock fractures; many fine black stains.

Oakland Series Profile Description

A11 0-3"--Dark brown (10YR 3/3) loam, brown (10YR 5/3) dry; moderate fine granular structure; slightly hard, friable, slightly sticky, plastic; common fine and very fine roots; many very fine tubular and common irregular pores; slightly acid (pH 6.4); abrupt smooth boundary (2 to 5 inches thick).

A12 3-6"--Dark brown (10YR 4/3) loam, pale brown (10YR 6/3) dry; moderate fine subangular blocky structure; slightly hard, friable, slightly sticky, plastic; common fine and very fine roots; many very fine tubular pores; medium acid (pH 5.6); clear smooth boundary (2 to 5 inches thick).

B1 6-16"--Brown (7.5YR 4/4) clay loam, light yellowish brown (10YR 6/4) dry; moderate fine subangular blocky structure; hard, friable, sticky, plastic; common fine and medium and few coarse roots; many very fine tubular pores; common pinkish gray (7.5YR 7/2) sand and silt coatings on vertical faces of peds; medium acid (pH 5.6); clear smooth boundary (6 to 14 inches thick).

B2t 16-29"--Brown (7.5YR 4/4) silty clay loam, light yellowish brown (10YR 6/4) dry; weak medium prismatic parting to moderate fine subangular blocky structure; hard, firm,

sticky, plastic; common fine and medium and few coarse roots; many very fine tubular pores; common moderately thick reddish brown (5YR 4/4) clay films; common pinkish gray (7.5YR 7/2) sand and silt coatings on vertical faces of peds; 10 percent weathered pebbles; strongly acid (pH 5.4); clear irregular boundary (8 to 20 inches thick).

C 29-32"--Sandstone saprolite; common thick reddish brown (5YR 4/4) clay films and common black staines in fractures.

APPENDIX 2. PLANT SULFUR ANALYSIS

Plant sulfur was determined using this modified procedure (Westermann, 1975).

A Dry-Ashing Procedure - similar to the AOAC method (Horwitz, 1975).

1. Place 0.5 g of plant sample into 50 ml beakers.
2. Add 2 ml 70% ETOH.
3. Add 3 ml of $Mg(NO_3)_2$ solution [950 g/l of $Mg(NO_3)_2\ 6H_2O$] If sample blows out during the dry ashing, decrease the amount of $Mg(NO_3)_2$ added, but must ash completely.
4. Heat until dry. Avoid excessive heating to prevent evolution of NO_2.
5. Hold the temperature at 500-550° for 2 hours.
6. Dissolve ash in excess HCl (10 ml of 3N). Heating aids dissolution.
7. Filter and bring to 50 ml volume. This can be done by weighing the beakers ahead of adding the HCl in which case 100 ml beakers are used. Sample dilution at this point = 1:100.

B. Turbidity Procedure - similar to Tabatabai and Bremner (1970).

1. Reagents

   a. 50% HCl + 50% HoAc. Add 50 ml of conc. HCl to

50 ml conc. acetic acid.

b. Turbidity solution. Heat 200 ml of distilled H_2O to 65° C, add 0. 6 g of gelatin, cool overnight and add 4 g $BaCl_2$. Store in refrigerator when not in use. Solution deteriorates with time and greater turbidity also develops with time.

2. Take a 10 ml aliquot of the dry-ashed sample and place into a 50 ml Erlenmeyer.

3. Add 1 ml of the acid solution (50% HCl + 50% HoAc), swirl and allow to set at least 1 hour.

4. Add 1 ml of the turbidity solution and swirly for 30 sec.

5. After 30 minutes, swirl for 15 sec and read turbidity at 500 mμ using a spectronic 20 spectrophotometer.

6. Percent sulfur is then calculated using the standard curve prepared with standards of 0, 10, 20, and 30 ppm SO_4 with the same amount of $Mg(NO_3)_2$ as samples.

APPENDIX 3. PLANT NITROGEN ANALYSIS

1. Weigh out 0. 3 g of oven dried plant sample into a digestion flask.
2. Add 10 ml of H_2SO_4 containing Salicylic Acid. (Ratio of 1 gram of Salicylic Acid to 30 ml conc. H_2SO_4).
3. Add 0. 5 g of digestion accelerator (100 g of K_2SO_4, 5 g of $CuSO_4$, 1 g of Se metal).
4. Add 1 g sodium thiosulfate.
5. Let set over night and once the solution has become clear digest at 350° C for 2 hours.
6. Transfer to Kjeldahl flask and distill into 10 ml of 0. 1N Boric Acid.
7. A titration is then run using 0. 1N HCl
8. Calculations:

$$\text{Total N} = \frac{\text{Normality of titrant x 1. 4}}{\text{sample weight}} \text{ x ml of titrant}$$

APPENDIX 4. McDOUGALL'S NUTRIENT-BUFFER SOLUTION

MODIFIED COMPOSITION OF NUTRIENT-BUFFER SOLUTION

Compound	grams/liter
$NaHCO_3$	9.80
$Na_2HPO_4 \cdot 7H_2O$	7.00
KCl	0.58
NaCl	0.48
$CaCl_2$	0.04
MgO	0.0115

APPENDIX 5. PLANT ANALYSIS

Yield Data and Species Composition

Treatment lbs/Acre	Rep	Yield lbs. DM/Acre	Percent Clover	Percent Grass
0 S	1	5149	38	62
	2	5049	50	50
	3	3936	44	56
	4	4766	50	50
	5	4577	30	70
10 S	1	4753	79	21
	2	4732	50	50
	3	6031	33	67
	4	4679	77	22
	5	3199	83	17
20 S	1	4663	83	17
	2	4527	92	8
	3	4933	69	31
	4	4627	75	25
	5	4384	86	14
40 S	1	4577	56	44
	2	5638	83	17
	3	5399	80	20
	4	5514	77	23
	5	5036	73	27
80 S	1	5330	85	15
	2	5520	67	33
	3	7228	78	22
	4	5145	80	20
	5	5251	45	54
160 S	1	5167	57	43
	2	5073	75	25
	3	5825	65	35
	4	4184	50	50
	5	4780	78	22

Sulfur Analysis

Treatment lbs/Acre	Rep	Percent Sulfur		
		Grass	Clover	Forage
0 S	1	0.099	0.107	0.107
	2	0.091	0.095	0.093
	3	0.063	0.083	0.067
	4	0.116	0.125	0.116
	5	0.087	0.095	0.095
10 S	1	0.135	0.135	0.135
	2	0.110	0.130	0.120
	3	0.103	0.140	0.121
	4	0.107	0.123	0.117
	5	0.080	0.090	0.090
20 S	1	0.125	0.154	0.149
	2	0.140	0.144	0.154
	3	0.112	0.140	0.140
	4	0.130	0.100	0.110
	5	0.103	0.103	0.107
40 S	1	0.130	0.144	0.135
	2	0.135	0.159	0.149
	3	0.135	0.144	0.144
	4	0.149	0.170	0.170
	5	0.159	0.149	0.154
80 S	1	0.159	0.170	0.159
	2	0.164	0.180	0.180
	3	0.192	0.197	0.197
	4	0.170	0.203	0.203
	5	0.149	0.170	0.159
160 S	1	0.186	0.209	0.209
	2	0.209	0.248	0.241
	3	0.191	0.234	0.215
	4	0.209	0.248	0.209
	5	0.192	0.221	0.192

Nitrogen Analysis

Treatment lbs/Acre	Rep	Percent Nitrogen		
		Grass	Clover	Mix
0 S	1	1.4	2.2	2.0
	2	1.2	2.2	1.7
	3	1.0	1.9	1.4
	4	1.3	2.3	1.8
	5	1.3	2.3	1.6
10 S	1	1.1	2.5	2.2
	2	1.5	2.5	2.0
	3	1.6	2.8	2.0
	4	1.3	2.4	2.0
	5	1.2	1.8	1.7
20 S	1	1.6	2.8	2.6
	2	1.6	2.8	2.7
	3	1.3	2.6	2.2
	4	1.3	2.1	1.9
	5	1.3	2.0	1.9
40 S	1	1.6	2.5	2.1
	2	1.4	2.6	2.4
	3	1.3	2.3	2.1
	4	1.4	2.7	2.4
	5	1.5	2.6	2.3
80 S	1	1.5	2.8	2.6
	2	1.6	2.8	2.4
	3	1.7	2.6	2.4
	4	1.4	2.9	2.6
	5	1.5	2.6	2.0
160 S	1	2.1	2.8	2.5
	2	1.8	3.0	2.7
	3	1.5	3.2	2.6
	4	2.0	2.0	2.5
	5	1.6	2.5	2.3

In Vitro Analysis

Treatment lbs/Acre	Rep	Average Percent Digestibility[1/]		
		Grass	Clover	Total Forage
0 S	1	39.6	52.6	47.5
	2	39.8	51.8	47.9
	3	36.2	50.1	39.2
	4	36.7	49.8	42.4
	5	36.2	48.1	40.8
10 S	1	39.2	53.8	50.2
	2	40.0	53.9	48.9
	3	35.9	47.5	43.9
	4	37.8	51.3	45.9
	5	36.3	50.0	40.7
20 S	1	38.6	52.7	48.9
	2	36.5	55.6	49.4
	3	36.1	48.1	34.5
	4	32.2	45.3	40.8
	5	28.9	42.4	39.2
40 S	1	34.5	44.8	43.4
	2	30.3	45.4	41.6
	3	30.8	46.3	40.9
	4	41.2	49.3	39.2
	5	31.2	48.5	44.2
80 S	1	37.8	52.2	44.7
	2	36.7	47.3	46.7
	3	34.8	48.1	43.5
	4	31.4	44.7	40.7
	5	33.4	47.0	41.6
160 S	1	36.6	54.7	48.7
	2	40.1	58.6	52.6
	3	30.4	53.9	47.8
	4	30.6	39.8	43.9
	5	32.6	45.2	47.7

[1/] Each tabulated value is the average of 3 determinations