# SELENIUM AND SULFUR NUTRITION OF BEEF CATTLE

by

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# INTRODUCTION

Few trace elements carry as many taboos against safe nutritional use as does selenium. This is primarily due to the fact that the earliest experience with this element was concerned with its very toxic effect in livestock feeds. As a result of this toxic characteristic plus the carcinogenic property of this element, governmental regulations have hampered the investigational use of this element in the production of meat animals.

Possibly the first positive nutritional effect noted with selenium was in the late 1930's and early 1940's. Work in South Dakota suggested an apparent chick growth stimulation from the addition of trace amounts of selenium to the ration. Following this observation, selenium was found to be a required factor for the prevention of muscular disease in young ruminant animals. In these investigations, it was discovered that vitamin E and selenium were interrelated in their metabolic functions and that this complicated the assessment of the selenium requirement of livestock.

Research has shown that the selenium requirement for the prevention of muscular disorders in ruminants is approximately 0.1 ppm. The requirement for selenium in cattle finishing rations has not been adequately demonstrated. Since the requirement for selenium in cattle rations is thought to be

small, the variation in response to supplemental selenium is probably due to the variation of the natural selenium content of feedstuffs and possibly a similar variation of vitamin E levels.

These studies were originally undertaken to determine if cattle require selenium in the rations for maximum feedlot performance. Various nutrients and additives were utilized in an attempt to place a stress upon the selenium and/or vitamin E nutrition of these animals in order to demonstrate the need for supplemental selenium.

Sulfur, a known antagonist of selenium, failed to interfere with the utilization of the natural or supplemental selenium in these trials but did appear to indicate that cattle rations containing urea may be deficient in sulfur. Since sulfur is an integral portion of the amino acids methionine and cystine, a source of sulfur must be available to rumen microorganisms for the synthesis of protein from urea or other nonprotein nitrogen sources and the protein of the natural feedstuffs. It has generally been believed that an adequate source of dietary sulfur is available to the microbial population of the rumen even when a large percent of the supplemental protein of the ration is obtained from urea. With these observations in mind, trials were conducted to determine the influence of supplemental sulfur upon feedlot performance, carcass characteristics and blood serum inorganic sulfate

values. Some rumen fermentation products and bacterial populations were studied when urea was fed in cattle finishing rations as the primary source of supplemental nitrogen.

# REVIEW OF LITERATURE

## Selenium Nutrition of Ruminants

The presence of selenium in plants has been known for many years. Investigations of the presence of selenium in plants have been of interest because of its toxic effects on grazing livestock. Selenium is one of the dispersed elements, found in minute amounts in all materials of the earth's crust and rarely concentrated in amounts above 100 ppm. Estimates of the abundance of selenium in igneous rocks are around 0.1 ppm and the earth's crust from 0.03 to 0.8 ppm. In some areas the soils contain abnormal amounts of selenium and this is concentrated by some of the plants grown upon such soils. Wheat, which normally contains less than 1 ppm selenium, may contain up to 50 times this amount on a seleniferous soil. Other plants seem able to grow only on soils of high selenium content -- a hint of some important biological function -- and may contain up to 0.2% selenium.

The form of selenium in plants has never been clarified. Rosenfeld and Beath (1964) have reviewed this subject and the possibility has been suggested that selenium may substitute for sulfur in plant metabolism. The proportions of organic and inorganic selenium in plants depend on the species. Inorganic selenium is almost entirely in the selenate form while numerous experiments have given presumptive evidence that the

organic selenium is present in protein-bound amino acids. Information from several studies, mainly by Scott (1958) and Hogue et al. (1959), has resulted in a summarization of selenium content in common feedstuffs. The selenium content of corn, oats and wheat varied from 0.02 to 0.32 ppm. The protein rich feeds seem to be somewhat higher in selenium than cereal grains. Plant grasses are somewhat lower in selenium content as compared with alfalfa hay.

Research which led to the discovery of selenium as a nutritional factor originated in studies of brewer's yeast as a protein supplement during World War II. Rats on a yeast diet developed liver necrosis. Wheat germ and wheat bran showed protective activity against this disorder. Upon fractionation, Schwarz (1944) showed that vitamin E was identified as the main protective agent. Further work in this area led to the isolation of an unidentified material referred to by Schwarz as "factor 3". Selenium was finally identified as the key component of "factor 3" by Schwarz and Foltz (1957). Since the discovery of the nutritional value of selenium in minute amounts and the fact that it can replace vitamin E as a preventative or cure for some conditions, it has been very difficult to separate these two factors in aminal nutrition. However, McLean et al. (1959) and Schwarz (1960) indicate that the two nutrients are interrelated in their metabolic function but that vitamin E cannot completely replace the need for

selenium; therefore selenium is a dietary essential in its own right.

Sheep

Muscular dystrophy has been recognized as a naturally occurring disease in sheep and cattle for many years. It is in this area of nutritional research where the great bulk of work with selenium has been done. Hartley et al. (1960) found that in New Zealand, where muscular dystrophy occurs in lambs, the administration of selenium not only reduced the incidence of muscular dystrophy in lambs but also increased the number of lambs born. Muscular dystrophy has been recorded in many countries throughout the world. Certain "dystrophic areas" appear in all of these countries in which there is a deficiency of selenium in the soil. Schubert et al. (1961) produced white muscle disease in lambs by feeding ewes during gestation on forages from affected areas. Analysis of forages showed no relationship between tocopherol content and the disease but there was a direct relationship between selenium content and white muscle disease. When ewe rations were supplemented with 0.1 ppm sodium selenite during gestation through weaning, protection against the disease was observed. When both selenium and vitamin E were given to the lambs, the former appeared to be more effective in preventing the disease as well as for supporting growth. Drake et al. (1960) admin-

istered vitamin E to ewes on farms where lamb losses were as high as 40-50% and 30-90% of the ewes were empty at lambing time. The vitamin E had no effect on incidences of white muscle disease in lambs, but oral dosage with 5 milligrams selenium reduced lamb mortality by 50% or more. In other trials, lambs were given vitamin E which gave some protection from white muscle disease, but selenium gave almost complete protection. Oldfield et al. (1960) fed 0.1 ppm selenium to ewes and administered a 1.4 milligram implant or 2000 I.U. of vitamin E orally to lambs at birth. They observed that all of the treatments prevented gross symptoms of white muscle disease. Vitamin E had no effect on growth but lambs receiving selenium showed greater growth than controls.

In addition to feeding forages, other types of rations have been observed to produce muscular dystrophy in sheep. Kidney beans are low in selenium and have been used in numerous experiments to experimentally produce muscular dystrophy. Hogue et al. (1962) reported that 24-60% of lambs became dystrophic from ewes fed kidney beans and mixed hay while a ration of mixed hay, corn, oats, wheat bran and linseed meal completely prevented the disease. Cooking of the basal ration prevented muscular dystrophy indicating that a heat labile antagonist may be present in feeds. In this work, selenium and vitamin E were equally effective in preventing muscular dystrophy, although neither gave complete protection when fed

to the ewe during gestation or early lactation. Apparently maternal transfer of either selenium or vitamin E was inadequate. A combination of these two factors was effective when given to the ewe. Both were effective when given directly to the lambs.

Welch et al. (1960) studied the effects of feeding fish oil, vitamin E and selenium to ewes. In three experiments, fish liver oil feeding during pregnancy and lactation was an effective means of producing muscular dystrophy in lambs. Vitamin E administration to the ewes was effective in counteracting the dystrophic effect of the oil as well as curing the dystrophy when given directly to the lambs. Selenium incorporation into the rations decreased but did not eliminate the occurrence of muscular dystrophy. Hopkins et al. (1964) fed infant lambs a diet containing torula yeast and stripped lard and symptoms of muscular dystrophy developed. Selenium stimulated growth greater than vitamin E early in the experiment but did not prevent the disease completely. Vitamin E prevented all symptoms and a combination showed positive effects of both selenium and vitamin E.

Borgman et al. (1963) studied the effects of injections of vitamin E and selenium upon the occurrence of muscular dystrophy in early weaned lambs. Levels of these nutrients used were as high as or higher than those used in other work; however no effects on the disease were observed. These authors

suggested that other factors may be involved in the production of this disease. High concentrations of sulfate have been observed to depress the uptake of selenium by crops. For example, Hartley and Grant (1961) reported that the addition of gypsum to pasture depressed the growth rate of selenium responsive lambs below that of similar lambs on untreated pasture. Other workers have reported that use of elemental sulfur alone as a fertilizer causes muscular dystrophy. Oregon workers describe frequent storms of this disease after fertilization with common sulfur-bearing fertilizers. Schubert et al. (1961) reported that muscular dystrophy was not always associated with a demonstrable forage selenium deficiency and reported that a sulfur antagonism can influence the biological availability of trace amounts of selenium. Muth et al. (1961) suggested a similar relationship. The addition of 0.053% sulfur as sulfate to the rations of ewes decreased the effectiveness of 0.1 ppm supplemental selenium in preventing nutritional muscular dystrophy in lambs. Hintz and Hogue (1964) tested the effect of the addition of selenium and sodium sulfate on the occurrence of muscular dystrophy. Selenium at 0.17 ppm during lactation had no effect on the disease. Sulfur at 0.33% increased the evidence of the disease and when it was given in combination with selenium, it prevented beneficial effects of selenium. Addition of supplemental cystine or methionine did not have any influence on selenium utilization. Paulson et al. (1966)

have recently demonstrated an antagonism upon selenium absorption due to the presence of sulfur in the rations of the lactating ewe. Administration of radioactive selenium resulted in a concentration of a greater amount of selenium in the small intestine than the rest of the G.I. tract. Addition of 0.5% sulfur resulted in an increased concentration of selenium in the small intestine suggesting interference with absorption.

McLean et al. (1959) was one of the first workers to observe a growth response to selenium. An increase in growth rates on 12-40% of the farms observed was noted with both oral and subcutaneous administration of selenium to lambs. Responses were noted in flocks where no cases of muscular dystrophy had been reported as well as in affected flocks. Slen et al. (1961) observed the effect of selenium on body weight gain and wool growth. Range ewes receiving daily doses of selenium in a 2 year study gained significantly faster and produced more wool than control animals. Robertson and During (1961) have also noted increased weight gains in lambs due to selenium supplementation. Young and Hawkins (1962) reported that on the basis of their work the effect of selenium in enhancing growth can largely be ascribed to a reversal of a depression in weight gain due to the presence of muscular disease. Shirley et al. (1966) observed no gain response due to selenium administration to lambs in the absence of muscular

dystrophy.

Cattle

Sheep in general seem to be more affected by white muscle disease than do cattle and as such more work with the selenium nutrition of sheep has been done. Calves are more susceptible to muscular dystrophy than adult cattle. Sharman et al. (1959) observed that five times more vitamin E is needed to prevent naturally occurring muscular dystrophy than that produced experimentally suggesting that other factors may also be involved. When no treatment, vitamin E or selenium was given in a field trial, calves developing muscular dystrophy were ten, two and zero, respectively. They concluded that selenium was more effective in preventing muscular dystrophy than was vitamin E but it was pointed out that selenium deficiencies might be masked because of ample tocopherols in forages. Maplesden and Loosli (1960) produced clinical symptoms of white muscle disease by feeding a vitamin E deficient ration containing cod liver oil. Daily doses of vitamin E with or without selenium added to the diet prevented this condition. However, selenium alone did not have any beneficial effects. Blaxter et al. (1961) treated beef calves with selenium both orally and by subcutaneous injection. Muscular dystrophy was essentially prevented by the selenium treatment. These workers concluded that selenium was as effective in reducing muscular dystrophy

as 20 milligrams of alpha tocopherol.

Schubert et al. (1961) reported a severe outbreak of white muscle disease of calves when a ration of alfalfa hay was fed to cows. This forage was from a ranch where white muscle disease had been a common occurrence. Of nine calves, four died spontaneously and showed cardiac lesions. At sacrifice three additional cases were evident. The forage analyzed 0.27% sulfur. This high level of sulfur may have been partially responsible for the severe outbreak of the disease. When forage in 2 successive years from the same source was fed, WMD incidence was two cases and none, respectively. However, sulfur content of the forage during these years was reduced.

Papers in the literature on the nutritional value of selenium in rations not dealing with muscular disease are rather limited. Jolly (1960) investigated the growth response from added selenium to calf rations. Twice monthly dosing with 10 milligrams of sodium selenate resulted in nearly 50% increased gain within a 4 week period as compared to control animals. Hartley and Grant (1961) have also reported superior gains in calves receiving supplemental selenium. Burroughs et al. (1963) added selenium and a combination of vitamin E and K to a finishing cattle ration low in vitamin E. Vitamins E and K supplementation resulted in 9% faster weight gains and an improvement of feed efficiency. Response from selenium was

as great as responses obtained from the supplemental vitamins. Barringer (1964) observed varying results with selenium additions to finishing cattle rations. In two of four trials, 0.1 ppm selenium tended to increase weight gains; in other trials, gains were the same or slightly depressed. Further work by Mukhtar (1966) indicated that 0.1 ppm selenium did not affect gains of feedlot cattle.

Further variable results due to selenium supplementation of cattle finishing rations have been obtained. Beeson et al. (1964) observed a 10% increase in weight gain and 3% improvement in feed efficiency with the daily addition of 1 milligram selenium to the ration. In a later trial at the same station reported by Smith et al. (1965), levels of 1, 2 and 4 milligrams selenium daily resulted in a slightly decreased gain and feed efficiency. Scott et al. (1965) fed rations supplemented with 0.1 ppm selenium to steer calves and reported increased gains and an improved feed efficiency. Carcass characteristics were not altered by the supplemental selenium. Shirley et al. (1966) studied the influence of supplemental selenium on percent calf crop and weaning weights of calves when the cows were on low selenium pastures. Selenium in a 2 year trial had no effect on calving percent or weaning weights. No symptoms of muscle disorders were observed in these calves.

## Factors Influencing Poisoning and Chronic Selenosis

In seleniferous areas of the country, the abundant distribution of selenium in natural feedstuffs often leads to toxicity problems. This has led to serious economic problems and numerous compounds have been tested for their effectiveness against selenium toxicity.

Early experiments on the protective effect of arsenic ($Na_2HAsO_3$) on chronic selenosis produced by inorganic selenium or seleniferous grain were reported by Moxon (1938). This was followed by experiments in which large numbers of inorganic and organic arsenicals were tested. Some of these have been reviewed by Rosenfeld and Beath (1964). Arsenite and arsenate were equally effective in preventing the toxic influence of seleniferous wheat, selenite and selenocystine. Organic arsenicals, arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acids have been found to give partial protection against chronic selenosis in rats. Arsanilic acid and p-hydroxyphenylarsonic acid gave 100% protection against 10 ppm selenium. Seven ppm of 3-nitro-r-hydroxyphenylarsonic acid was also effective.

Wahlstrom et al. (1955) studied the effectiveness of arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid against chronic selenosis in pigs. Excellent protection against 10 ppm selenium (selenite) was given at levels of 0.02 and 0.05% of the respective arsenicals. Wahlstrom et al. (1956) and

Wahlstrom and Olson (1959) in further studies investigated the effects of organic arsenicals and linseed meal on chronic selenosis. Arsanilic acid at 0.01% and 3-nitro-4-hydroxy-phenylarsonic acid at 0.005% gave best protection when used in combination with linseed meal. Minyard et al. (1957, 1960) investigated the effectiveness of arsanilic acid in counteracting selenium poisoning in cattle grazing and feedlot trials. Arsanilic acid levels of 0.005% and 0.01% resulted in increased gains. When 12 ppm of selenium were added to feedlot rations, a 0.01% arsanilic acid addition appeared to increase gain and reduce slightly the selenium toxicity symptoms. Toxicity alleviation was observed in all trials even though gains were not always stimulated. Moxon et al. (1944) had previously observed that steers consuming hay with up to 5.6 ppm selenium and pastures up to 13.0 ppm selenium gained more rapidly and had fewer incidences of selenium poisoning when allowed access to a salt mixture containing 25 ppm arsenic as sodium arsenite.

Sulfur has also been shown to be an antagonist to the utilization of selenium. Shrift (1961) has recently reviewed some of the interrelationships between selenium and sulfur in plants and microorganisms. Halverson and Monty (1960) observed that the addition of 0.29, 0.58 and 0.87% inorganic sulfate to diets containing 10 ppm selenium as selenate or selenite alleviated from 20 to 40% of the selenium-produced

growth depression in rats. Sulfate, however, did not substantially prevent liver degeneration due to selenium. Ganther and Bauman (1962) fed diets to rats containing 5 ppm selenium and noted that 1% sodium sulfate modified or partially alleviated chronic selenosis produced by selenate. Sodium sulfate was less effective against selenite. The sulfate increased the urinary excretion of selenium but had no effect on fecal excretion. Halverson et al. (1962) noted that sulfate did not reduce selenite toxicity in rats nor did it reduce toxicity from feeding seleniferous wheat containing 20 ppm selenium.

Sulfur has also been observed to antagonize selenium as a preventative factor of muscular dystrophy; however this has been reported elsewhere in this review.

## The Effects of Organic and Inorganic Sulfur in Ruminant Rations

### General

Late in the 1800's it was demonstrated that rumen microorganisms were capable of synthesizing microbial proteins from non-protein nitrogen. Since that time a great deal of work has been done in attempting to establish proper methods of utilizing non-protein nitrogen in the most economical way.

One nutrient which has received a great deal of attention in the utilization of non-protein nitrogen has been the element

of sulfur. Sulfur is an integral portion of the amino acids methionine and cystine which in turn are some of the building blocks of protein. It has been well established that rumen microorganisms are capable of synthesizing these sulfur amino acids in the rumen when sufficient sulfur is fed in the diet.

Block et al. (1951) demonstrated that the microorganisms of the rumen were capable of incorporating non-protein nitrogen into amino acids. When radioactive sulfur was fed to a goat, milk protein showed considerable activity within 3 hours after ingestion of $S^{35}$. Peak activity appeared approximately 24 hours after initial feeding. When $Na_2S^{35}O_4$ was fed to a ewe, the radioactive sulfur was found to be in the protein contents of the rumen material. This was equally distributed between methionine and cystine suggesting that they were synthesized at approximately equal rates in the rumen and were used by the tissues to make new protein. This confirmed earlier work by Block and Stekol (1950) that the ruminant can utilize and convert inorganic forms of sulfur into wool and milk proteins.

Hale and Garrigus (1953) fed elemental $S^{35}$ and $Na_2S^{35}O_4$ and observed the presence of $S^{35}$ in the cystine of the wool proteins. The sulfate source of sulfur appeared to be better utilized than did the elemental sulfur. It was suggested that this may be due to solubility of the two compounds. Radioactivity also appeared in the blood and urine. Later work

by Kulwich et al. (1957) with lambs indicated that the absorption of radioactive sulfur into the blood stream was rapid, occurring within 6 hours. Fractionation studies on liver, spleen, skin and wool revealed that most of the tracer was in the form of cystine and methionine.

Lewis (1954) postulated that since the microorganisms in the rumen of sheep were capable of reducing nitrate and nitrite to ammonia, a similar reduction of sulfate to hydrogen sulphide might occur. In vivo and in vitro experiments confirmed this theory. Further work on the utilization of sulfate and protein sulfur was reported by Anderson (1956). He demonstrated that inorganic sulfate is reduced to sulfide which is shared by the animal host and microorganisms, the latter synthesizing the sulfur amino acids. When small amounts of sulfate are present in the ration, a very efficient utilization of sulfide is observed. Further, when the supply of inorganic sulfate is exhausted, then the microorganisms degrade food protein with the release of sulfide which is utilized for the synthesis of new microbial protein.

Using labeled inorganic sulfate, Emery et al. (1957a, b) observed that microorganisms from grain fed cows utilized inorganic sulfur to a greater extent than did those from cows fed a high roughage ration. In a 3 hour incubation period, the formation of cystine was twice as great as the formation of methionine. In observing microorganisms, it was noted

that most of them did not incorporate the radioactive sulfate. In further studies with pure cultures of ten rumen microorganisms, it was reported that only five of these utilized a significant amount of radioactive sulfates. In the presence of cysteine in the media, only three incorporated inorganic sulfate into microbial protein, thus indicating a rather specific requirement of sulfur for individual microorganisms.

Sheep

Numerous workers have demonstrated the need for sulfur by ruminant animals. Thomas et al. (1951) clearly demonstrated this requirement. They noted that lambs on sulfur deficient purified rations lost weight and were always in negative balance for both sulfur and nitrogen. Lambs which were fed urea-sulfur diets were in positive balance and gained weight. Wool growth was decreased but did continue to grow at a reduced rate up to 5 months on sulfur deficient diets even though the animals lost weight. This suggests that wool growth has greater priority on nutrients from the metabolic pool than does tissue growth or maintenance.

Early in the era of urea feeding to ruminants, it was observed that supplemental sulfur may increase the value of urea in ruminant rations. Willman et al. (1946) fed sodium sulfate to lambs consuming shelled corn, corn silage and urea

and observed increased weight gains. The addition of sulfur to urea rations does not always increase weight gains. In an experiment using a semi-purified ration, Loosli and Harris (1945) reported results of adding sulfur to a low nitrogen basal ration. Sulfate sulfur additions did not increase weight gains but there was an increase in nitrogen retention of these lambs. Sulfur additions in the form of the amino acid methionine did improve both weight gains and nitrogen retention similar to that noted by the addition of linseed meal. The value of elemental sulfur in rations deficient in methionine was studied by Garrigus et al. (1950). They noted an improvement in the growth rate of lambs with the addition of methionine. The improvement in gain by the addition of elemental sulfur was not as great as that of methionine suggesting a better utilization of the organic form of sulfur than the inorganic form. Albert et al. (1955) observed variable results with the addition of elemental sulfur to lamb rations. In one trial, sulfur only slightly improved gains while in a second trial, gains were significantly increased. Nitrogen balance and wool growth improved with sulfur but was not statistically significant. Morris (1958) in drought feeding studies on sheep consuming native grass hay and sorghum silage observed large losses in body weight. When these rations were supplemented with urea and sodium sulfate, significant reductions in body weight losses were ob-

served.

Starks et al. (1953) observed improvement of the utilization of dietary nitrogen in lambs by the addition of elemental sulfur to a purified ration. The lambs fed the basal ration were in negative sulfur and nitrogen balance. Addition of sulfur to the basal rations put the lambs in positive sulfur and nitrogen balance. The lambs fed the basal ration as well as the sulfur supplemented rations lost weight in this 90 day trial. This was due to the fact that the sulfur supplemented lambs were pair fed with the unsupplemented ones, thus resulting in a very low feed intake. The S:N ratios of these experimental rations were 1:40 and 1:3.5. In a later experiment, Starks et al. (1954) compared the addition of various levels of elemental sulfur (0.2, 0.4, 0.6%), sodium sulfate (0.89, 1.33, 1.78%) and methionine (0.2, 0.5, 0.7%) to the ration. The lambs on the basal ration lost weight, but the addition of sulfur in the three forms used increased body weight gain and wool growth. The lowest levels of each source of sulfur furnished adequate or nearly adequate sulfur as judged by performance of the lambs. The medium levels of sulfur promoted the most rapid and efficient gains while the highest levels appeared to be least effective although neither of these observations were statistically supported. Albert et al. (1956) studied the sulfur requirement of lambs using graded levels and various sources of sulfur. Addition of

sulfur to the rations improved gains in this experiment. The optimum level of sulfur for gains was very consistent with that observed by Starks et al. (1954). Similarly, it was observed that high levels of sulfur resulted in depressed performance. On the basis of total sulfur, about 70% less sulfur was needed as methionine than as elemental sulfur, or about 50% less than as sulfate sulfur. This suggests that sodium sulfate may be used more efficiently than elemental sulfur and agrees with the work of Starks et al. (1953).

Lofgreen et al. (1947) observed that the addition of 0.2% methionine to a lamb ration of timothy hay and corn, in which urea furnished 40% of the dietary nitrogen, increased nitrogen retention as compared to the basal ration. In a later study, Lofgreen et al. (1953) did not improve gains or feed utilization of lambs by adding 0.2% sodium sulfate to a ration with urea furnishing 40% of the dietary nitrogen. In the earlier trial, the N:S ratios were 15:1 while in the latter one the ratios were 9:1 and 7:1.

The response with methionine supplementation varied with different constituents in the rations in work reported by Klosterman et al. (1951a) even though they did observe increased nitrogen retention. Little effect due to methionine supplementation of field peas was noted, however, an increased response was observed when it was added to an alfalfa ration. In other work by Klosterman et al. (1951b), there was no

improvement in protein utilization in lambs by the addition of 0.2% methionine to rations containing primarily wheat straw and field peas.

Variable responses to methionine supplementation have also been seen by Oklahoma workers. No effect from additions of methionine was observed by Gallup et al. (1952) in digestion and nitrogen balance studies when lambs were fed a 7.2% protein ration. When rations with 10.2% protein containing urea were used, methionine supplementation resulted in increased crude fiber and organic matter digestion and increased nitrogen retention. These differences were not statistically significant. Noble et al. (1953) supplemented corn and prairie hay with combinations of soybean meal and urea. Methionine additions had only slight effects on the growth rate of these lambs. Urea and methionine did not produce the growth rate observed with the soybean meal protein. In another trial, Noble et al. (1954) reported on methionine additions to urea rations with a N:S ratio of 59:1. Methionine reduced the ratio to 15:1. There was no significant difference in body weight gain but there was a trend for the methionine to improve the urea ration.

Other workers have also shown variable responses when utilizing methionine as a sulfur source in lamb rations. Jordan (1957) fed a combination of orotic acid and methionine and noted increased weight gains in one trial and no

effect in another trial. Trenkle (1958) fed methionine to lambs on both purified and natural rations. Methionine did result in increased weight gains on the purified rations but was of no value in rations with natural feed ingredients. Oltjen et al. (1962) supplemented purified lamb rations containing urea as the only source of nitrogen with 0.2% and 0.4% methionine. They observed a nonsignificant increase in weight gains over the lambs on a control ration.

Most of the work with sulfur was reported in the mid-1950's, however, recently there has been a revival of interest in this area. Smith et al. (1964) fed lambs rations supplemented with 0.30% and 1.30% elemental sulfur. There was no effect on gains at the lower level of sulfur but a drastic reduction in gain was noted with the higher level of sulfur. This was attributed to ration unpalatability. Generally carcass measurements were not affected with the exception that the low level of sulfur resulted in more youthful and higher grading carcasses. Teuscher et al. (1965) supplemented corn silage and ground corn rations for lambs with 0.25% elemental sulfur in a 54 day trial. No differences in body weight gains were observed due to sulfur alone; however there was a significant increase in gain of sulfur fed lambs when implanted with diethylstilbesterol.

Cattle

Reports in the literature concerning sulfur utilization in urea type cattle rations are much less numerous than those with sheep and as such the sulfur requirements of cattle are not very well known. One possible reason that there has been more work done with sulfur nutrition of sheep is that they may have a higher sulfur requirement than cattle because of the high cystine content of the wool.

Early work with sulfur and urea in cattle rations was reported by Jones and Haag (1946) and consisted of trials began in 1944. The rations of dairy heifers receiving poor quality rations and urea were supplemented with 1% sodium sulfate. In 12 of 16 pairs of animals, an increase in gain due to sulfur supplementation of the rations was observed. Bailey and Ross (1948) found that cattle could use inorganic sulfates and that methionine had no apparent advantage over sodium sulfate when used in the rations.

Jones et al. (1952) conducted two studies with lactating cows by feeding a low sulfur ration and supplementing with sulfur. The addition of 1% sodium sulfate or 0.5% methionine to the grain mixture did not improve milk production or feed utilization. Thompson et al. (1952) supplemented dairy lactation rations containing 2% urea with 22 grams daily of methionine. The additional methionine did not improve milk production or ration digestibility. Wing (1955, 1957) has

observed a stimulation in body weight gain and improved feed efficiency with dairy calves and 9-month old dairy heifers by the addition of a combination of orotic acid and methionine. Neither the orotic acid nor the methionine was effective when used alone.

Levels of supplemental sulfur in urea and silage rations were tested in a 90-day dairy heifer growth trial by Brown et al. (1960). All levels of added sulfate significantly increased body weight gains over the urea control but did not promote as great a growth rate as did a soybean meal supplement. Methionine hydroxy analogue also stimulated gains in this trial. Growth of dairy heifers appeared to be stimulated by sodium sulfate additions to rations containing urea as reported by Lassiter et al. (1958b). Sulfate addition to rations in which 50% of the nitrogen was from urea supported growth equal to that observed when urea furnished 30% of the nitrogen. Sulfate added to rations in which 70% of the nitrogen was from urea did not stimulate growth. In an earlier trial by Lassiter et al. (1958a), no sulfate had been added to the ration and growth was depressed with the two higher levels of urea.

Davis et al. (1954) observed no lactation stimulation when comparing soybean meal with urea and urea plus sodium sulfate as supplementary protein sources. The evidence suggests that soybean meal was superior to urea as a nitrogen

source and that added sulfate was of questionable value. Feed intakes were very similar and no marked differences in utilization for milk production were noted.

Very little is found in the literature concerning the addition of sulfur to the ration of beef cattle. Workers at the Illinois Agricultural Experiment Station (1952) fed steer calves rations containing corn silage. Urea replaced soybean meal at the rate of 40% of the total nitrogen in the rations of a portion of the experimental animals. Sulfur supplemented at the rate of 2 milligrams daily per 100 pounds of body weight improved gain over the no sulfur-urea controls but gain was not equivalent to that of steers fed soybean meal. Perry et al. (1953) tested modifications of the soybean meal base Purdue Supplement A with calves receiving corncob rations for 161 days. They reported that urea incorporation into Purdue Supplement A was satisfactory but incorporation of additional sulfur was of no value.

The value of methionine and methionine hydroxy analog as a sulfur source in cattle finishing rations was studied by Gossett et al. (1962). Methionine supplementation improved gain slightly with a protein supplement based on urea but not as great as did lysine. Methionine analog had no effect on gain at one level and depressed gains when fed at a higher level. This is in disagreement with the work of Brown et al. (1960) who had observed increased gains of dairy heifers when

fed the methionine analog.

The value of supplemental sulfur in beef cattle rations has been studied at Minnesota recently and variable results have been obtained. Kolari et al. (1963) fed corn silage and ground ear corn to heifers for 84 days. Elemental sulfur addition to either linseed oil meal or urea supplements increased gains and improved efficiency of gain. The greatest growth response was obtained on the urea diet. In a later study by Kolari et al. (1964), no influence of supplemental sulfur on feedlot performance with either of the protein source supplements was observed. Heifers in this report were fed a corn ration for 84 days and steers a corn silage ration for 118 days. In a third trial, Meiske et al. (1966) fed supplemental elemental sulfur in a finishing ration to steers for 152 days. Again no increased gain due to the sulfur supplementation of urea protein supplements was noted. Additional sulfur to linseed oil rations resulted in decreased gains.

Hatfield et al. (1967) fed corn silage which was supplemented with urea and biuret with and without sulfur at the time of ensiling. In a 138-day trial with steers, there was no apparent effect on performance due to the sulfur supplementation. Tolman and Woods (1966) observed that feeding supplemental sulfur to calves consuming a full feed of corn silage with urea supplying 100% of the supplemental nitrogen did not improve the performance over rations in which no sulfur was

fed.

Serum inorganic sulfate

Weir and Rendig (1954) reported that blood serum inorganic sulfate levels were found to be closely correlated with the total sulfur content of the ration. When rations containing 0.26% sulfur were fed to lambs, the serum inorganic sulfate levels were 2.0 to 4.0 milligrams/100 milliliters. When rations containing 0.16% sulfur were fed, serum sulfate dropped below 1.0 milligrams/100 milliliters. These workers observed that when animals refused to eat that the sulfate level of the blood dropped initially and then rose to a normal level which was probably due to the result of the breakdown of body tissue. It was further suggested that serum inorganic sulfate levels of the blood might be used to determine whether the addition of sulfur to the ration of ruminants is warranted. Further work by these authors (Rendig and Weir, 1957) confirmed the earlier results. When hay rations containing 0.28% and 0.26% sulfur were fed to lambs over a 147 day period, the serum inorganic sulfate levels tended to increase. A decrease in serum sulfate levels was noted when hay rations containing 0.21% sulfur or lower was fed; however no difference in rate of gain was observed with the reduced serum sulfate levels.

Rendiq and Weir (1957) and Lofgreen et al. (1953) have

observed that the serum inorganic sulfate level of the blood is not proportional to sulfur intake at least at higher intake levels. The latter workers reported no change or slightly lower levels of serum sulfate in lambs receiving rations containing 0.29% sulfur as compared with lambs fed the control ration (0.23% sulfur). Whiting et al. (1954) has presented evidence which suggests that sulfur intake of sheep does not necessarily influence the serum inorganic sulfate levels. Rations containing levels of sulfur from 0.08% to 0.17% had very little effect on the serum sulfate levels.

Kroger and Carroll (1964) have found that feeding high levels of sulfur as gypsum resulted in higher levels of inorganic sulfate in the blood of cattle. However, the work reported by Dale et al. (1954) suggests that the serum sulfate level in cattle may have little value in determining sulfur intake. When cattle were all fed the same ration, up to 300% difference in the serum sulfate levels of individual animals was observed.

Mineral interactions

When urea replaces a portion or all of the vegetable protein source in ruminant rations, mineral imbalances may become important. This has recently been reviewed by Goodrich (1965). He states that 6 parts of corn and 1 part of urea supplies as much nitrogen as an equal amount of soybean

meal, but only 13.5, 38.1 and 9.1% as much calcium, phosphorus and sulfur as soybean meal. Meiske and Goodrich (1966) have given a brief review of the value of trace mineral supplementation in some urea-containing rations.

Goodrich and Tillman (1966a) reported some rather interesting interactions of minerals involving the utilization of both urea and sulfur. Growth and mineral balance studies using purified rations with lambs were conducted to determine the effect of sulfur source, nitrogen source and copper levels on growth and balance of nitrogen, sulfur, copper, phosphorus and calcium. Additions of sulfur as sulfate decreased retention of copper; added sulfate resulted in the greater sulfur digestibility but because of urinary losses, retention of sulfur fed as elemental sulfur was greater; and both urea and sulfate lowered the retention of calcium. In a second paper, Goodrich and Tillman (1966b) reported reduced plasma phosphorus levels and a decrease in liver copper stores when feeding high levels of sulfur. These two reports suggest that a critical evaluation of ration mineral balances should be made when urea is fed in ruminant rations.

Rumen microbial activity

A requirement for sulfur by rumen microorganisms has been demonstrated in several experiments. It has been shown that rumen microorganisms utilize elemental sulfur in the synthesis of microbial proteins. This is documented elsewhere in this

review by the evidence of increased performance of animals with the incroporation of sulfur in the rations. Hunt et al. (1954) using an in vitro system observed increased cellulose digestion, urea utilization, riboflavin and vitamin $B_{12}$ synthesis when sulfur either as the sulfate or methionine was added to the media. Burroughs et al. (1951) had previously shown that sulfur was one of several minerals required for maximum urea utilization and cellulose digestion. Hubbert et al. (1958) further demonstrated by in vitro techniques that sulfur was required and that the microorganisms were quite tolerant of high levels of sulfur in the media. More recently, Martin et al. (1964) has demonstrated a need for sulfur by the rumen microorganisms. In vivo cellulose digestion of a purified ration by steers was reduced by 70% in the absence of sulfur while in vitro cellulose digestion was reduced by 95%. Evans and Davis (1966) observed that sulfur increased microbial activity for digestion of cellulose. Sixty-five micrograms of sulfur per milliliter of rumen fluid was optimum. This was produced by feeding 0.29% sulfur as sodium sulfate.

The sulfur needed for the formation of sulfur-containing amino acids can be supplied to the mixed rumen bacteria as sulfate, sulfide or elemental sulfur. Elemental sulfur was shown by Starks et al. (1953) to be utilized by lambs fed a semi-synthetic diet otherwise deficient in sulfur since

control lambs became ill and died. The work of Emery et al. (1957a, b), Block et al. (1951) and Anderson (1956) showed that sulfur from sulfate was incorporated into mixed rumen microbial protein as cystine and methionine, but the results of Müller and Krampitz (1955) suggested that only the bacteria incorporate sulfur. It is most likely that sulfur from sulfate is not directly incorporated into the microogranisms but is first reduced to sulfide as suggested by Lewis (1954). Anderson (1956) reported that sulfide is also produced by mixed rumen bacteria from sulfur amino acids in feed protein.

Reports in the literature concerning the effect of sulfur on the rumen microbial population are very scarce. Williams and Moir (1951) made ruminal flora studies in lambs and compared the total number of free microorganisms in the rumen of lambs fed different sources of dietary nitrogen. Variation in numbers of microorganisms was observed; however the bacteria increased dramatically with the addition of the sulfur containing amino acid methionine to a urea ration over that observed in a ration containing urea alone. Gall et al. (1951) made similar observations feeding urea and sulfate sulfur in purified rations to sheep. They observed that a urea plus sulfur ration supported a bacterial population of about double that found in rumen contents of animals fed urea without sulfur. Although Whanger and Matrone (1965) did not make cell counts in rumen fluid, a greater harvest

of cells per unit volume was observed from sheep fed a urea-sulfur purified ration than sheep fed a sulfur free ration.

Little could be found in the literature concerning the effects of sulfur on the end products of microbial fermentation in the rumen. Gzhitskii et al. (1961) fed milk cows rations containing urea and sodium sulfate. Total volatile fatty acids and protein sulfur increased in the rumen of cows given urea and sulfur. A predominance of propionic over butyric acid was noted in these cows while no change in lactic or acetic acid was observed. Kolesov et al. (1961) observed no change in rumen function when elemental sulfur or sodium sulfate was added to urea rations of sheep; however blood urea appeared lower in sulfur supplemented animals. In observing the rumen fermentation of sheep fed either sulfur adequate or sulfur deficient purified diets, Whanger and Matrone (1965, 1966) noted marked differences. The levels of propionate, butyrate and higher fatty acids in rumen fluid were greater from sulfur fed animals. Isotope studies indicated that microorganisms from sulfur-fed animals could synthesize butyrate and higher fatty acids from acetate, whereas those from the sulfur deficient animals could not. Only traces of lactic acid were produced in rumen fluid of sulfur-fed animals whereas large amounts were produced in sulfur-deficient sheep.

# EXPERIMENTAL PROCEDURES

## General

The feedlot trials reported in this dissertation are on file in the Ruminant Nutrition Section of the Animal Science Department, Iowa State University, Ames, Iowa. They are numbered as Cattle Experiments 727, 734, 748, 751 and 759. Experiments 727, 734 and 751 deal primarily with supplemental vitamin E and selenium additions to rations; Experiment 748 was a trial in which supplemental selenium and sulfur was fed and in Experiment 759 supplemental sulfur was the principal variable in the experimental rations.

These experiments were conducted at the University Beef Nutrition Farm. The cattle were housed in groups of five or six animals in a shed open to the south with small outside paddocks surfaced with concrete. Broken corncobs were provided as bedding. The cattle had access to noniodized block salt and fresh water at all times.

The experimental animals in these trials were weighed individually on 3 consecutive days at the beginning and end of each trial with the initial and final weights being averages of these 3-day weights. Periodically at 2- or 3-week intervals throughout the feeding trials, the cattle were weighed. Average daily gain and feed efficiency was calculated for all experimental lots every 3 or 4 weeks. Data

collected at slaughter from the experimental animals included carcass weight, carcass grade, dressing percent and fat thickness and loin eye area at the 12th rib.

Statistical analyses of the data collected in this research were conducted according to the methods of Snedecor (1956).

## Vitamin E and Arsanilic Acid Additions to Cattle Rations

### Experiment 727

A vast literature in the area of selenium toxicity has been developed over the years. One method whereby selenium toxicity has been combatted has been the feeding of arsenicals in an attempt to block the toxic influence of the naturally occurring selenium in feedstuffs.

There has also been a considerable amount of work reported in the literature concerning the interrelationship between vitamin E and selenium nutrition. Previous work at Iowa State has shown improved liveweight gain and feed utilization when additions of either vitamin E or selenium were made to no-hay cattle finishing rations low in vitamin E. These experiments were unique in that no previous experiments had revealed a need for supplemental sources of this vitamin or this mineral in cattle finishing rations.

Based upon the above observations, it was suggested that

one might demonstrate the need for selenium in beef cattle by supplementing the rations with arsanilic acid. Thus, the primary purpose of this experiment was to determine the influence of a small amount of arsanilic acid upon the response of supplemental vitamin E in the finishing ration.

Seventy-two good to choice yearling cattle weighing 775 pounds per animal were divided into 12 lots of six animals each upon the basis of randomization within each general breed classification. Six lots of animals were fed rations containing no arsanilic acid and six lots were fed rations containing 400 milligrams of arsanilic acid daily. Within each of these two groups, three of the lots received no supplemental vitamin E and the other three received 200 I.U. of vitamin E daily per animal. This experiment contained three replicates of each treatment. The experimental design is outlined in Table 1.

The cattle received a full feed of rolled corn, 4 pounds of ground corncobs and 2 pounds of supplement per animal per day. The supplemental mixtures fed the experimental animals are described in Table 2.

At the time that this experiment was planned, it was anticipated that the trial would be conducted for approximately 5 months at which time the animals would be weighed off trial, the arsanilic acid removed from the rations and the animals retained in the feedlot for a 2-week period prior to slaughter to allow the excretion of any residual arsanilic acid from

Table 1. Design for Experiment 727

| No arsanilic Acid | | 400 mg. arsanilic Acid | |
|---|---|---|---|
| No vitamin E | 200 I.U. vitamin E | No vitamin E | 200 I.U. vitamin E |
| Lot 1 | Lot 2 | Lot 3 | Lot 4 |
| Lot 5 | Lot 6 | Lot 7 | Lot 8 |
| Lot 9 | Lot 10 | Lot 11 | Lot 12 |

Table 2. Supplemental mixtures fed to cattle in Experiment 727 (all values expressed in lb.)

| Ingredients | Lots 1,5,9 | Lots 2,6,10 | Lots 3,7,11 | Lots 4,8,12 |
|---|---|---|---|---|
| Soybean oil meal | 1007.4 | 1001.4 | 1004.8 | 998.8 |
| Urea | 84.0 | 84.0 | 84.0 | 84.0 |
| Dicalcium phosphate | 42.0 | 42.0 | 42.0 | 42.0 |
| Limestone | 48.0 | 48.0 | 48.0 | 48.0 |
| Trace mineral premix[a] | 5.0 | 5.0 | 5.0 | 5.0 |
| Stilbesterol[b] | 12.0 | 12.0 | 12.0 | 12.0 |
| Vitamin A premix[c] | 1.6 | 1.6 | 1.6 | 1.6 |
| Vitamin E premix[d] | -- | 6.0 | -- | 6.0 |
| Arsanilic acid premix[e] | -- | -- | 2.6 | 2.6 |
| | 1200.0 | 1200.0 | 1200.0 | 1200.0 |

[a]The percent composition of the trace mineral premix was as follows: manganese - 4.7, iron - 8.0, zinc - 0.2, copper - 0.5 and cobalt - 0.2.

[b]Stilbesterol premix contained 1 gm. diethylstilbesterol per lb. and provided 20 mg. per animal per day.

[c]Vitamin A premix contained 2.3 million I.U. of vitamin A per lb. and provided 6,132 I.U. per animal per day.

[d]Vitamin E premix contained 20,000 I.U. of vitamin E per lb. and provided 200 I.U. per animal per day.

[e]Arsanilic acid premix contained 90 gm. arsanilic acid per lb.

the tissues. Due to an unfortunate misunderstanding which developed with the Food and Drug Administration, the feeding of arsanilic acid was required to be terminated at an early date. Consequently, this additive was in the ration for a period of 112 days from October 10, 1963, to January 30, 1964.

From January 30, 1964, to April 29, 1964, the experimental procedure was altered so that six lots were fed a control ration of no supplemental vitamin E (Lots 1,3,5,7,9 and 11) while six lots were fed 200 I.U. of supplemental vitamin E per animal per day (Lots 2,4,6,8,10 and 12). This was a period of 90 days.

The cattle were weighed every 14 days throughout the trial. Average daily gains and feed efficiency values were calculated for all experimental lots each 28-day period.

## Selenium and Arsanilic Acid Additions to Cattle Rations

### Experiment 734

Data from Experiment 727 with arsanilic acid in the ration indicated that this additive resulted in a depressed rate of gain. Vitamin E additions partially alleviated this depressed rate of gain suggesting that the arsanilic acid may have had the effect of blocking the nutritional action of the selenium in the natural feedstuffs, and the supplemental vitamin E may have had substitution value for the selenium. There-

fore, the purpose of this trial was to feed two levels of arsanilic acid to determine a level of this additive which would most effectively block the nutritional action of the naturally occurring selenium in the available feedstuffs as well as the supplemental selenium which replaced the vitamin E used in the first trial.

Sixty-six steers of mixed breeding were placed in the lots upon the basis of randomization within two weight groups. Six lots contained five steers each of the heavier weight cattle while the remaining six lots contained six steers each which weighed approximately 100 pounds less than the heavier steers. The average weight of the 66 steers used in this trial was 710 pounds.

The experimental treatments in this trial consisted of four lots of animals receiving no arsanilic acid, four lots receiving 200 milligrams of arsanilic acid per steer per day and four lots receiving 400 milligrams of arsanilic acid per steer per day. In addition, one-half of all the experimental rations were supplemented with selenium at the rate of 0.1 ppm. The experimental design is outlined in Table 3.

The daily ration consisted of a full feed of rolled shelled corn, 4 pounds of ground corncobs and 2 pounds of the supplemental mixture which is outlined in Table 4. The arsanilic acid supplement was removed from the experimental rations 2 weeks prior to slaughter of these animals.

Table 3. Design for Experiment 734[a]

| | No selenium | 0.1 ppm selenium |
|---|---|---|
| No arsanilic acid | Lot 1 (5)<br>Lot 3 (6) | Lot 2 (5)<br>Lot 4 (6) |
| 200 mg. arsanilic acid | Lot 5 (5)<br>Lot 7 (6) | Lot 6 (5)<br>Lot 8 (6) |
| 400 mg. arsanilic acid | Lot 9 (5)<br>Lot 11 (6) | Lot 10 (5)<br>Lot 12 (6) |

[a]Numbers in parentheses indicate the number of animals in the lots.

Table 4. Supplemental mixtures fed to cattle in Experiment 734 (all values expressed in lb)

| Ingredients | Lots 1,3 | Lots 2,4 | Lots 5,7 | Lots 6,8 | Lots 9,11 | Lots 10,12 |
|---|---|---|---|---|---|---|
| Soybean oil meal | 1013.4 | 1013.4 | 1012.1 | 1012.1 | 1010.8 | 1010.8 |
| Urea | 84.0 | 84.0 | 84.0 | 84.0 | 84.0 | 84.0 |
| Dicalcium phosphate | 42.0 | 42.0 | 42.0 | 42.0 | 42.0 | 42.0 |
| Limestone | 48.0 | 36.0 | 48.0 | 36.0 | 48.0 | 36.0 |
| Trace mineral[a] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Stilbesterol[b] | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| Vitamin A[c] | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 |
| Arsanilic acid[d] | -- | -- | 1.3 | 1.3 | 2.6 | 2.6 |
| Selenium premix[e] | -- | 12.0 | -- | 12.0 | -- | 12.0 |
| | 1200.0 | 1200.0 | 1200.0 | 1200.0 | 1200.0 | 1200.0 |

[a]The percent composition of the trace mineral premix was as follows: manganese - 4.7, iron - 8.0, zinc - 0.2, copper - 0.5, and cobalt - 0.2.

[b]Stilbesterol premix contained 2 gm diethylstilbesterol per lb. and provided 20 mg. per animal per day.

[c]Vitamin A premix contained 2.3 million I.U. of vitamin A per lb. and provided 6,132 I.U. per animal per day.

[d]Arsanilic acid premix contained 90 gm. arsanilic acid per lb.

[e]Selenium premix contained 0.183 gm. $Na_2SeO_3 \cdot 5H_2O$ per lb. of limestone.

This trial began on June 2, 1964, and was in progress until November 17, 1964, or a total of 168 days. Cattle were weighed every 14 days and average daily gain and feed efficiency computed at 28-day periods.

## Red Kidney Beans and Selenium in Beef Heifer Rations

### Experiment 751

Raw kidney beans are known to be low in selenium. These kidney beans when fed in the ration of pregnant ewes have resulted in the occurrence of muscular dystrophy in their lambs. It has been suggested that in addition to their low selenium that they contain tocopherol inhibitory factors which enhance the development of muscular dystrophy.

With this in mind, the present experiment was designed to further study the effect of supplemental selenium in finishing rations of beef cattle.

Thirty Hereford heifers averaging approximately 550 pounds were randomly allotted into six lots with five heifers per lot. Two experimental treatments were used in this trial. Three replicate lots served as controls with the remaining three lots receiving supplemental selenium at the rate of approximately 0.1 ppm of the total ration. The experimental design is outlined in Table 5.

The daily ration consisted of cracked, raw kidney beans in an amount which the heifers would consume, a full feed of

Table 5. Design for Experiment 751

| No selenium | 0.1 ppm selenium |
|---|---|
| Lot 1 | Lot 2 |
| Lot 3 | Lot 4 |
| Lot 5 | Lot 6 |

rolled shelled corn, 4 pounds of ground corncobs and 2 pounds of a supplemental mixture which is described in Table 6.

This trial was initiated on April 15, 1965, and continued to October 6, 1965, or a total of 174 days. The animals were weighed every 14 days throughout the trial and average daily gains and feed efficiency calculated at 28-day intervals.

## Selenium, Sulfur and Unsaturated Oil Additions to Cattle Rations

### Experiment 748

In the preceding experiments when cattle finishing rations were supplemented with arsanilic acid, an apparent depressed rate of gain due to the feeding of this compound was observed. If the addition of arsanilic acid was blocking the nutritional action of selenium, it was not apparent since supplemental vitamin E or selenium did not significantly improve gains over the control animals. Based on these observations, another approach in studying the significance of selenium in feedlot rations appeared appropriate.

Table 6. Supplemental mixtures fed to cattle in Experiment 751 (all values expressed in lb.)

| Ingredients | Lots 1,3,5 | Lots 2,4,6 |
|---|---|---|
| Corn | 900.0 | 900.0 |
| Urea | 25.0 | 25.0 |
| Dicalcium phosphate | 30.0 | 30.0 |
| Limestone | 35.0 | 26.0 |
| Trace mineral premix[a] | 4.2 | 4.2 |
| Stilbesterol premix[b] | 2.5 | 2.5 |
| Vitamin A premix[c] | 3.3 | 3.3 |
| Selenium premix[d] | -- | 9.0 |
| | 1000.0 | 1000.0 |

[a]The percent composition of the trace mineral premix was as follows: manganese - 4.7, iron - 8.0, zinc - 0.2, copper - 0.5 and cobalt - 0.2.

[b]Stilbesterol premix contained 2 gm. diethylstilbesterol per lb. and provided 10 mg. per heifer per day.

[c]Vitamin A premix contained 2.3 million I.U. of vitamin A per lb. and provided 15,180 I.U. per heifer per day.

[d]Selenium premix contained 0.183 gm. $Na_2SeO_3 \cdot 5H_2O$ per lb. of limestone.

In the seleniferous areas of the northern Great Plains, the addition of sulfur to livestock rations to alleviate selenium toxicities has been utilized. Sulfur has further been implicated in playing a role in antagonizing selenium absorption which results in muscular dystrophy in calves and lambs. Thus, the primary purpose of this experiment was to observe the influence of supplementing cattle rations with sulfur in

an attempt to block the nutritional effect of the natural and supplemental selenium in the rations.

The sulfur added in this experiment was a crude source of Glauber's salt, a sodium sulfate compound ($Na_2SO_4 \cdot 10H_2O$), and was fed at the rate of one-third pound per animal per day. This compound contained approximately 9.25% sulfur.

Vegetable oils high in polyunsaturated fatty acids have been shown to increase the animal's requirement for vitamin E. Therefore, it was postulated that a vegetable oil in the ration might place a stress upon the selenium and vitamin E needs of the steers in this experiment. Soybean oil was added to a portion of the rations in this trial to evaluate supplemental selenium as a replacement for vitamin E.

A crude source of soybean oil was used in this trial. Since vegetable oils are rich sources of tocopherols, an attempt was made to destroy them by heat treatment and lauryl peroxide by the method of Machlin (1961).

Seventy-two Holstein steers with an average weight of approximately 600 pounds were allotted into twelve groups of six animals each. Four lots of cattle received a basal ration containing no supplemental sulfur, four lots received the supplemental sulfur compound alone and four lots received a conbination of the supplemental sulfur compound and soybean oil. When soybean oil was included in the ration it was mixed with only the corn portion of the ration at the rate of 8% of

the weight of the corn. Superimposed upon these treatments was a treatment of supplemental selenium at the level of 0.2 ppm of the ration. The experimental design is outlined in Table 7.

The daily rations consisted of a full feed of rolled shelled corn, 6 pounds of ground corncobs and 2 pounds of the supplemental mixtures which are outlined in Table 8.

This trial began on February 25, 1965. After 127 days, the soybean oil treatment was discontinued and the lots of animals which had been receiving this treatment received two-thirds of a pound of the supplemental sulfur compound per animal per day for the balance of the trial. The cattle were weighed every 14 days throughout the experiment and average

Table 7. Design for Experiment 748

| | No selenium | 0.2 ppm selenium |
|---|---|---|
| Basal ration | Lot 1<br>Lot 2 | Lot 3<br>Lot 4 |
| Basal and sulfur[a] | Lot 5<br>Lot 6 | Lot 7<br>Lot 8 |
| Basal, sulfur[a] and soybean oil[b] | Lot 9<br>Lot 10 | Lot 11<br>Lot 12 |

[a]Glauber's salt was mixed with a portion of the corn in the ration to provide one-third of a pound of the sulfur compound per animal per day.

[b]Soybean oil was mixed with the corn portion of the ration at the rate of 8% of the weight of the corn.

Table 8. Supplemental mixtures fed to cattle in Experiment 748 (all values expressed in lb.)

| Ingredients | Lots 1,2,5,6,9,10 | Lots 3,4,7,8,11,12 |
|---|---|---|
| Corn | 500.0 | 500.0 |
| Soybean meal | 253.6 | 253.6 |
| Urea | 100.0 | 100.0 |
| Dicalcium phosphate | 60.0 | 60.0 |
| Limestone | 75.0 | 57.0 |
| Trace mineral premix[a] | 4.2 | 4.2 |
| Stilbesterol premix[b] | 5.0 | 5.0 |
| Vitamin A premix[c] | 2.2 | 2.2 |
| Selenium premix[d] | -- | 18.0 |
| | 1000.0 | 1000.0 |

[a]The percent composition of the trace mineral premix was as follows: manganese - 4.7, iron - 8.0, zinc - 0.2, copper - 0.5 and cobalt - 0.2.

[b]Stilbesterol premix contained 2 gm. diethylstilbesterol per lb. and provided 20 mg. per animal per day.

[c]Vitamin A premix contained 2.3 million I.U. of vitamin A per lb. and provided 10,120 I.U. per animal per day.

[d]Selenium premix contained 0.183 gm. $Na_2SeO_3 \cdot 5H_2O$ per lb. of limestone.

daily gain and feed efficiency computed at 28-day intervals. A heavy weight lot of cattle from each experimental treatment was removed from the experiment August 24, 1965, after 180 days. The lighter weight cattle remained on experiment until September 22, 1965, or a total of 209 days.

Sulfur analyses of feedstuffs used in this and subsequent

experiments were made by the method described by the Parr Instrument Company (1960).

## Supplemental Sulfur Additions to Cattle Finishing Rations

### Experiment 759

Because of the inability of obtaining a growth response with supplemental selenium in any of the trials as had been observed earlier by Barringer (1964) and an interesting but unexpected observation made in Experiment 748, this experiment was designed to further observe the influence of supplementing cattle finishing rations with inorganic sulfur. It had been generally believed that cattle finishing rations were adequate in sulfur content. The results noted earlier suggested that a reappraisal of sulfur nutrition was appropriate.

Eighty steers averaging about 625 pounds were allotted into 15 lots of five or six animals upon the basis of randomization within each feeder grade group. Five experimental rations were used in this trial with three replicates per treatment, one each of: Angus, mixed breeding and Holstein steers. The experimental treatments used were a control group, three treatments of varying levels of elemental sulfur and one treatment of sulfur as a crude source of Glauber's salt, a sodium sulfate compound. The experimental design and

treatments are outlined in Table 9.

The daily ration consisted of a full feed of rolled shelled corn, 4 pounds of ground corncobs and 2 pounds of the supplemental mixtures outlined in Table 10. This mixture provided 0.25 pound of urea in the daily ration of these steers.

The experiment was started on September 30, 1965, and was completed on April 28, 1966, with a feeding period of 216 days. The cattle were weighed every 21 days throughout the trial and average daily gains and feed efficiency calculated. At the time of slaughter, blood samples were collected for serum inorganic sulfate analysis. The method for this analysis was that of Berglund and Sörbo (1960).

## Blood Serum Inorganic Sulfate Study

### Experiment 124

The values of the serum inorganic sulfate analyses of blood samples collected from animals slaughtered in Experiment 759 were variable although there was a trend for increased serum sulfate with increasing levels of sulfur in the ration. The primary purpose of this experiment was to further study the result of varying levels of supplemental elemental sulfur in the ration upon the blood serum inorganic sulfate levels of beef cattle.

Four Hereford steer calves weighing from 500-600 pounds

Table 9. Design for Experiment 759[a]

| Control | Elemental Sulfur (lb./day) | | | Glauber's salt |
|---|---|---|---|---|
| | 0.015 | 0.030 | 0.060 | 0.33 lb./day |
| Lot 1 (6) | Lot 2 (5) | Lot 3 (5) | Lot 4 (6) | Lot 5 (5) |
| Lot 6 (5) | Lot 7 (6) | Lot 8 (5) | Lot 9 (5) | Lot 10 (6) |
| Lot 11 (5) | Lot 12 (5) | Lot 13 (6) | Lot 14 (5) | Lot 15 (5) |

[a]Numbers in parentheses indicate the number of animals in the lot.

Table 10. Supplemental mixtures fed to cattle in Experiment 759 (all values expressed in lb.)

| Ingredients | Lots 1,6,11 | Lots 2,7,12 | Lots 3,8,13 | Lots 4,9,14 | Lots 5,10,15 |
|---|---|---|---|---|---|
| Ground corn | 783.0 | 775.5 | 768.0 | 753.0 | 618.0 |
| Urea | 125.0 | 125.0 | 125.0 | 125.0 | 125.0 |
| Dicalcium phosphate | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 |
| Limestone | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Stilbesterol premix[a] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Trace mineral premix[b] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Vitamin A premix[c] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Elemental sulfur | -- | 7.5 | 15.0 | 30.0 | -- |
| Glauber's salt | -- | -- | -- | -- | 165.0 |
| | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 |

[a]Stilbesterol premix contained 2 gm. diethylstilbesterol per lb. and provided 20 mg. per animal per day.

[b]The percent composition of the trace mineral premix was as follows: manganese - 4.4, iron - 6.6, zinc - 12.0, copper - 1.3 and cobalt - 0.2, magnesium - 20.0 and iodine - 0.3.

[c]Vitamin A premix contained 2.3 million I.U. of vitamin A per lb. and provided approximately 22,500 I.U. per animal per day.

were used in this experiment. The supplemental mixtures fed were those used in Experiment 759 (Table 10) except that urea was reduced to 10% of the mixture with corn added. Because of low feed consumption, only 1.5 pounds of this mixture was fed. This resulted in a daily intake of 0.15 pound of urea and 0.011, 0.023, and 0.045 pound of elemental sulfur. Rolled shelled corn and ground corncobs were also fed.

In the initial portion of this trial, the steers were individually fed in stanchions twice daily. Prior to starting the experimental treatments, the steers were allowed to accustom themselves to the stanchions and the control ration for a 3-week period. During this time, feed consumption was measured and the level of intake of the animal consuming the lowest amount of feed was selected as the level of intake for the experimental periods. A 4 X 4 Latin-square design was used. Each experimental ration was fed for a 1-week period with blood samples taken just prior to the morning feed at the beginning and the end of each period. At the conclusion of each period, the steers were all fed the control supplemental mixture for 1 week prior to the beginning of the next experimental treatment.

The second portion of this trial was a study of the blood serum inorganic sulfate levels at various times after feeding. In this study the steers were group fed. Only two treatments were used in this portion of the trial and they were the

control supplement and low level of supplemental elemental sulfur as described previously. Blood samples for analyses were drawn at 0, 3, 6, 9 and 12 hours after the morning feed after the steers had been on the experimental treatments for a period of 1 week.

All blood samples in this experiment were taken from the jugular vein and serum inorganic sulfate determined by the method of Berglund and Sörbo (1960).

## Effect of Sulfur Upon Rumen Fermentation and *In Vitro* Starch Digestion

The objective of this experiment was to determine the effects of supplemental sulfur in the cattle rations which contained urea as the sole source of supplemental protein. In Experiments 748 and 759, the addition of sulfur to the finishing ration had resulted in an initial stimulation of feedlot gains of 127 and 83 days, respectively. Thus, this experiment was planned to observe some of the fermentation processes occurring in the rumen over an extended period of time with the addition of sulfur to urea rations.

Two yearling steers weighing approximately 700 pounds were fistualted and 4-inch permanent plastic cannulae were inserted. These steers were housed at the Ruminant Nutrition Laboratory on the campus. The rations used in this trial were rolled shelled corn, ground corn cobs and a

modification of the protein supplement found in Table 10 (Experiment 759). The urea concentration of these mixtures was reduced from 12.5% to 10.0% with an equivalent amount of corn replacing the urea. The steers received the control urea mixture for a 2-week period to allow them to become accustomed to the diet before sampling began. During this period, feed consumption was observed and the level of intake of the steer consuming the lowest amount of feed was selected as the level for the balance of the experiment. Because of poor feed consumption, only 1.5 pounds of the protein supplement mixtures were fed. The sulfur supplements used in these trials were the medium level of elemental sulfur and the supplement containing Glauber's salt. These supplements provided a daily intake of 0.023 pound of elemental sulfur and 0.25 pound of Glauber's salt.

This experiment was in progress for a total of 146 days. The animal designated as Steer B received the control supplement for half of this time and was then fed the supplement containing Glauber's salt. The animal designated as Steer S received the Glauber's salt supplement for 42 days, was then fed the control supplement for 13 days and then received the elemental sulfur supplement for the balance of the experiment.

Samples of rumen fluid were obtained from the steers through the fistula at various intervals after feeding and periodically throughout the trial for measurement of ammonia

levels, pH, volatile fatty acid levels, total viable bacterial numbers and for use in *in vitro* starch digestion studies. Digesta was filtered through four layers of cheesecloth to obtain the rumen fluid for these determinations.

Ruminal ammonia

Samples for ammonia determination were collected at 1, 2, 3 and 4 hours after feeding every 7 to 10 days throughout this trial. These samples were immediately centrifuged and analyzed for ammonia using the Modified Conway dish as described by Öbrink (1955). Ammonia values were determined after 3 hours incubation at 39°C.

Volatile fatty acids and pH

Samples for volatile fatty acid determination were collected at 0, 1, 2, 3, 4, 6 and 8 hours after feeding every 14 to 21 days during the trial. To 10 milliliters of rumen fluid, 0.5 milliliter of a 5% solution of mecuric chloride was added. After centrifugation at 8,000 RPM for 30 minutes, the supernatant was collected and frozen. Preparation at the time of analysis included the addition of 1 milliliter of a freshly prepared 25% solution of metaphosphoric acid to 5 milliliters of the supernatant. After centrifugation of this solution at 12,000 RPM for 10 minutes, total and molar proportions of volatile fatty acids were determined by gas-liquid chromatography as described by Baumgardt (1964). A slight modifi-

cation of this procedure was used in our laboratory. This was that an oven temperature of 136°C. and an injector temperature of 176°C. was maintained on the chromatograph for the analysis of these samples.

Measurement of pH was made on all samples obtained for volatile fatty acid analysis immediately upon collection and prior to any preparation for analysis.

Total viable bacterial numbers

In the course of this trial, counts of the total viable bacterial numbers present in the rumen fluid of these steers were made. These counts were usually made at 7- or 10-day intervals and samples for making the counts were collected at 4 hours after the morning feed. Twenty milliliters of rumen fluid were collected from each steer into tubes which had previously been gassed with carbon dioxide. These tubes were then placed in a prewarmed insulated container and taken to the laboratory where total viable bacterial counts were made using the procedure described by Bryant and Robinson (1961).

In vitro starch digestion

Several in vitro studies were made to determine the effect of sulfur in the medium upon starch digestion by rumen microorganisms. Rumen fluid was obtained via fistula from a steer receiving the basal ration used in these trials. The

contents were collected 2 hours after feeding and strained through four layers of cheesecloth into a prewarmed insulated container for transport to the laboratory. The procedure used was that described by Loper et al. (1966). The composition of the nutrient medium of Loper et al. (1966) was altered by replacing sulfur containing compounds with the corresponding chlorides to insure a nutrient medium which was free of sulfur. The contents of the basal medium which was used in these trials is shown in Table 11. The treatments used were sulfur to nitrogen (S:N) ratios of 0, 1:16, 1:12, 1:8 and 1:4. Solutions of sodium sulfate were prepared to contain 0, 0.5825, 0.7767, 1.1650 and 2.3300 milligrams of sulfur per 0.5 milliliter of solution. A 0.5 milliliter aliquot of these solutions was added to 20 milliliters of the basal medium containing 0.5% corn starch and microorganisms in the incubation tubes. This provided 0, 29, 39, 58 and 117 micrograms of sulfur per milliliter of the incubation medium and the desired S:N ratios. Each tube was fitted with a rubber stopper containing inlet and outlet glass tubings for bubbling the solution with a constant flow of carbon dioxide. Each tube was connected directly to the carbon dioxide source to insure that there would be no movement of hydrogen sulfide from one tube to another. Cultures were incubated for 8 hours in a 39°C. waterbath and all treatments were ran in triplicate within an experiment.

Table 11. Incubation medium for *in vitro* rumen microorganism fermentation

| Constituent | Grams/liter |
|---|---|
| $KH_2PO_4$ | 0.600 |
| $Na_2HPO_4 \cdot 7H_2O$ | 1.198 |
| $NaHCO_3$ | 3.500 |
| KCl | 4.000 |
| NaCl | 4.000 |
| $MgCl \cdot 6H_2O$ | 0.507 |
| $FeCl_3 \cdot 6H_2O$ | 0.073 |
| $CaCl_2$ | 0.550 |
| Urea | 2.000 |

Upon termination of incubation, bacterial action was stopped by addition of 1 milliliter of saturated mercuric chloride per tube. The tubes were centrifuged and an aliquot of fluid from each tube within a treatment group was pooled for analyses of ammonia and volatile fatty acid levels. The percent of starch digested in each tube was then determined using the gravimetric method described by Loper *et al.* (1966). Ammonia and volatile fatty acid levels were determined as described previously.

# RESULTS

## Vitamin E and Arsanilic Acid Additions to Cattle Rations

### Experiment 727

The results of the effect of the addition of vitamin E and arsanilic acid supplementation on feedlot performance appear in Tables 12 and 13. For this experiment and subsequent experiments, the summary tables appear in the Results section of each experiment with all other tables appearing in the Appendix.

Arsanilic acid was fed only during the initial 112 days of this trial and these results are found in Table 12. Even though differences in performance were seen in treatment averages, statistical analysis indicates that these were not statistically significant. Addition of vitamin E to the basal ration increased average daily gain and improved feed efficiency by 2.5% and 3.0%. When arsanilic acid was added to the rations, gain and feed efficiency was improved by 5.5% and 4.5% in the presence of supplemental vitamin E but gains were not as great as without arsanilic acid. The greater response from vitamin E addition to the ration in the presence of arsanilic acid suggests that the arsenical may have had the desired effect of suppressing the utilization of the natural occurring selenium of the feeds and that vitamin E

Table 12. Experiment 727: Cattle performance supplementing the ration with vitamin E and arsanilic acid

| Added to ration/steer/day: | No arsanilic acid | | 400 mg. arsanilic acid | |
|---|---|---|---|---|
| | No vitamin E | 200 I.U. vitamin E | No vitamin E | 200 I.U. vitamin E |
| | | 112 day experiment | | |
| No. steers | 18 | 18 | 18 | 18 |
| Initial weight, lb. | 781 | 782 | 784 | 781 |
| Final weight, lb. | 1126 | 1135 | 1114 | 1127 |
| Av. daily gain, lb. | 3.07 | 3.15 | 2.92 | 3.09 |
| Av. daily ration, lb. | | | | |
| Rolled corn | 16.5 | 16.4 | 16.2 | 16.3 |
| Corncobs | 4.0 | 4.1 | 4.0 | 4.1 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 22.5 | 22.5 | 22.2 | 22.4 |
| Feed/100 lb. gain, lb. | 735 | 712 | 759 | 725 |

| Added to ration/steer/day: | No arsanilic acid | 400 mg. arsanilic acid | No vitamin E | 200 I.U. vitamin E |
|---|---|---|---|---|
| | | 112 day experiment | | |
| No. steers | 36 | 36 | 36 | 36 |
| Initial weight, lb. | 782 | 783 | 783 | 782 |
| Final weight, lb. | 1130 | 1119 | 1119 | 1131 |
| Av. daily gain, lb. | 3.11 | 3.01 | 3.00 | 3.12 |
| Av. daily ration, lb. | | | | |
| Rolled corn | 16.4 | 16.2 | 16.3 | 16.3 |
| Corncobs | 4.1 | 4.1 | 4.1 | 4.1 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 22.5 | 22.3 | 22.4 | 22.4 |
| Feed/100 lb. gain, lb. | 722 | 742 | 746 | 719 |

Table 13. Experiment 727: Cattle performance supplementing the ration with vitamin E and arsanilic acid

| Added to ration/steer/day: | No arsanilic acid No vitamin E | No arsanilic acid 200 I.U. vitamin E | 400 mg. arsanilic acid[a] No vitamin E | 400 mg. arsanilic acid 200 I.U. vitamin E |
|---|---|---|---|---|
| | | 202 day experiment | | |
| No. steers | 17 | 17 | 17 | 17 |
| Initial weight, lb. | 789 | 773 | 777 | 776 |
| Final weight, lb. | 1297 | 1302 | 1293 | 1305 |
| Av. daily gain, lb. | 2.55 | 2.62 | 2.56 | 2.62 |
| Av. daily ration, lb. | | | | |
| Rolled corn | 16.9 | 17.3 | 16.9 | 16.8 |
| Corncobs | 4.0 | 4.0 | 4.0 | 4.1 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 22.9 | 23.3 | 22.9 | 22.9 |
| Feed/100 lb. gain, lb. | 901 | 890 | 895 | 874 |
| Carcass measurements | | | | |
| Chilled carcass wt., lb. | 805 | 805 | 805 | 810 |
| Loin eye area, sq. in. | 12.5 | 12.7 | 12.9 | 12.7 |
| Loin fat thickness, in. | 0.82 | 0.85 | 0.88 | 0.85 |
| Carcass grade[b] | 7.8 | 7.7 | 7.6 | 7.6 |
| Dressing percent[c] | 62.1 | 61.8 | 62.2 | 61.4 |

[a]Arsanilic acid was removed from the ration after 112 days.

[b]Carcass grade was given a numerical value as follows: low good - 4, average good - 5, high good - 6, low choice - 7, average choice - 8 and high choice - 9.

[c]Dressing percent based on full weights off experiment and warm carcass weight shrunk 2.5%.

Table 13 (Continued)

| Added to ration/steer/day: | No arsanilic acid | 400 mg. arsanilic acid[a] | No vitamin E | 200 I.U. vitamin E |
|---|---|---|---|---|
| | | 202 day experiment | | |
| No. steers | 34 | 34 | 34 | 34 |
| Initial weight, lb. | 781 | 776 | 783 | 775 |
| Final weight, lb. | 1299 | 1299 | 1295 | 1303 |
| Av. daily gain, lb. | 2.58 | 2.59 | 2.55 | 2.62 |
| Av. daily ration, lb. | | | | |
| Rolled corn | 17.1 | 16.9 | 16.9 | 17.1 |
| Corncobs | 4.0 | 4.0 | 4.0 | 4.0 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 23.1 | 22.9 | 22.9 | 23.1 |
| Feed/100 lb. gain, lb. | 896 | 885 | 898 | 882 |
| Carcass measurements | | | | |
| Chilled carcass wt., lb. | 805 | 808 | 805 | 807 |
| Loin eye area, sq. in. | 12.6 | 12.8 | 12.7 | 12.7 |
| Loin fat thickness, in. | 0.84 | 0.87 | 0.85 | 0.85 |
| Carcass grade[b] | 7.8 | 7.6 | 7.7 | 7.7 |
| Dressing percent[c] | 62.0 | 62.2 | 62.2 | 61.9 |

substituted for selenium and resulted in improved performance.

Overall, the addition of arsanilic acid to the ration resulted in a reduction in gain by 3.2% and feed efficiency by 2.7%. The presence of vitamin E in the ration improved gain and feed efficiency by 3.8% and 3.6%, nonsignificant difference.

Due to requirements of the Food and Drug Administration, the arsanilic acid was removed from the ration after 112 days. The performance of the cattle for the entire feeding trial of 202 days is found in Table 13. During the last 90 days of this trial, the depressing effect of arsanilic acid on performance was completely lost. The final data indicate that there was no difference in performance between those formerly receiving arsanilic acid in the ration and the control animals. The presence of vitamin E in the ration resulted in an increased rate of gain of 2.7% over the controls; however, this was not statistically different.

Only slight differences in carcass measurements were observed among treatments in this trial and these could not be attributed to the treatment.

## Selenium and Arsanilic Acid Additions to Cattle Rations

### Experiment 734

Results of the influence of arsanilic acid and 0.1 ppm supplemental selenium upon feedlot performance and carcass

measurements are shown in Table 14.

All cattle in this experiment gained very well. Statistical analysis indicated that there was no difference due to any of the experimental treatments used in this trial. The addition of arsanilic acid to the rations apparently had no influence upon the performance of these cattle as had been observed in Experiment 727. With arsanilic acid in the ration in this trial, there appeared to be a trend for decreased performance. Gain was only very slightly influenced but there was an increase in feed required for gain with increasing levels of arsanilic acid in the ration.

Apparently the rations used in this trial were adequate in selenium and/or vitamin E content since there was no response to the addition of 0.1 ppm selenium to the ration. This was true both in the absence and presence of arsanilic acid.

Supplemental selenium had little effect upon carcass characteristics. The addition of arsanilic acid in the ration tended to decrease carcass weights and dressing percent. The higher level of arsanilic acid appeared to decrease loin eye area and increase the fat covering as measured over the 12th rib. None of these effects on carcass traits were observed in Experiment 727.

Table 14. Experiment 734: Cattle performance supplementing the ration with selenium and arsanilic acid

| Added to ration/steer/day: | No a.a.[a] No Se[b] | No a.a. 0.1 ppm Se | 200 mg. a.a. No Se | 200 mg. a.a. 0.1 ppm Se | 400 mg. a.a. No Se | 400 mg. a.a. 0.1 ppm Se |
|---|---|---|---|---|---|---|
| | | | 168 day experiment | | | |
| No. steers | 11 | 11 | 11 | 11 | 11 | 11 |
| Initial weight, lb. | 706 | 704 | 703 | 698 | 705 | 700 |
| Final weight, lb. | 1240 | 1243 | 1230 | 1228 | 1242 | 1229 |
| Av. daily gain, lb. | 3.18 | 3.21 | 3.14 | 3.15 | 3.20 | 3.15 |
| Av. daily ration, lb. | | | | | | |
| Rolled corn | 16.9 | 17.9 | 17.2 | 17.8 | 18.6 | 17.0 |
| Corncobs | 4.0 | 3.9 | 4.0 | 3.9 | 4.0 | 3.9 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 22.9 | 23.8 | 23.2 | 23.7 | 24.6 | 22.9 |
| Feed/100 lb. gain, lb. | 720 | 743 | 738 | 752 | 769 | 728 |
| Carcass measurements | | | | | | |
| Chilled carcass wt., lb. | 764 | 753 | 745 | 742 | 740 | 743 |
| Loin eye area, sq. in. | 12.9 | 12.8 | 13.0 | 12.9 | 12.2 | 12.1 |
| Loin fat thickness, in. | 0.65 | 0.69 | 0.68 | 0.66 | 0.74 | 0.73 |
| Carcass grade[c] | 8.8 | 8.6 | 8.4 | 8.6 | 8.7 | 8.3 |
| Dressing percent[d] | 61.6 | 60.6 | 60.6 | 60.4 | 59.6 | 60.5 |

[a]Arsanilic acid.

[b]Selenium.

[c]Carcass grade was given a numerical value as follows: low good - 4, average good - 5, high good - 6, low choice - 7, average choice - 8 and high choice - 9.

[d]Dressing percent based on full weights off experiment and warm carcass weights shrunk 2.5%.

Table 14 (Continued)

| Added to ration/steer/day: | No selenium | 0.1 ppm selenium | No arsanilic acid | 200 mg. arsanilic acid | 400 mg. arsanilic acid |
|---|---|---|---|---|---|
| | | | 168 day experiment | | |
| No. steers | 33 | 33 | 22 | 22 | 22 |
| Initial weight, lb. | 705 | 701 | 705 | 701 | 703 |
| Final weight, lb. | 1237 | 1233 | 1241 | 1229 | 1236 |
| Av. daily gain, lb. | 3.17 | 3.17 | 3.19 | 3.15 | 3.17 |
| Av. daily ration, lb. | | | | | |
| Rolled corn | 17.6 | 17.6 | 17.4 | 17.5 | 17.8 |
| Corncobs | 3.9 | 3.9 | 3.9 | 3.9 | 4.0 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 23.5 | 23.5 | 23.3 | 23.4 | 23.8 |
| Feed/100 lb. gain, lb. | 742 | 741 | 731 | 745 | 749 |
| Carcass measurements | | | | | |
| Chilled carcass wt., lb. | 750 | 746 | 759 | 743 | 741 |
| Loin eye area, sq. in. | 12.7 | 12.6 | 12.8 | 12.9 | 12.1 |
| Loin fat thickness, in. | 0.69 | 0.69 | 0.67 | 0.67 | 0.74 |
| Carcass grade[c] | 8.6 | 8.5 | 8.7 | 8.5 | 8.5 |
| Dressing percent[d] | 60.6 | 60.5 | 61.2 | 60.5 | 60.0 |

## Red Kidney Beans and Selenium in Beef Heifer Rations

Experiment 751

The effect of kidney beans in the ration of beef heifers upon selenium nutrition is found in Table 15.

There was no apparent stress placed upon the selenium requirement of the animals in this trial due to the incorporation of kidney beans into the ration. Average daily liveweight gain and feed efficiency were not changed with supplemental selenium at the rate of 0.1 ppm. The kidney beans were fed at a very low level in this trial because of the tendency to promote digestive disturbances at higher levels. This low level of intake may be a reason that there was no stress placed upon the selenium requirement.

Selenium in the ration appeared to reduce dressing percent, fat covering over the 12th rib and carcass grade; but the loin eye area tended to be increased with supplemental selenium. The relatively small number of animals used in this trial would cast doubt upon the validity of these observations even though these differences were noted within all lots in each treatment group.

Table 15. Experiment 751: Cattle performance supplementing the ration with kidney beans and selenium

| Added to ration/heifer/day: | No selenium | 0.1 ppm selenium |
|---|---|---|
| | 174 day experiment | |
| No. heifers | 30 | 30 |
| Initial weight, lb. | 546 | 548 |
| Final weight, lb. | 924 | 928 |
| Av. daily gain, lb. | 2.17 | 2.19 |
| Av. daily ration, lb. | | |
| Rolled corn | 10.0 | 10.0 |
| Corncobs | 4.0 | 3.9 |
| Rolled kidney beans | 2.3 | 2.3 |
| Supplement | 2.2 | 2.2 |
| Total | 18.5 | 18.4 |
| Feed/100 lb. gain, lb. | 850 | 846 |
| Carcass measurements | | |
| Chilled carcass wt., lb. | 535 | 532 |
| Loin eye area, sq. in. | 10.8 | 11.2 |
| Loin fat thickness, in. | 0.58 | 0.50 |
| Carcass grade[a] | 6.9 | 6.2 |
| Dressing percent[b] | 57.9 | 57.3 |

[a]Carcass grade was given numerical a value as follows: low good - 4, average good - 5, high good - 6, low choice - 7, average choice - 8 and high choice - 9.

[b]Dressing percent based on full weights off experiment and warm carcass weights shrunk 2.5%.

## Selenium, Sulfur and Unsaturated Oil Additions to Cattle Rations

Experiment 748

The effect of the addition of selenium, sulfur fed in the form of Glauber's salt and soybean oil upon cattle performance during the initial 127 days of this trial is shown in Table 16.

The addition of sulfur as Glauber's salt in the absence of supplemental selenium resulted in an unexpected increase in weight gain of 4.9% which was significantly greater ($P < 0.05$) than the controls. An improvement of feed efficiency of 3.9% which was not significantly different was also observed. Addition of sulfur in the presence of 0.2 ppm selenium also resulted in a significant ($P < 0.05$) increase in liveweight gain. Feed efficiency was nonsignificantly improved by 2.6%. When Glauber's salt plus soybean oil was fed in the presence or absence of supplemental selenium, no response in growth rate was noted. There was, however, a nonsignificant improvement of approximately 2% in feed efficiency.

Selenium addition to the ration did not influence feedlot gains. There was a nonsignificant improvement of feed efficiency of 2% with the addition of selenium. Since selenium in the ration had no influence on performance, one

Table 16. Experiment 748: Cattle performance supplementing the ration with selenium, Glauber's salt and soybean oil

| Added to ration/steer/day: | | | | | | |
|---|---|---|---|---|---|---|
| Selenium (ppm) | 0 | 0 | 0 | 0.2 | 0.2 | 0.2 |
| Glauber's salt (lb.) | 0 | 0.33 | 0.33 | 0 | 0.33 | 0.33 |
| Soybean oil | -- | -- | +[a] | -- | -- | +[a] |
| | | | 127 day experiment | | | |
| No. steers | 12 | 12 | 11 | 12 | 12 | 12 |
| Initial weight, lb. | 609 | 613 | 615 | 614 | 609 | 607 |
| Final weight, lb. | 1003 | 1026 | 1009 | 1009 | 1028 | 1001 |
| Av. daily gain, lb. | 3.10 | 3.26 | 3.10 | 3.11 | 3.29 | 3.11 |
| Av. daily ration, lb. | | | | | | |
| Rolled corn | 14.4 | 14.8 | 14.0 | 14.0 | 14.8 | 13.5 |
| Corncobs | 5.8 | 5.7 | 5.7 | 5.8 | 5.7 | 5.7 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 22.2 | 22.8 | 22.0 | 21.8 | 22.8 | 21.5 |
| Feed/100 lb. gain, lb. | 718 | 690[b] | 702[b] | 700 | 682[b] | 684[b] |

[a]Soybean oil was added as 8% of the corn portion of the ration.

[b]Value does not include weight of Glauber's salt.

Table 16 (Continued)

| Added to ration/steer/day: | | | | | |
|---|---|---|---|---|---|
| Selenium (ppm) | 0 | 0.2 | | | |
| Glauber's salt (lb.) | | | 0 | 0.33 | 0.33 |
| Soybean oil | | | -- | -- | +[a] |
| | | | 127 day experiment | | |
| No. steers | 35 | 36 | 24 | 24 | 23 |
| Initial weight, lb. | 612 | 610 | 611 | 611 | 611 |
| Final weight, lb. | 1013 | 1013 | 1006 | 1029 | 1005 |
| Av. daily gain, lb. | 3.15 | 3.17 | 3.10 | 3.28 | 3.10 |
| Av. daily ration, lb. | | | | | |
| Rolled corn | 14.4 | 14.1 | 14.2 | 14.8 | 13.8 |
| Corncobs | 5.8 | 5.7 | 5.8 | 5.7 | 5.8 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 22.4 | 22.0 | 22.0 | 22.8 | 21.8 |
| Feed/100 lb. gain, lb. | 703[b] | 689[b] | 709 | 687[b] | 693[b] |

may compare the average effect of the sulfur source and sulfur plus soybean oil during this 127-day period. Glauber's salt alone resulted in a significant ($P < 0.05$) increase in liveweight gain of 5.8% and improved efficiency of feed conversion by 3.1%. A conbination of soybean oil and Glauber's salt had no influence on gain but tended to improve feed efficiency.

The cattle in this experiment were marketed at two different times and a summary of the effect of selenium on feedlot performance for the total trial is shown in Table 17. As in the two previous trials, selenium supplementation had no significant effect on the feedlot performance or on carcass measurements.

A summary of the influence of sulfur as Glauber's salt and Glauber's salt plus soybean oil upon feedlot performance of the cattle marketed at two different times is shown in Table 18. Cumulative average daily gains of the cattle on the control and Glauber's salt treatment are shown graphically in Figure 1.

In the initial 127-day period of the trial, the daily intake of urea was 0.20 pound per steer. Average ration protein was 10.3% and 10.1% with urea supplying 24% and 23% of the total protein equivalent for the light and heavy weight groups of cattle, respectively. Total sulfur content of the rations for the light weight group of cattle was 0.09%, 0.21%

Table 17. Experiment 748: Cattle performance supplementing the ration with selenium

| Added to ration/steer/day: | No selenium | 0.2 ppm selenium | No selenium | 0.2 ppm selenium |
|---|---|---|---|---|
| | 209 day experiment | | 180 day experiment | |
| No. steers | 17 | 18 | 18 | 18 |
| Initial weight, lb. | 566 | 564 | 656 | 656 |
| Final weight, lb. | 1196 | 1183 | 1191 | 1198 |
| Av. daily gain, lb. | 3.01 | 2.96 | 2.97 | 3.01 |
| Av. daily ration, lb. | | | | |
| Rolled corn | 16.0 | 15.4 | 16.3 | 16.2 |
| Corncobs | 5.7 | 5.7 | 6.0 | 6.0 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 23.7 | 23.1 | 24.3 | 24.2 |
| Feed/100 lb. gain, lb. | 786 | 781 | 819 | 804 |
| Carcass measurements | | | | |
| Chilled carcass wt., lb. | 676 | 666 | 661 | 661 |
| Loin eye area, sq. in. | 11.3 | 11.2 | 11.7 | 11.4 |
| Loin fat thickness, in. | 0.25 | 0.24 | 0.23 | 0.20 |
| Carcass grade[a] | 5.6 | 5.9 | 4.7 | 4.4 |
| Dressing percent[b] | 56.5 | 56.3 | 55.5 | 55.2 |

[a]Carcass grade was given a numerical value as follows: low good - 4, average good - 5, high good - 6, low choice - 7, average choice - 8, and high choice - 9.

[b]Dressing percent based on full weights off experiment and warm carcass weights shrunk 2.5%.

Table 18. Experiment 748: Cattle performance supplementing the ration with selenium, Glauber's salt and soybean oil

| Added to ration/steer/day: | | | | | | |
|---|---|---|---|---|---|---|
| Glauber's salt (lb.) | 0 | 0.33 | 0.33[a] | 0 | 0.33 | 0.33[a] |
| Soybean oil[b] | -- | -- | +[c] | -- | -- | +[c] |
| | (Light weight group) | | | (Heavy weight group) | | |
| | Initial 127 day experiment | | | | | |
| No. steers | 12 | 12 | 11 | 12 | 12 | 12 |
| Initial weight, lb. | 566 | 566 | 564 | 657 | 656 | 654 |
| Final weight, lb. | 970 | 995 | 964 | 1042 | 1059 | 1043 |
| Av. daily gain, lb. | 3.18 | 3.38 | 3.15 | 3.03 | 3.17 | 3.06 |
| Av. daily ration, lb. | | | | | | |
| Rolled corn | 13.9 | 14.6 | 13.3 | 14.6 | 14.9 | 14.2 |
| Corncobs | 5.5 | 5.5 | 5.5 | 6.0 | 6.0 | 6.0 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 21.4 | 22.4 | 21.1 | 22.6 | 23.2 | 22.5 |
| Feed/100 lb. gain, lb. | 675 | 654[d] | 659[d] | 745 | 721[d] | 724[d] |

[a]Glauber's salt increased to 0.67 lb. per steer per day at the end of 127 days.

[b]Soybean oil was added as 8% of the corn portion of the ration.

[c]Soybean oil was discontinued at the end of 127 days.

[d]Value does not include weight of Glauber's salt.

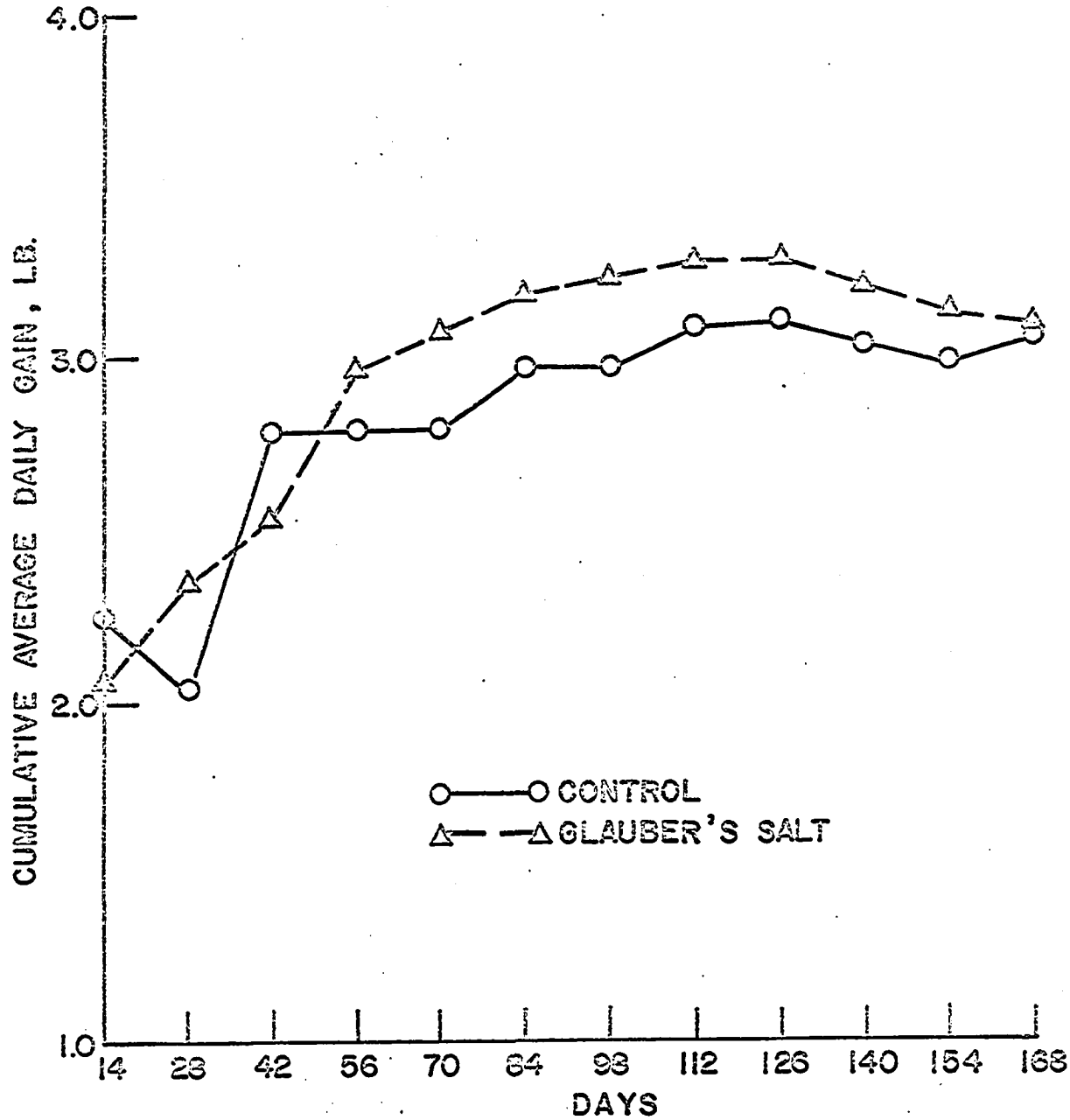
Table 18 (Continued)

| Added to ration/steer/day: | | | | | | |
|---|---|---|---|---|---|---|
| Glauber's salt (lb.) | 0 | 0.33 | 0.33[a] | 0 | 0.33 | 0.33[a] |
| Soybean oil[b] | -- | -- | +[c] | -- | -- | +[c] |
| | (Light weight group) | | | (Heavy weight group) | | |
| | Final 82 day experiment | | | Final 53 day experiment | | |
| No. steers | 12 | 12 | 11 | 12 | 12 | 12 |
| Initial weight, lb. | 970 | 995 | 964 | 1042 | 1059 | 1043 |
| Final weight, lb. | 1186 | 1185 | 1197 | 1193 | 1206 | 1183 |
| Av. daily gain, lb. | 2.65 | 2.32 | 2.84 | 2.86 | 2.77 | 2.65 |
| Av. daily ration, lb. | | | | | | |
| Rolled corn | 18.7 | 18.2 | 18.4 | 21.3 | 20.0 | 20.1 |
| Corncobs | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 26.7 | 26.5 | 27.0 | 29.3 | 28.3 | 28.7 |
| Feed/100 lb. gain, lb. | 1011 | 1126[d] | 927[d] | 1023 | 1013[d] | 1057[d] |
| Carcass measurements | | | | | | |
| Chilled carcass wt., lb. | 663 | 674 | 677 | 651 | 662 | 670 |
| Loin eye area, sq. in. | 11.1 | 11.1 | 11.7 | 11.5 | 11.4 | 11.7 |
| Loin fat thickness, in. | 0.24 | 0.31 | 0.18 | 0.20 | 0.22 | 0.22 |
| Carcass grade[e] | 5.8 | 5.8 | 5.7 | 4.3 | 4.8 | 4.7 |
| Dressing percent[f] | 55.9 | 56.9 | 56.6 | 54.6 | 54.9 | 56.6 |

[e]Carcass grade was given a numerical value as follows: low good - 4, average good - 5, high good - 6, low choice - 7, average choice - 8 and high choice - 9.

[f]Dressing percent based on full weights off experiment and warm carcass weights shrunk 2.5%.

Figure 1. Experiment 748: Cumulative average daily gain of light and heavy weight steers fed a supplemental source of sulfur (Glauber's salt) in the ration


4.0
3.0
2.0
1.0
CUMULATIVE AVERAGE DAILY GAIN, LB.
CONTROL
GLAUBER'S SALT
14
28
42
56
70
84
98
112
126
140
154
168
DAYS

and 0.22% for the control, sulfur and sulfur plus soybean oil treatment groups, respectively. Corresponding S:N ratios were 1:18.5, 1:7.6 and 1:7.4. Total sulfur content of the rations for the heavy weight group of cattle was 0.09%, 0.21% and 0.21% and S:N ratios were 1:18.4, 1:7.7 and 1:7.6 for the respective treatments.

Daily urea intake was 0.20 pound per steer during the final phase of the trial. Ration protein was 9.9% with urea supplying 20% and 18.7% of the ration protein equivalent for the light and heavy groups of steers. Total ration sulfur content for the light weight cattle was 0.09%, 0.20% and 0.30% and S:N ratios were 1:17.6, 1:8.1 and 1:5.3 for the respective treatments. Corresponding values for the heavy group of cattle were 0.09%, 0.19% and 0.29% sulfur and S:N ratios of 1:17.1, 1:8.3 and 1:5.6.

During the initial protion of the trial with the lighter weight cattle, supplemental sulfur alone resulted in an increased rate of gain ($P < 0.05$) of 5.9% over the control and sulfur plus soybean oil groups. Feed efficiency was not statistically different but was improved by 3.1% with supplemental sulfur. A combination of Glauber's salt and soybean oil in the ration did not improve gain but feed efficiency was improved as compared with the control treatment.

After 127 days, soybean oil was removed from the ration and the level of Glauber's salt in the ration was doubled.

In the final 82 days of the trial, the lower level of sulfur resulted in a 12.5% and 10.2% nonsignificant reduction in gain and feed efficiency as compared with the control. Removing soybean oil from the ration and increasing the sulfur level tended to increase gain and significantly ($P < 0.05$) reduced feed required for gain. The lack of statistical differences in gains during the latter portion of the trial with the light group of cattle was probably due to the fact that response and depression due to sulfur was not at all uniform within treatment groups.

The effect of sulfur, supplied as Glauber's salt, on performance was less pronounced in the heavy group of cattle both in the initial and final portions of the trial. Initially, sulfur alone nonsignificantly improved gain by 4.4% while feed efficiency was improved by 3.2% over the control group. Sulfur plus soybean oil did not increase gain but did improve feed efficiency. As with the light cattle, sulfur alone in the final 53 day period resulted in a nonsignificant decrease in gain of 3%, but this was much less severe and may be due to the shorter final feeding period. Feed efficiency was not influenced. Removing the soybean oil and increasing the Glauber's salt in the ration did not stimulate gains as had been observed with the lighter weight cattle. In this case, gains were 7.3% less than the control animals. This was a nonsignificant difference.

The effect of the treatments on carcass characteristics in this trial was variable. In the lighter group of cattle, the Glauber's salt treatment tended to increase dressing percent and carcass weight. The combination of soybean oil and Glauber's salt appeared to decrease fat thickness and increase the area of the loin eye. In the heavier lots of cattle, the sulfur source again appeared to increase dressing percent and carcass weight. With both weight groups of cattle the combination of soybean oil and supplemental sulfur appeared to promote the heaviest carcass weights. Carcass grades were not influenced.

## Supplemental Sulfur Additions to Cattle Finishing Rations

### Experiment 759

The influence of supplemental sulfur in the ration upon feedlot performance during the initial 83-day period and final 133-day period of this trial is found in Table 19.

Daily urea intake in this trial was 0.25 pound per animal. Average ration protein was 10.9% and 10.7% with urea supplying 30% and 28% of the total protein equivalent during the initial and final phases of this trial, respectively. Total ration sulfur for the control, elemental sulfur (low, medium and high levels) and Glauber's salt treatments was 0.09%, 0.16%, 0.24%, 0.39% and 0.24% respectively, in the initial 83

Table 19. Experiment 759: Cattle performance with supplemental sulfur in finishing rations

| Added to ration/steer/day: | No sulfur | 0.015 lb. sulfur | 0.03 lb. sulfur | 0.06 lb sulfur | 0.33 lb. Glauber's salt |
|---|---|---|---|---|---|
| | | | Initial 83 day experiment | | |
| No. steers | 16 | 14 | 16 | 16 | 14 |
| Initial weight, lb. | 617 | 617 | 616 | 617 | 620 |
| Final weight, lb. | 841 | 843 | 852 | 865 | 862 |
| Av. daily gain, lb. | 2.70 | 2.72 | 2.85 | 2.99 | 2.92 |
| Av. daily ration, lb. | | | | | |
| Rolled corn | 13.1 | 13.6 | 13.6 | 14.1 | 14.1 |
| Corncobs | 3.9 | 4.0 | 4.0 | 4.0 | 4.0 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 19.0 | 19.6 | 19.6 | 20.1 | 20.1 |
| Feed/100 lb. gain, lb. | 705 | 720 | 686 | 672 | 677 |
| | | | Final 133 day experiment | | |
| No. steers | 16 | 14 | 16 | 16 | 14 |
| Initial weight, lb. | 841 | 843 | 852 | 865 | 862 |
| Final weight, lb. | 1150 | 1165 | 1153 | 1133 | 1153 |
| Av. daily gain, lb. | 2.32 | 2.42 | 2.26 | 2.02 | 2.19 |
| Av. daily ration, lb. | | | | | |
| Rolled corn | 16.7 | 16.6 | 15.7 | 15.3 | 16.6 |
| Corncobs | 4.0 | 4.0 | 4.0 | 4.0 | 3.9 |
| Supplement | 2.0 | 1.9 | 2.0 | 2.0 | 2.0 |
| Total | 22.7 | 22.5 | 21.7 | 21.3 | 22.5 |
| Feed/100 lb. gain, lb. | 977 | 933 | 964 | 1056 | 1016 |

day period. Ratios of S:N for the respective treatments were 1:19.9, 1:10.6, 1:7.3, 1:4.5 and 1:7.2. During the final 133 days of the trial, total ration sulfur was 0.09%, 0.15%, 0.23% 0.37% and 0.23% and S:N ratios for the respective treatments were 1:18.8, 1:10.9, 1:7.5, 1:4.6 and 1:7.5.

During the initial 83-day period of this trial, supplemental sulfur resulted in a nonsignificant increase in rate of gain at all levels fed. Increased rates of gain for the respective treatments were 0.7%, 5.5%, 10.7% and 8.1%. Feed consumption was increased with sulfur in the ration. Feed efficiency was generally improved during this period although not significantly. Improvement in feed efficiency over the control ration was 2.7%, 4.7% and 4.0% when sulfur was included in the ration at the rate of 0.03 and 0.06 pound of elemental sulfur and 0.33 pound Glauber's salt daily; the lower level of sulfur appeared to reduce feed efficiency. When sulfur was supplemented in the ration at approximately equal levels as different sources (0.03 pound elemental sulfur and 0.33 pound Glauber's salt), feedlot performance appeared very similar.

Stimulation in gain due to the sulfur treatments was not observed until the 42nd day of the trial. With each successive period, sulfur further stimulated gain up to 83 days. Subsequent to 83 days, sulfur at the rate of 0.03 and 0.06 pound elemental sulfur per day and the Glauber's salt began

to depress performance. The high level of elemental sulfur appeared to depress gain to a greater degree and more rapidly than other treatments. The cumulative average daily gains by 21-day periods throughout the trial are shown graphically in Figure 2.

Feedlot performance in the final 133 days of this trial are also found in Table 19. Analysis of variance of these data indicated that the gains during this period were not statistically different, however, there was a very definite trend as was earlier noted in Experiment 748. Average gains were 2.6%, 12.9% and 5.6% less than the controls when daily supplements of 0.03 and 0.06 pound of elemental sulfur and 0.33 pound of Glauber's salt were fed. Feed efficiency was reduced by 8.1 and 4.0% for the latter two treatments. Feed consumption also was reduced. The lowest level of sulfur fed in this trial did not result in adverse performance during this period but improved rate of gain and feed utilization by approximately 4.5% over that of the control ration.

The results of supplemental sulfur in the ration over the total 216-day feeding period are in Table 20. Even though there was an initial stimulation from sulfur followed by a depression of feedlot performance, the net results appear to be one of cancellation of the effects. Over the entire trial, the lowest level of sulfur resulted in an improvement in average daily gain and feed efficiency of 2%. The highest level

Figure 2. Experiment 759: Cumulative average daily gain of steers fed supplemental sulfur (elemental sulfur and Glauber's salt) in the ration

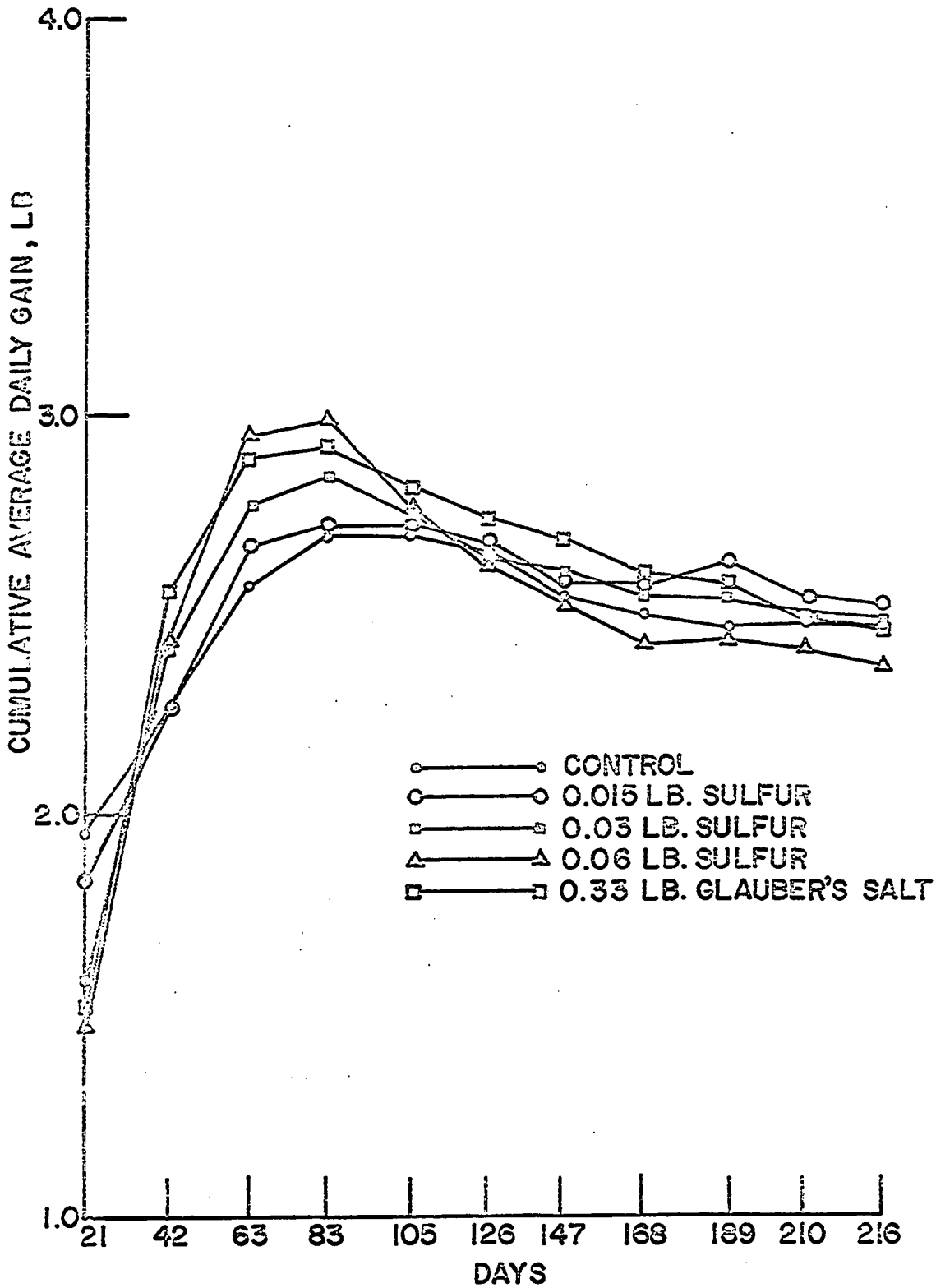

4.0
3.0
2.0
1.0
CUMULATIVE AVERAGE DAILY GAIN, LB
21
42
63
83
105
126
147
168
189
210
216
DAYS
CONTROL
0.015 LB. SULFUR
0.03 LB. SULFUR
0.06 LB. SULFUR
0.33 LB. GLAUBER'S SALT

Table 20. Experiment 759: Cattle performance with supplemental sulfur in finishing rations

| Added to ration/steer/day: | No sulfur | 0.015 lb. sulfur | 0.03 lb. sulfur | 0.06 lb. sulfur | 0.33 lb. Glauber's salt |
|---|---|---|---|---|---|
| | | | 216 day experiment | | |
| No. steers | 16 | 14 | 16 | 16 | 14 |
| Initial weight, lb. | 617 | 617 | 616 | 617 | 620 |
| Final weight, lb. | 1150 | 1165 | 1153 | 1133 | 1153 |
| Av. daily gain, lb. | 2.47 | 2.53 | 2.48 | 2.39 | 2.47 |
| Av. daily ration, lb. | | | | | |
| Rolled corn | 15.4 | 15.4 | 14.9 | 14.9 | 15.6 |
| Corncobs | 3.9 | 4.0 | 4.0 | 4.0 | 4.0 |
| Supplement | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Total | 21.3 | 21.4 | 20.9 | 20.9 | 21.6 |
| Feed/100 lb. gain, lb. | 863 | 845 | 841 | 872 | 861 |
| Carcass measurements | | | | | |
| Chilled carcass wt., lb. | 684 | 691 | 684 | 677 | 696 |
| Loin eye area, sq. in. | 11.5 | 12.1 | 11.8 | 11.8 | 11.4 |
| Loin fat thickness, in. | 0.54 | 0.57 | 0.52 | 0.51 | 0.69 |
| Carcass grade[a] | 8.1 | 7.8 | 7.9 | 8.3 | 8.0 |
| Dressing percent[b] | 59.5 | 59.3 | 59.3 | 59.8 | 60.4 |
| Serum inorganic sulfate[c] | 5.96 | 7.43 | 7.40 | 8.18 | 7.15 |

[a]Carcass grade was given a numerical value as follows: low good - 4, average good - 5, high good - 6, low choice - 7, average choice - 8 and high choice - 9.

[b]Dressing percent based on full weights off experiment and actual cold carcass weights.

[c]Expressed as mg. per 100 ml. of blood serum.

of elemental sulfur reduced average daily gain by 3.2%.

The effect of sulfur upon carcass characteristics are shown in Table 20. Only slight differences were observed. The high level of elemental sulfur and Glauber's salt tended to produce slightly heavier carcasses and increased dressing percent. Similar observations upon the carcass weight had been observed in Experiment 748. The animals receiving the low level of sulfur appeared to have the greatest loin eye area while cattle receiving Glauber's salt had the smallest loin eye and the thickest fat covering over the 12th rib. No influence upon carcass grade was observed.

Blood serum inorganic sulfate was increased with sulfur additions to the ration. These values are also shown in Table 20. Statistical analysis of these values indicated that there were no differences due to treatments; however the highest level of serum sulfate was observed with the highest level of supplemental sulfur in the ration. Values for the other sulfur treatments were essentially the same. A large variation of individual serum inorganic sulfate values within treatment groups probably resulted in lack of statistical significance among treatments.

## Blood Serum Inorganic Sulfate Study

Experiment 124

Feed consumption during this trial was very poor. Average daily feed intake for these steers was 9.0 pounds. Daily urea intake was 0.15 pound per steer and the ration contained 10.9% protein. Treatment levels of daily sulfur intake were 0.011, 0.023 and 0.045 pound per steer per day. The total ration sulfur for the control and three levels of elemental sulfur was 0.07%, 0.20%, 0.32% and 0.57%; corresponding S:N ratios were 1:23.0, 1:8.6, 1:5.3 and 1:3.0.

The results of the initial portion of this study appear in Table 21. As was noted in Experiment 759, the serum inorganic sulfate levels were extremely variable. Sulfur added to the ration did tend to increase the level of serum sulfate after a 7 day treatment period, however, these differences were not statistically significant since there was also an increase after the period on the control ration. The apparent reason for the relatively small differences between the initial and final values with the two higher levels of sulfur is animal number 38. This steer did not perform well and usually had a higher serum sulfate level than the other animals, and in the latter two treatment periods, a decrease in serum sulfate levels was observed with supplemental sulfur in the ration.

Table 21. Blood serum inorganic sulfate levels of steers fed various amounts of elemental sulfur (Samples taken just prior to feeding)

| Added to ration/steer/day: | Elemental sulfur (lb.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 0.011 | | 0.023 | | 0.045 | |
| | Day | | Day | | Day | | Day | |
| Steer | 0 | 7 | 0 | 7 | 0 | 7 | 0 | 7 |
| | (Serum sulfate, mg. per 100 ml.) | | | | | | | |
| 38 | 3.64 | 6.50 | 2.32 | 6.66 | 7.62 | 6.26 | 7.94 | 4.74 |
| 48 | 2.44 | 4.66 | 2.54 | 4.74 | 3.16 | 5.36 | 5.00 | 4.90 |
| 90 | 3.60 | 4.90 | 1.26 | 9.60 | 1.92 | 5.44 | 1.80 | 5.72 |
| 101 | 1.24 | 1.40 | 6.22 | 3.34 | 2.18 | 5.06 | 3.78 | 4.74 |
| Av. | 2.73 | 4.37 | 3.09 | 6.09 | 3.72 | 5.53 | 4.63 | 5.03 |
| Av. Inc. | | 1.64 | | 3.00 | | 1.81 | | 0.40 |

The results of the second portion of this study appear in Table 22. The two treatments used were the control ration and a low level of elemental sulfur which gave 0.07% and 0.20% sulfur in the respective rations. Added sulfur gave a nonsignificant increase in serum inorganic sulfate levels. This was observed with two of the three steers in this trial. Serum sulfate levels were increased at the various times after feeding. This increase with time was statistically significant ($P < 0.01$). The increase from the initial observation to 12 hours after feeding was greater when sulfur was fed in the ration.

Table 22. Blood serum inorganic sulfate levels at various times after feeding

| Added to ration/steer/day: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Elemental sulfur (lb.) | | | | | | | |
| Steer no. | 0 | | | | 0.011 | | | |
| | 48 | 90 | 101 | Av. | 48 | 90 | 101 | Av. |
| | (Serum sulfate, mg. per 100 ml.) | | | | | | | |
| Hours after feeding | | | | | | | | |
| 0 | 4.12 | 1.20 | 1.66 | 2.33 | 3.74 | 1.74 | 2.44 | 2.64 |
| 3 | 4.60 | 1.22 | 1.66 | 2.49 | 4.12 | 1.78 | 2.72 | 2.87 |
| 6 | 5.08 | 1.54 | 1.92 | 2.85 | 4.66 | 1.96 | 3.12 | 3.25 |
| 9 | 6.60 | 2.18 | 2.36 | 3.71 | 5.48 | 4.20 | 3.42 | 4.37 |
| 12 | 8.62 | 2.16 | 2.50 | 4.43 | 8.92 | 4.24 | 3.44 | 5.53 |
| Av. | | | | 3.16 | | | | 3.73 |

## Effect of Sulfur Upon Rumen Fermentation and *In Vitro* Starch Digestion

The daily feed consumption of the fistulated steers used in these trials was 10 pounds of rolled shelled corn, 4 pounds of ground corncobs and 1.5 pounds of the protein supplement mixture. The ration protein content was 9.7%; the daily urea consumption was 0.15 pound and supplied 26% of the ration protein equivalent. The control ration contained 0.08% sulfur. Daily additions of 0.023 pound elemental sulfur or 0.25 pound Glauber's salt to the ration increased the ration sulfur content to 0.23%. The S:N ratios of the control and sulfur treat-

ment rations were 1:19.0 and 1:6.8, respectively.

Ruminal ammonia

The results of the experiment of the influence of Glauber's salt and elemental sulfur upon ruminal ammonia production with Steer S is found in Table 23.

Ruminal ammonia production was found to be quite variable when Glauber's salt was added to the ration. Ammonia levels were highest at 1 hour after feeding with a decrease at subsequent hours. At 1 hour after feeding, the initial control period was significantly ($P < 0.01$) greater than all other periods except at 7 days after Glauber's salt was added to the ration. Ammonia levels were nonsignificantly reduced after the 7 day observation. When Glauber's salt was removed from the ration after 42 days, there was a further reduction in the ammonia level. This level was significantly different only from the ammonia level at the observation made at 7 days on the Glauber's salt ration ($P < 0.05$). Similar patterns were observed at 2 hours after feeding; significant differences among periods were noted ($P < 0.01$). Ammonia levels were significantly reduced ($P < 0.01$) from the initial control period to 7 and 13 days after Glauber's salt was added to the ration. A further reduction ($P < 0.01$) was noted when Glauber's salt was removed from the ration. At 3 and 4 hours after feeding, significant differences ($P < 0.01$ and $P < 0.05$,

Table 23. Ruminal ammonia production in Steer S fed a urea type ration supplemented with Glauber's salt and elemental sulfur (Ammonia expressed in mg. per 100 ml. of rumen fluid)

| Treatment[a] | Control | Glauber's salt | | | | | | Control | | Glauber's |
|---|---|---|---|---|---|---|---|---|---|---|
| Days on treatment | 13 | 7 | 13 | 20 | 27 | 34 | 42 | 6 | 13 | salt av. |
| Hours after feeding | | | | | | | | | | |
| 1 | 63.73 | 50.64 | 41.64 | 41.68 | 44.11 | 45.89 | 43.87 | 38.82 | 35.61 | 44.64 |
| 2 | 37.86 | 27.81 | 27.07 | 34.32 | 27.11 | 32.38 | 32.26 | 21.60 | 21.31 | 30.16 |
| 3 | 14.81 | 14.28 | 14.80 | 21.23 | 15.97 | 13.99 | 19.34 | 10.63 | 10.58 | 16.60 |
| 4 | 5.08 | 4.19 | 5.65 | 11.98 | 6.43 | 5.71 | 9.10 | 5.84 | 4.63 | 7.18 |
| Av. | 30.37 | 24.23 | 22.29 | 27.30 | 23.41 | 24.49 | 26.14 | 19.22 | 18.03 | |

| Treatment[a] | Control | | Elemental sulfur | | | | | | | Control | Elemental sulfur av. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Days on treatment | 6 | 13 | 10 | 17 | 25 | 39 | 47 | 54 | 61 | 8 | |
| Hours after feeding | | | | | | | | | | | |
| 1 | 38.82 | 35.61 | 39.38 | 33.39 | 33.21 | 32.78 | 41.09 | 38.47 | 31.06 | 17.72 | 35.70 |
| 2 | 21.60 | 21.31 | 23.96 | 20.45 | 21.23 | 23.57 | 23.76 | 23.47 | 23.96 | 8.76 | 22.91 |
| 3 | 10.63 | 10.58 | 13.87 | 8.57 | 13.28 | 13.44 | 11.59 | 11.40 | 17.43 | 3.41 | 12.80 |
| 4 | 5.84 | 4.63 | 6.58 | 3.76 | 6.33 | 7.69 | 5.06 | 4.48 | 8.76 | 9.15 | 6.09 |
| Av. | 19.22 | 18.03 | 20.95 | 16.67 | 18.51 | 19.37 | 20.38 | 19.46 | 20.30 | 9.76 | |

[a]Daily intake of Glauber's salt or elemental sulfur was 0.25 lb. and 0.023 lb., respectively.

respectively) were noted among periods but these differences could not be attributed to treatments except that there tended to be a reduction of ammonia when Glauber's salt was removed from the ration.

When elemental sulfur was fed in the ration, there were significant differences ($P < 0.01$) in ammonia levels among periods of observation at all hours after feeding. Generally these differences were not due to treatment except that there tended to be an increase in ammonia levels at the initial observation after sulfur was added to the ration and a significant reduction ($P < 0.01$) at 1, 2 and 3 hours after sulfur was removed from the ration.

Average ruminal ammonia levels were higher when the ration was supplemented with Glauber's salt than elemental sulfur. This difference was statistically significant ($P < 0.01$) at 1 and 2 hours and ($P < 0.05$) at 3 hours after feeding. No statistical difference was noted at 4 hours.

Ruminal ammonia levels as influenced by Glauber's salt in the ration of Steer B is found in Table 24.

In the initial portion of this trial, no source of supplemental sulfur was present in the ration. No significant differences were found among periods at 1 hour after feeding. More variation was observed at 2, 3 and 4 hours. Differences among periods were statistically different ($P < 0.05$, $P < 0.05$ and $P < 0.01$) at these hours. With the addition of Glauber's

Table 24. Ruminal ammonia production in Steer B fed a urea type ration supplemented with Glauber's salt (Ammonia expressed in mg. per 100 ml. of rumen fluid)

| Treatment | Control | | | | | | | | Control |
|---|---|---|---|---|---|---|---|---|---|
| Days on Treatment | 13 | 25 | 32 | 39 | 46 | 53 | 61 | 68 | av. |
| Hours after feeding | | | | | | | | | |
| 1 | 30.75 | 35.54 | 30.58 | 34.04 | 32.95 | 37.06 | 28.91 | 30.95 | 32.60 |
| 2 | 20.32 | 20.04 | 21.72 | 24.01 | 17.29 | 28.01 | 28.40 | 21.66 | 22.68 |
| 3 | 9.72 | 8.12 | 11.30 | 13.24 | 5.55 | 18.25 | 18.76 | 11.79 | 12.09 |
| 4 | 5.37 | 2.05 | 4.09 | 7.11 | 3.82 | 11.25 | 11.34 | 5.87 | 6.36 |
| Av. | 16.54 | 16.44 | 16.92 | 19.60 | 14.90 | 23.64 | 21.85 | 17.57 | |

| Treatment[a] | Glauber's salt | | | | | | | | Control | Glauber's salt |
|---|---|---|---|---|---|---|---|---|---|---|
| Days on treatment | 6 | 18 | 25 | 33 | 47 | 55 | 62 | 69 | 8 | av. |
| Hours after feeding | | | | | | | | | | |
| 1 | 42.26 | 30.56 | 30.97 | 33.01 | 25.42 | 34.47 | 30.97 | 30.58 | 38.95 | 32.28 |
| 2 | 26.71 | 22.79 | 21.79 | 19.67 | 25.12 | 23.86 | 17.14 | 23.96 | 31.36 | 22.63 |
| 3 | 12.80 | 11.76 | 8.71 | 11.10 | 14.80 | 13.34 | 6.53 | 13.01 | 22.40 | 11.50 |
| 4 | 5.21 | 5.49 | 4.19 | 3.80 | 8.47 | 4.09 | 2.34 | 4.68 | 12.76 | 4.78 |
| Av. | 21.74 | 17.65 | 16.42 | 16.90 | 18.45 | 18.94 | 14.25 | 18.06 | 26.37 | |

[a]Daily intake of Glauber's salt was 0.25 lb.

salt to the ration, ruminal ammonia levels initially increased. Ammonia levels varied more among periods during the sulfur portion of this trial than during the control portion. This was evident at all hours after feeding. Generally no trend upon ammonia levels with time on trial was evident, but there were significant differences ($P < 0.01$) among periods at all hours. At 6 days on the Glauber's salt diet, the ammonia level at 1 hour after feeding was significantly greater ($P < 0.01$) than all other periods. The differences at other hours could not be attributed to the treatment. Contrary to observations made with the other steer in these trials, ruminal ammonia levels appeared to be increased when the Glauber's salt was removed from the ration at the end of 69 days.

Average ammonia levels were not different at 1, 2 and 3 hours after feeding due to the addition of Glauber's salt to the ration of Steer B. The average level at 4 hours appeared to be slightly reduced as compared with the average during the control period.

Volatile fatty acids and pH

The influence of Glauber's salt and elemental sulfur upon volatile fatty acid production and pH in the trial with Steer S is found in Table 25.

In the initial portion of this trial, the addition of

Table 25. Ruminal pH and volatile fatty acid production in Steer S fed a urea type ration supplemented with Glauber's salt and elemental sulfur[a]

| Treatment[b] | Days on treat-ment | pH | μM V.F.A. per ml. | $C_2/C_3$ | Molar percentage | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $C_2$ | $C_3$ | $iC_4$ | $C_4$ | $iC_5$ | $C_5$ |
| Control | 16 | 6.61 | 113.2 | 3.47 | 65.5 | 18.9 | 0.97 | 11.6 | 1.6 | 1.5 |
| Glauber's salt | 16 | 6.41 | 113.1 | 3.24 | 65.4 | 20.2 | 0.79 | 11.0 | 1.4 | 1.2 |
| Glauber's salt | 30 | 6.72 | 92.1 | 2.43 | 60.6 | 24.9 | 1.07 | 10.0 | 2.0 | 1.4 |
| Glauber's salt | 42 | 6.53 | 109.5 | 4.02 | 67.6 | 16.8 | 0.76 | 12.6 | 1.4 | 0.9 |
| Control | 13 | 6.55 | 106.0 | 3.90 | 67.8 | 17.4 | 0.75 | 11.0 | 1.9 | 1.1 |
| Elemental sulfur | 19 | 6.46 | 99.9 | 3.28 | 66.0 | 20.1 | 0.85 | 9.2 | 2.4 | 1.4 |
| Elemental sulfur | 38 | 6.50 | 99.4 | 4.20 | 70.2 | 16.7 | 0.68 | 9.4 | 2.0 | 1.1 |
| Elemental sulfur | 52 | 6.68 | 98.2 | 3.25 | 64.9 | 20.0 | 0.74 | 11.5 | 1.6 | 1.2 |
| Control | 7 | 6.64 | 96.3 | 3.19 | 65.1 | 20.4 | 0.87 | 10.0 | 2.1 | 1.2 |
| Control av. | | 6.60 | 104.8 | 3.50 | 66.2 | 18.9 | 0.88 | 10.9 | 1.9 | 1.3 |
| Glauber's salt av. | | 6.55 | 104.9 | 3.12 | 64.5 | 20.7 | 0.92 | 11.2 | 1.6 | 1.2 |
| Elemental sulfur av. | | 6.55 | 99.2 | 3.54 | 67.0 | 18.9 | 0.79 | 10.0 | 2.0 | 1.2 |

[a]Each value is the mean of measurements on samples collected at 0, 1, 2, 3, 4, 5 and 8 hours after feeding.

[b]Daily intake of Glauber's salt or elemental sulfur was 0.25 and 0.023 lb., respectively.

Glauber's salt to the ration appeared to have the effect of reducing the molar percent of acetate and butyrate. Propionate increased which narrowed the acetate to propionate ratio. No trend in the levels of isobutyrate, isovalerate and valerate over this period of time was observed. The concentration of the total volatile fatty acids in the rumen also appeared to be reduced with supplemental sulfur as Glauber's salt in the ration. At the final observation of this period (42 days), the Glauber's salt resulted in an increase in the molar percent of acetate and butyrate along with a decrease in propionate. The relatively wide acetate to propionate ratio was narrowed only slightly when Glauber's salt was removed from the diet. The molar percent of propionate increased slightly but butyrate decreased drastically.

In the latter portion of this trial, the addition of elemental sulfur to the ration initially resulted in an increase in propionate with a reduction in both acetate and butyrate. At 38 days on trial, acetate production was increased with a decrease in propionate. An opposite pattern with respect to acetate and propionate was observed at 52 days on trial; the molar percent of butyrate increased. When sulfur was removed from the ration, the proportions of acetate and propionate were not changed but there was a decrease in butyrate.

The average influence of Glauber's salt and elemental

sulfur upon volatile fatty acids in this trial is also found in Table 25. Supplemental sulfur as Glauber's salt decreased the average molar percent of acetate. This difference was not statistically significant. Elemental sulfur resulted in a molar percent of acetate which was greater than both the control and Glauber's salt average. Only the elemental sulfur and Glauber's salt averages were statistically different ($P < 0.05$). The molar percent of propionate was greatest with Glauber's salt in the ration but this was not statistically significant. The molar percent of butyrate during the Glauber's salt period was greatest. Only the Glauber's salt and elemental sulfur averages were significantly different ($P < 0.05$). In the case of the minor volatile fatty acids, significant differences due to treatment were noted only with isobutyrate and isovalerate. The molar percent of isobutyrate during the control and Glauber's salt periods was greater ($P < 0.05$ and $P < 0.01$, respectively) than during the elemental sulfur feeding period. A significant difference ($P < 0.01$) in the molar percent of isobutyrate with time after feeding was also noted. The molar percent of this acid was greatest at 0 hours with a decline to 6 hours and then an increase at 8 hours. The molar percent of isovalerate was lowest when Glauber's salt was in the ration. This was statistically different ($P < 0.05$ and $P < 0.01$, respectively) from the control and elemental sulfur periods. The molar percent of isovalerate was also in-

fluenced with time after feeding; the molar percent was greatest prior to feeding with a decline to a low at 4 hours.

Results of the trial with Steer B on the influence of Glauber's salt upon volatile fatty acid production is found in Table 26. In the control portion of this trial, the molar percent of acetate appeared to increase with time on the ration. The greatest change in the volatile fatty acid pattern during this period was observed with propionate and butyrate. The molar percent of propionate was greatly increased from 34 to 48 days with a subsequent drop at 60 days on the control ration. This variation in molar percent of propionate resulted in a drastic fluctuation in the acetate to propionate ratio. Butyrate varied inversely with propionate during this period. The total concentration of volatile fatty acids remained fairly constant with the exception of the observation at 48 days when it was low. This corresponded with the greatest molar percent of propionate and lowest molar percent of butyrate.

When sulfur as Glauber's salt was added to the ration, the molar percent of both acetate and propionate increased with the increase in propionate being greater, thus narrowing the acetate to propionate ratio. At this observation, 7 days after Glauber's salt was added to the diet, the molar percent of butyrate was decreased. At 27 days, propionate was further increased with a reduction in both acetate and butyrate. At

Table 26. Ruminal pH and volatile fatty acid production in Steer B fed a urea type ration supplemented with Glauber's salt[a]

| Treatment[b] | Days on treatment | pH | μM V.F.A. per ml. | $C_2/C_3$ | Molar percentage | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $C_2$ | $C_3$ | $iC_4$ | $C_4$ | $iC_5$ | $C_5$ |
| Control | 16 | 6.49 | 115.1 | 3.16 | 64.2 | 20.3 | 0.95 | 11.5 | 1.8 | 1.3 |
| Control | 34 | 6.29 | 114.2 | 3.22 | 65.4 | 20.3 | 0.79 | 10.9 | 1.4 | 1.3 |
| Control | 48 | 6.66 | 82.7 | 2.79 | 65.2 | 23.4 | 0.92 | 6.8 | 2.1 | 1.6 |
| Control | 60 | 6.59 | 116.1 | 4.19 | 67.1 | 16.0 | 0.88 | 12.8 | 1.9 | 1.3 |
| Glauber's salt | 7 | 6.43 | 113.3 | 3.52 | 68.2 | 19.4 | 0.79 | 9.2 | 1.4 | 1.1 |
| Glauber's salt | 27 | 6.36 | 100.8 | 2.67 | 64.7 | 24.2 | 0.72 | 7.8 | 1.4 | 1.2 |
| Glauber's salt | 48 | 6.63 | 112.9 | 3.39 | 67.7 | 20.0 | 0.77 | 9.3 | 1.3 | 1.0 |
| Glauber's salt | 62 | 6.64 | 96.4 | 3.31 | 67.5 | 20.4 | 0.84 | 8.2 | 1.4 | 1.7 |
| Control | 7 | 6.74 | 86.2 | 3.21 | 66.7 | 20.8 | 0.90 | 8.3 | 1.9 | 1.4 |
| Control av. | | 6.56 | 102.5 | 3.26 | 65.8 | 20.2 | 0.89 | 10.0 | 1.8 | 1.4 |
| Glauber's salt av. | | 6.52 | 105.6 | 3.18 | 67.0 | 21.1 | 0.78 | 8.6 | 1.4 | 1.3 |

[a]Each value is the mean of measurements on samples collected at 0, 1, 2, 3, 4, 6 and 8 hours after feeding.

[b]Daily intake of Glauber's salt was 0.25 lb.

subsequent observations, the molar percent of acetate increased and propionate decreased and remained fairly stable thereafter. The level of butyrate tended to be variable at the last two observations. The molar percents of isobutyrate, isovalerate and valerate did not vary greatly during the latter portion of this trial with the exception that valerate was increased at 62 days. No great change in volatile fatty acid proportion was observed when Glauber's salt was removed from the ration. The total concentration of the acids was decreased with only a slight narrowing of the acetate to propionate ratio being noted. The molar percent of isobutyrate and isovalerate increased after Glauber's salt was removed from the ration.

The average influence of sulfur addition as Glauber's salt to the ration may be seen in Table 26. The total concentration of volatile fatty acids appeared to be increased slightly, however, the variation of the total acids within treatment periods suggests that this was not a true difference. The supplemental sulfur source significantly ($P < 0.01$) increased the molar percent of acetate. Propionate was also increased but this was not significant. The molar percent of butyrate was decreased by the addition of Glauber's salt to the ration. This was a significant depression ($P < 0.01$). The molar percent of acetate, propionate and butyrate was not significantly different at the various times after

feeding. Although the difference was small, Glauber's salt depressed ($P < 0.01$) the average molar percent of isobutyrate and was found to be changed ($P < 0.01$) at various hours after feeding. The molar percent of this acid was greatest prior to or at 1 hour after feeding with a decline to 6 hours. Isovalerate was depressed ($P < 0.01$) when Glauber's salt was in the ration but the molar percent of valerate was not influenced.

Total viable bacterial numbers

The effect of Glauber's salt and elemental sulfur upon total viable bacterial numbers in rumen fluid collected from Steer S is found in Table 27.

Supplemental sulfur as Glauber's salt in the ration did not appear to influence total viable ruminal bacterial numbers. In the initial portion of this trial, bacterial numbers were significantly different ($P < 0.01$) among periods of observation; however the bacterial numbers differed as widely during the period of Glauber's salt feeding as between the control and Glauber's salt periods. Elemental sulfur added to the ration appeared to initially reduce the bacterial numbers. However, further differences ($P < 0.01$) in the bacterial numbers during this period of sulfur supplementation suggest that this difference may not be due to the experimental treatment. The average viable bacterial numbers during periods of

Table 27. Effect of Glauber's salt and elemental sulfur upon viable bacterial numbers in the rumen of Steer S

| Treatment[a] | Control | | Glauber's salt | | | | Control | |
|---|---|---|---|---|---|---|---|---|
| Days on treatment | 9 | 18 | 20 | 27 | 38 | 45 | 7 | 14 |
| Bacteria[b] | 49.8 | 35.4 | 33.2 | 32.6 | 45.8 | 42.0 | 45.8 | 44.0 |

| Treatment | Control | | Elemental sulfur | | | | | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|
| Days on treatment | 7 | 14 | 14 | 21 | 31 | 42 | 50 | 56 | 63 | 7 |
| Bacteria[b] | 45.8 | 44.0 | 28.8 | 51.6 | 46.2 | 32.2 | 40.4 | 41.8 | 31.8 | 40.0 |

| | Control av. | Glauber's salt av. | Elemental sulfur av. |
|---|---|---|---|
| Bacteria[b] | 43.0 | 38.4 | 38.9 |

[a]Daily intake of Glauber's salt or elemental sulfur was 0.25 and 0.023 lb., respectively.

[b]Viable bacteria per ml. of rumen fluid (X $10^8$).

Glauber's salt and elemental sulfur addition to the ration were essentially the same in this trial.

The results of the trial of the effect of Glauber's salt upon bacterial numbers which was conducted with Steer B are found in Table 28.

Statistical analyses indicated that there were significant differences ($P < 0.01$) among periods of observation within each phase of the total experiment but that there was no difference in the average number of bacteria on the two different treatments. As was observed earlier with elemental sulfur, Glauber's salt appeared to depress the number of viable bacteria, but this again may be questioned since further variation in numbers was observed during the sulfur treatment. When Glauber's salt was removed from the diet of this steer, viable bacterial numbers appeared to increase.

## In vitro starch digestion

The results of *in vitro* starch digestion by rumen microorganisms in four trials as influenced by various levels of sulfur are found in Table 29.

In all of the trials, starch digestion was measured after an 8-hour incubation period. As was expected, variation in starch digestion was observed on a given treatment among the different trials. This was noted particularly in the incubation tubes where no sulfur was included in the medium. In

Table 28. Effect of Glauber's salt upon viable bacterial numbers in the rumen of Steer B

| Treatment | Control | | | | | | | | Control |
|---|---|---|---|---|---|---|---|---|---|
| Days on treatment | 0 | 9 | 18 | 38 | 45 | 56 | 63 | 70 | av. |
| Bacteria[a] | 43.8 | 56.4 | 41.2 | 25.6 | 25.6 | 44.8 | 42.0 | 47.2 | 40.8 |

| Treatment[b] | Glauber's salt | | | | | | | | Control | Glauber's salt |
|---|---|---|---|---|---|---|---|---|---|---|
| Days on treatment | 7 | 20 | 27 | 37 | 48 | 56 | 62 | 70 | 7 | av. |
| Bacteria[a] | 29.2 | 31.8 | 46.6 | 45.8 | 35.8 | 25.8 | 35.2 | 42.6 | 47.2 | 36.6 |

[a]Viable bacteria per ml. of rumen fluid (X $10^8$).

[b]Daily intake of Glauber's salt was 0.25 lb.

Table 29. Effect of various sulfur to nitrogen (S:N) ratios upon *in vitro* starch digestion by ruminal bacteria[a]

| | S:N ratios[b] | | | |
|---|---|---|---|---|
| Control | 1:16 | 1:12 | 1:8 | 1:4 |
| | Percent digestion per 8 hours | | | |
| Trial 1 | 21.4 | 66.3 | 63.3 | 64.2 | 62.2 |
| | 24.2 | 64.7 | 65.2 | 62.6 | 62.7 |
| | 27.0 | 64.4 | 67.0 | 64.3 | 62.3 |
| | 24.2 | 65.1 | 65.2 | 63.7 | 62.4 |
| Trial 2 | 42.5 | 64.7 | 61.5 | 62.8 | 62.0 |
| | 42.7 | 62.9 | 63.2 | 61.3 | 61.9 |
| | 42.4 | 62.4 | 64.4 | 66.6 | 62.2 |
| | 42.5 | 63.3 | 63.0 | 63.6 | 62.0 |
| Trial 3 | 8.5 | 56.6 | 54.3 | 52.0 | 54.9 |
| | 8.7 | 50.0 | 50.9 | 52.1 | 49.4 |
| | 8.3 | 52.8 | 57.7 | 51.9 | 53.5 |
| | 8.6 | 53.1 | 54.3 | 52.0 | 52.6 |
| Trial 4 | 16.3 | 63.2 | 60.0 | 73.6 | 64.9 |
| | 12.5 | 70.1 | 66.7 | 75.1 | 72.8 |
| | 10.2 | 71.9 | 71.6 | 62.9 | 72.4 |
| | 13.0 | 68.4 | 66.1 | 70.5 | 70.0 |
| Av. | 22.1 | 62.5 | 62.2 | 62.5 | 61.8 |

[a]Bacteria collected from 2 liters of ruminal fluid were added to 1 liter of the incubation medium.

[b]Incubation medium contained 0, 29, 39, 58 and 117 mcg. of sulfur per ml. for the control and S:N ratio treatments of 1:16, 1:12, 1:8 and 1:4, respectively.

all trials there was a significant ($P < 0.01$) increase in starch digestion with the addition of sulfur to the incubation tubes. There was no significant difference in starch digestion in any of the trials among the various treatment levels of sulfur that were added. In three of the four trials, the sulfur supplied to give a S:N ratio of 1:4 resulted in an average starch digestion of 1 to 2% less than the lower levels of added sulfur. This was not observed in the fourth trial. The average results of the four trials indicate that under the conditions of this experiment sulfur in the incubation medium was required for optimum starch digestion ($P < 0.01$). Increasing the levels of added sulfur did not further influence starch digestion by the rumen microorganisms in these trials.

In the first three trials in which the influence of sulfur upon starch digestion was observed, analysis of the incubation medium for ammonia levels was made after the 8-hour incubation period. The results of these observations appear in Table 30.

In all trials within this experiment, sulfur added to the <u>in vitro</u> incubation medium resulted in a significantly reduced level of ammonia ($P < 0.05$, $P < 0.05$ and $P < 0.01$ for Trials 1, 2 and 3, respectively) following the 8-hour incubation period. In the combined trial, the presence of added sulfur in the incubation medium resulted in a significant

Table 30. Effect of various sulfur to nitrogen (S:N) ratios upon ammonia levels after *in vitro* starch digestion by ruminal bacteria[a]

| | Control | S:N ratios[b] 1:16 | 1:12 | 1:8 | 1:4 |
|---|---|---|---|---|---|
| | | Mg. ammonia per 100 ml. | | | |
| Trial 1 | 38.91 | 32.00 | 30.77 | 29.60 | 31.65 |
| Trial 2 | 38.76 | 32.86 | 30.97 | 30.77 | 32.89 |
| Trial 3 | 44.07 | 31.22 | 30.63 | 31.30 | 30.71 |
| Av. | 40.58 | 32.02 | 30.79 | 30.56 | 31.75 |

[a]Analyses for ammonia were conducted on a pooled sample from the three starch digestion tubes within each treatment.

[b]Incubation medium contained 0, 29, 39, 58 and 117 mcg. of sulfur per ml. for the control and S:N ratio treatments of 1:16, 1:12, 1:8 and 1:4, respectively.

reduction ($P < 0.01$) of the level of ammonia following the period of starch digestion.

No statistical difference in ammonia levels due to the various levels of sulfur added to the medium was noted. In all trials and in the combined trial, sulfur added to give S:N ratios of 1:12 and 1:8 tended to result in slightly lower levels of ammonia in the incubation medium than the treatments in which the S:N ratios were 1:16 and 1:4. This suggests that under the conditions of this experiment, the former levels of sulfur were adequate for optimum utilization of the urea in the incubation medium.

The higher level of ammonia found in the control tubes in Trial 3 than in other trials corresponded with the reduced level of starch digestion. This observation may have been due to a reduced number of microorganisms added to the incubation medium or some slight error in technique in this particular trial. No trend between starch digestion and ammonia levels was detected in Trials 1 and 2.

Results of volatile fatty acid analyses on the incubation medium following *in vitro* starch digestion is found in Table 31.

The addition of sulfur to the incubation medium increased ($P < 0.01$) the total concentration of volatile fatty acids following an 8-hour period of incubation. This increase was observed within all four trials. Levels of sulfur higher than that added to give a S:N ratio of 1:16 did not significantly influence the total concentration of volatile fatty acids. The molar percent of acetate and propionate was not significantly influenced by the sulfur treatment. Acetate and propionate over the four trials tended to be increased by approximately 1 molar percent, but there was no difference with increased levels of sulfur in the medium. The ratio of acetate to propionate was influenced only very slightly. The molar percent of butyrate was significantly reduced ($P < 0.01$) by the addition of the lowest level of sulfur to the incubation medium (S:N ratio of 1:16). The

Table 31. Effect of various sulfur to nitrogen (S:N) rations upon volatile fatty acid levels after *in vitro* starch digestion by ruminal bacteria[a]

| Trial | S:N[b] ratio | μM V.F.A./ml. | $C_2/C_3$ | Molar percentage | | |
|---|---|---|---|---|---|---|
| | | | | $C_2$ | $C_3$ | $C_4$ |
| 1 | Control | 16.4 | 1.15 | 51.3 | 44.5 | 4.2 |
| | 1:16 | 34.4 | 1.05 | 50.1 | 47.9 | 2.0 |
| | 1:12 | 36.9 | 1.04 | 50.2 | 48.1 | 1.7 |
| | 1:8 | 36.7 | 1.05 | 50.4 | 48.0 | 1.6 |
| | 1:4 | 37.0 | 1.02 | 49.8 | 48.6 | 1.6 |
| 2 | Control | 26.9 | 0.90 | 45.6 | 50.5 | 3.9 |
| | 1:16 | 37.7 | 0.89 | 45.9 | 51.7 | 2.4 |
| | 1:12 | 37.9 | 0.94 | 47.3 | 50.2 | 2.5 |
| | 1:8 | 37.2 | 0.92 | 46.7 | 50.9 | 2.4 |
| | 1:4 | 38.6 | 0.87 | 45.6 | 52.2 | 2.2 |
| 3 | Control | 13.8 | 1.65 | 58.4 | 35.3 | 6.3 |
| | 1:16 | 36.7 | 1.33 | 55.5 | 41.8 | 2.7 |
| | 1:12 | 37.7 | 1.31 | 55.2 | 42.2 | 2.6 |
| | 1:8 | 38.8 | 1.31 | 55.4 | 42.4 | 2.2 |
| | 1:4 | 38.3 | 1.29 | 55.1 | 42.6 | 2.3 |
| 4 | Control | 8.8 | 0.88 | 45.1 | 51.3 | 3.6 |
| | 1:16 | 25.8 | 1.09 | 51.0 | 46.9 | 2.1 |
| | 1:12 | 30.5 | 1.00 | 49.4 | 49.2 | 1.4 |
| | 1:8 | 21.7 | 1.12 | 52.2 | 46.7 | 1.1 |
| | 1:4 | 26.0 | 1.09 | 51.4 | 47.2 | 1.3 |
| Av. | Control | 16.5 | 1.08 | 49.6 | 45.9 | 4.4 |
| | 1:16 | 33.6 | 1.07 | 50.6 | 47.1 | 2.3 |
| | 1:12 | 35.8 | 1.07 | 50.6 | 47.3 | 2.1 |
| | 1:8 | 33.6 | 1.09 | 51.1 | 47.0 | 1.9 |
| | 1:4 | 35.0 | 1.06 | 50.4 | 47.7 | 1.9 |

[a]Analyses for volatile fatty acids were conducted on a pooled sample from the three starch digestion tubes within each treatment.

[b]Incubation medium contained 0, 29, 39, 58 and 117 mcg. of sulfur per ml. for the control and S:N ratio treatments of 1:16, 1:12, 1:8 and 1:4, respectively.

proportion of butyrate was reduced further with larger additions of sulfur, but this was not statistically significant.

The volatile fatty acid production and starch digestion in the first three trials appeared to follow a pattern since with a low starch digestion there tended to be a low concentration of volatile fatty acids. In light of this, it is difficult to explain the results obtained in the fourth trial. In the control tubes, starch digestion was low with a correspondingly low production of volatile fatty acids. With the addition of sulfur to the medium, starch digestion was increased to levels greater than in the other trials. However, the level of total acids was low as well as a greater variation was observed in acid production among the various sulfur treatments than had been observed in previous trials.

# DISCUSSION

Nutrition research has progressed to the point where the researcher must now encounter problems of great complexity. Often these problems are compounded and confounded by numerous interrelationships with other nutrients and the presence of very minute amounts of the factor being studied. This is particularly true in the area of minerals and a prime example of this is selenium. Selenium, as a mineral, has been recognized for many years; only recently has it been implicated as a required factor for proper nutrition of livestock.

A portion of this research has been an attempt to evaluate the need for selenium in rations for finishing beef cattle. Rations containing no hay were fed in this research because of the relatively large amount of vitamin E usually present in this feedstuff.

Two levels of supplemental selenium in the ration have been used in this research. In Experiments 734 and 751, 0.1 ppm selenium was fed while 0.2 ppm selenium was fed in Experiment 748. No feedlot response to selenium supplementation was observed in any of these trials. These observations were both in agreement and contradiction with results noted by other workers in ruminant nutrition.

Much of the work reported in which selenium supplementation has produced increased growth of ruminants has been in

areas where muscular dystrophy is a problem. Slen et al. (1961) observed over a 2-year period that selenium given to range ewes significantly increased body weight gain and appeared to increase wool growth as well. Hopkins et al. (1964) reported that selenium given to lambs moderated the incidence of nutritional muscular dystrophy and resulted in increased weight gains. Increased growth rates of 12-40% with lambs receiving selenium either orally or intramuscularly have been reported by McLean et al. (1959). A response from selenium was observed in flocks in which no history of muscular disease was found as well as where it was prevalent. Jolly (1960) and Hartley (1961) reported that selenium treated calves gained up to 50% faster than untreated controls. Most of these preceding observations have been made where the animals were pasture fed and do not relate directly to feedlot performance.

In the feedlot, Barringer (1964) observed that selenium in cattle rations gave a growth response in two of four trials. Beeson et al. (1964) and Scott et al. (1965) also reported improved feedlot performance with supplemental selenium in the ration. Smith et al. (1965) and Mukhtar (1966) noted a slight depression or no difference in gain due to selenium.

The minute amount of selenium which is suspected to be required in ruminant rations leads to problems in interpretation of results. Slight changes in the selenium content

of natural feedstuffs from year to year or one geographical area to another may be an explanation for the variation in response to selenium supplementation. That this variation in selenium content of feedstuffs exists has been documented by Allaway and Hodgson (1964). These workers observed a wide variability in the selenium content of alfalfa hay in different counties in Iowa. These samples ranged in selenium content from 0.03 to 0.64 ppm. In a study on selenium levels of plants, mostly grasses, Gardiner and Gorman (1963) have implicated the annual rainfall of an area in determining the selenium content. Heavy rainfall has been noted to decrease the selenium content of plants. Fertilization practices where gypsum, a sulfur containing compound, and elemental sulfur are used as fertilizer may play a role in determining the selenium content of plants. Sulfate sulfur appears to depress the uptake of selenium by the crop (Shrift, 1961). Muth et al. (1959) have described frequent storms of White Muscle Disease after use of common sulfur-bearing fertilizers. The factors mentioned above may have played a role in determining the selenium content of feedstuffs fed in this research and other research and as a result a variation in response to supplemental selenium in the ration has been noted.

A vitamin E - selenium interrelationship has been observed in muscular diseases in ruminants by many workers. Burroughs et al. (1963) observed a growth response of the

same magnitude with either vitamin E or selenium and that a combination of the two did not give an additive effect. Therefore, it is conceivable that variation in vitamin E content of corn from year to year and area to area may also play a role in selenium response. Herting and Drury (1963) have reported variations in tocopherols of corn oil and the corn grain in samples from different areas. If rations are adequate in vitamin E, then one may not necessarily expect additional gain with supplemental selenium. At this point one may compare the results obtained with supplemental vitamin E in Experiment 727 and the results obtained by Mukhtar (1966) when supplemental selenium was fed. These two trials were both in progress at approximately the same time, yet a growth response was obtained with vitamin E but selenium had no effect. This suggests that the two factors are not completely interchangeable and verifies the theory of McLean et al. (1959) and Schwarz (1960) that the two nutrients may be interrelated in their metabolic functions but that they cannot completely replace each other.

The use of various substances in this research in an attempt to inhibit the utilization of naturally occurring selenium appeared to be generally without success. Only in Experiment 727 was there any indication that this may have occurred. Supplemental vitamin E in the ration resulted in a greater gain response in the presence of arsanilic acid

than in its absence. This suggests that the arsenical may have had the effect of antagonizing the utilization of selenium and that vitamin E then substituted for the selenium in promoting the greater gain. This supposition, however, must be accepted with caution since no such observation was made in Experiment 734. In this trial two levels of arsanilic acid were fed in conjunction with selenium. Generally, a slight depression in gain was observed with arsanilic acid in the ration; however this reduction in gain was not alleviated with the addition of supplemental selenium. Arsenic compounds and arsenicals have been used by several researchers in attempts to reduce the effects of toxic levels of selenium in both monogastric and ruminant animals. Moxon et al. (1944) had observed that cattle on hay or pasture containing high levels of selenium gained faster when they were allowed access to sodium arsenite. Minyard et al. (1957, 1960) reported that cattle receiving seleniferous feeds gained faster and exhibited fewer symptoms of selenium toxicity when fed arsanilic acid. Similar results have not been observed in the research reported in this dissertation. The arsenicals may reinforce the natural detoxication mechanisms of the body to get rid of excess selenium but appear to have had little or no effect on the metabolism of ordinary levels of selenium.

Experiment 751 was designed to determine the influence of red kidney beans on selenium utilization. Kidney beans

are known to be low in selenium content and have been used in the production of muscular dystrophy in lambs, (Hogue et al., 1962 and Hintz and Hogue, 1964). Hogue (1958) has also reported that the kidney bean has a tocopherol inhibitory factor since the value of alpha tocopherol acetate in preventing exudative diathesis in chicks was reduced in the presence of kidney beans. No effect of the kidney beans in the ration of beef heifers upon selenium utilization was observed in the present studies since feedlot performance was unchanged with supplemental selenium. Since the kidney beans appeared to be unpalatable to the heifers, possibly the consumption of the beans was so low as to not allow a manifestation of any inhibitory factor or there may have been an ample supply of either selenium and/or vitamin E in the rest of the ration components. The kidney beans in the ration appeared to be very conducive to producing digestive disturbances in these heifers.

Two additional attempts to elicit a response from selenium supplementation were made in Experiment 748. One method was to include in the ration a source of unsaturated oil as soybean oil. The purpose of the oil was to place a stress upon the vitamin E needs of the animal in the anticipation of fulfilling a portion of this requirement with supplemental selenium. In the production of muscular dystrophy in calves and lambs, unsaturated fats, particularly fish liver oils,

have been used (Maplesden and Loosli, 1960; Welch et al., 1960 and Hogue et al., 1959). Addition of vitamin E and/or selenium to the rations either decreased or prevented the incidence of muscular dystrophy. Blaxter (1962) reported that the presence of unsaturated fats in the diet may increase the tocopherol requirement 10 to 100 times. Since soybean oil is known to be rich in tocopherols, it was necessary to destroy them. This was attempted by the method of Machlin (1961) by heating with lauryl peroxide. Machlin (1961) had found 90% destruction of the alpha tocopherols. When this treated source of unsaturated oil was fed in these studies, no effect upon the feedlot performance of the cattle was noted either in the presence or absence of 0.2 ppm supplemental selenium. Although no change in feedlot performance of cattle on the soybean oil rations was observed, it was noted at the time of slaughter that there was a higher level of blood tocopherols than in other treatment groups in this trial (Mukhtar, 1966). This suggests that there may have been some tocopherols in the oil which escaped destruction from the heat and peroxide treatment. Apparently the feedstuffs used in this trial were adequate in selenium and/or vitamin E content since no influence upon feedlot performance was observed with supplemental selenium in the ration.

Sulfur added to the rations used in Experiment 748 had

absolutely no influence upon the selenium requirement of these steers. In fact this provided the most interesting observation of these experiments since a response in feedlot performance due to the added sulfur was observed. It has been demonstrated by several workers that sulfur has an antagonistic effect upon selenium utilization at normal levels which led to the development of muscular dystrophy in lambs and calves. Muth et al. (1961) reported that addition of 0.053% sulfur as sulfate to ewe rations decreased the effectiveness of selenium in preventing muscular dystrophy in their lambs. Hintz and Hogue (1964) obtained similar results when a ration containing 0.33% sulfur was fed to ewes. Paulson et al. (1966) found an increased concentration of selenium in the small intestine of sheep when the ration contained 0.5% sulfur. This implied that sulfur interfered with selenium absorption. Other workers have observed an alleviation of selenium toxicity symptoms in rats with sulfate addition to the diet (Halverson and Monty, 1960; Ganther and Bauman, 1962 and Halverson et al., 1962).

The experiments in this research were originally undertaken to study the selenium requirement of feedlot cattle. This objective has not been realized for two reasons. First, all attempts to elicit a response to supplemental selenium in the rations failed. Secondly, laboratory analyses for selenium in biological materials was unsuccessful. A great

deal of time and effort was utilized in working on these analyses.

It has been shown in this research and that reported in the literature that the selenium requirement of ruminants is very low, probably 0.1 ppm or less. This has been done very capably in areas where livestock are susceptible to muscular dystrophy. Further developments in selenium nutrition of feedlot cattle will need to await the development of very sensitive analytical techniques for this elusive element. This is essential since it has been shown that the selenium content of forages varies considerably within the state of Iowa. Very possibly this same pattern exists in corn which makes up the major portion of the ration for feedlot cattle. Additional research will also need to be done to further understand the role of vitamin E and its interrelationship with selenium.

The addition of sulfur to the rations in Experiments 748 and 759 resulted in an increased gain and feed efficiency for a portion of the trial and then resulted in a depression in feedlot performance. The rations used in these trials contained a high urea protein supplemental mixture with urea supplying 20-25% of the protein equivalent in the former trial and approximately 29% in the latter. Since sulfur is an integral portion of the amino acids methionine and cystine, a source of sulfur is essential for the conversion of non-

protein nitrogen into amino acids and protein by the microorganisms of the rumen. However, it has generally been accepted that adequate sulfur is present in natural feedstuffs for maximum microbial utilization of non-protein nitrogen and animal growth.

The finding of a growth response from sulfur is not without precedence. This observation had generally been made with sheep fed purified or semi-purified rations which were low in sulfur and contained a non-protein nitrogen source. Thomas et al. (1951), Albert et al. (1955), Starks et al. (1953) and other workers have observed that lambs were in negative nitrogen and sulfur balance and lost weight without a supplemental source of sulfur in the ration. Improvement in performance with sulfur additions to natural rations has been less noticeable. Loosli and Harris (1945) and Lofgreen et al. (1953) observed no increase in gains of lambs with additions of sodium sulfate to natural rations. Methionine, a sulfur amino acid, addition to rations has resulted in improved performance with lambs (Garrigus et al., 1950; Starks et al., 1954; Albert et al., 1956; Trenkle, 1958 and Oltjen et al., 1962); but methionine likewise has had little or no effect on performance in other trials (Klosterman et al., 1951b; Noble et al., 1953, 1954 and Trenkle, 1958). Similar variations in response to inorganic sulfur have been observed with cattle. Perry et al. (1953), Kolari et al. (1964), Meiske et al.

(1966), Tolman and Woods (1966) and Hatfield et al. (1967) have not observed improved feedlot performance with sulfur while Gossett et al. (1962), Kolari et al. (1963) and Wolf (1967) have reported a gain stimulation with a sulfur source in the ration.

The improved feedlot performance observed in this research appears to be a true response since it has been observed in three trials: Experiment 748 and 759 and the trial conducted by Wolf (1967). The puzzling portion of this research, however, was the initial response from sulfur followed by the rather drastic depression during the latter portion of the trial. This phenomenon has not previously been reported in any of the trials conducted with sulfur. The total levels of sulfur fed in Experiment 748 were approximately 0.20% to 0.22% with the basal ration containing 0.09%. The sulfur levels in Experiment 759 were 0.09%, 0.16%, 0.23%, 0.38% and 0.23% for the control and the respective treatment graded levels of sulfur. These levels were not considered excessively high since Starks et al. (1954) and Albert et al. (1956) had observed improved performance of lambs with levels of sulfur up to 0.4% of the ration. These workers as well as Smith et al. (1964) reported a reduction in gain with sulfur above this level. This was further complicated by an observation made during the latter portion of Experiment 748. Two groups of steers

which had previously received 0.22% sulfur were placed on rations containing 0.30% sulfur and a stimulation in gain was observed in one instance while a depression was observed in the second.

That the gain response due to supplemental sulfur in these rations was a true gain may be further verified by the fact that dressing percent and carcass weights were generally greater when sulfur was added to the rations. This was true in both Experiment 748 and 759 which indicated that there was an actual increase in marketable carcass. No difference in carcass grade was observed. Smith et al. (1964) had observed that the carcasses from lambs fed sulfur appeared more youthful and graded higher. Other carcass characteristics did not appear to be influenced by the presence of sulfur. This was in agreement with observations made in the present research as well as those reported by Kolari et al. (1964).

An explanation for the initial response to sulfur followed by a depression in feedlot performance is difficult. It is suggested that the initial response in performance was due to the improved utilization of the non-protein nitrogen by the microorganisms in the presence of the sulfur. The two trials in this research were in progress for approximately 200 days. This was a longer feeding period that used in sheep experiments as well as the cattle trials reported in the literature. The growth response to sulfur in Experiments

748 and 759 were 127 and 83 days, respectively. Trials in the literature have been in progress for longer than this, yet no such depression has previously been reported. One possible contributing factor to the depression in performance may lie in the mineral nutrition of these animals. Goodrich (1965) has reported that six parts of corn and one part of urea supplies as much nitrogen as an equal amount of soybean meal but only 13.5% and 38.1% as much calcium and phosphorus. Thus, the need for mineral supplements may arise. The rations used in Experiments 748 and 759 contained 0.41% calcium and 0.29% phosphorus and 0.26% calcium and 0.29% phosphorus, respectively, which is adequate by National Research Council (1963) standards. The level of calcium in Experiment 748 was higher because of feeding soybean oil in a portion of the trial. Goodrich and Tillman (1966a, b) have reported that both urea and sulfur reduced the body retention of calcium and phosphorus while sulfate reduced plasma phosphorus. In light of the above, possibly the urea and sulfur fed in these two trials placed a stress upon the calcium and phosphorus balance of these animals leading to a marginal deficiency state which resulted in a depressed growth rate. Further circumstantial evidence for this theory is that a higher level of urea and lower level of calcium was fed in Experiment 759 than 748. The growth stimulation from sulfur disappeared earlier in the trial and declined more rapidly in

Experiment 759 lending support to this proposal. It is not suggested that this is the sole reason for the depression in gain and feed efficiency with added sulfur in the ration, but this observation indicates that a critical reevaluation in the area of mineral requirements is warranted when both sulfur and urea are included in cattle finishing rations.

In contrast to the two higher levels of sulfur which were fed in Experiment 759, the low level of elemental sulfur (0.015 pound per day or 0.16% sulfur in the ration) did not result in the initial gain stimulation followed by the depression. This level did, however, result in improved performance over the control diet and higher levels of sulfur for the entire feeding period.

Work with sheep has indicated that sulfur as the sulfate was more efficiently utilized than in the elemental form (Hale and Garrigus, 1953; Starks et al., 1954 and Albert et al., 1956). These observations do not agree completely with the present research. In Experiment 759, equivalent levels of sulfur in these two forms appeared to be nearly equal in the rate of improved feedlot performance which they promoted. Early in the trial, the sulfate appeared to be slightly better utilized; but during the latter portion of the trial, the elemental sulfur did not appear to depress performance as rapidly as did the sulfate as Glauber's salt.

Several workers have suggested that the blood serum

inorganic sulfate level of ruminants might be used to determine whether the addition of sulfur to the ration is warranted. Weir and Rendig (1954) and Rendig and Weir (1957) have observed that serum sulfate levels of sheep increase with increased sulfur in the rations. A similar observation has been made by Kroger and Carroll (1964) with cattle. The results obtained in these studies confirm the above reports. This was noted in both Experiment 759 and 124. In these trials, serum inorganic sulfate was nonsignificantly increased with supplemental sulfur in the ration but did not increase proportionately to the increased level of sulfur. This observation is supported by those of Rendig and Weir (1957) who suggested that this is particularly true at higher levels of sulfur intake. They further noted that there is a critical level of intake required to maintain a normal serum sulfate value. Serum inorganic sulfate values for cattle found in the literature range from approximately 3.5 to 10 milligrams per 100 milliliters. The serum sulfate values obtained in these studies fall in this rather broad range. A great deal of variation in the values of serum sulfate was observed between treatment levels of sulfur and among individuals on a given treatment. Part of this variation may be attributed to a low feed intake. Weir and Rendig (1954) reported that low intakes result in an initial drop in serum sulfate followed by a rise to a normal level or above, pre-

sumably as a result of the breakdown of body tissue. Other variation may be due to animal differences. Dale et al. (1954) have observed up to 300% variation in serum sulfate values of animals receiving the same ration. Lofgreen et al. (1953) and Whiting et al. (1954) have also observed that the serum sulfate levels do not necessarily reflect the level of dietary sulfur intake.

The results of Experiment 124 on the level of serum inorganic sulfate at various hours after feeding are difficult to explain. The increase in values up to 12 hours after feeding was unexpected and no reports could be found in the literature confirming or denying these observations. It appears that with two of the steers the serum sulfate levels had reached a plateau at 9 and 12 hours, but this was not true with the third steer. Anderson (1956) has shown that there is a linear disappearance of sulfate from the rumen liquor in a 10-hour period. Concurrently there is a rise in sulfide levels, peaking at 2 to 6 hours and then gradually declining. He reported that the sulfide which is not utilized by the microorganisms for protein synthesis is rapidly absorbed through the rumen epithelium. It is known that sulfide is oxidized to sulfate in the blood (Young and Maw, 1958). Thus, this mechanism might explain the rise in serum sulfate levels with time after feeding which was noted in this experiment.

Although these studies have shown that serum sulfate is increased with supplemental sulfur in the ration, it does not appear that this criterion could be used as a very sensitive detector of the sulfur adequacy of a ration.

Results obtained with the studies using the fistualted steers did not contribute to understanding the observations made in the feedlot studies with supplemental sulfur. It has been shown by numerous workers that sulfur in ruminant rations containing urea improves growth rate and nitrogen balance. It is known that urea is degraded to ammonia very rapidly in the rumen. This ammonia is then converted to microbial protein, presumably more efficiently if adequate sulfur is present. The results of the in vitro experiments in this research support the observations that a sulfur source is required for utilization of the ammonia produced from urea. However, the various levels of added sulfur did not greatly influence this ammonia utilization. Based on the results obtained with the fistualted steers, apparently there was adequate sulfur present in the natural feeds. Generally, there was no decrease in rumen ammonia levels with sulfur additions to the ration. An exception to this observation was noted with one steer when initially the addition of Glauber's salt resulted in a reduction of ammonia levels. However, later sampling did not confirm this. The situation was further confounded by noting that removing sulfur from

the ration appeared to further decrease ammonia levels in the rumen. Opposite results were obtained with the other animal used in these studies. Sulfur in the ration appeared to result in an increase in the level of ammonia. This was followed by a decline with a later increase when sulfur was removed from the ration. No supporting data could be found in the literature for any of these observations on ruminal ammonia levels. However, Kolesov et al. (1961) reported no change in rumen function of sheep fed elemental or sulfate sulfur but did note that blood urea was lower in sulfur supplemented animals. The preferred source and requirement of sulfur for the rumen microorganisms is not well known. Müller and Kramptiz (1955) have found that only bacteria incorporate sulfur and Emery et al. (1957a) reported that of ten different bacteria, five were able to use inorganic sulfate and only three used sulfate in the presence of organic sulfur. Thus, ammonia levels in the rumen may not be influenced greatly enough in the presence of supplemental sulfur to be used as an indicator for utilization of this mineral. It was thought that the level of ruminal ammonia over an extended period of time might be used as a measure of the need for sulfur in a urea supplemented diet.

Although a few reports have indicated that sulfur in the ration has an influence upon the bacterial population in the rumen, no conclusions concerning the effect of sulfur in the

ration upon viable bacterial numbers in these studies can be drawn. Bacterial numbers appeared to be reduced with the addition of either elemental sulfur or Glauber's salt to the ration. This was followed by an increase in numbers within a 2- to 3-week period. However, variation in bacterial numbers during the sulfur treatment periods does not allow one to conclude that the initial depression was a true effect. Possibly, the initial decrease in the bacteria may be due to an adjustment in numbers due to the dietary change. Williams and Moir (1951) suggest that 2 weeks are required for this change. Gall et al. (1951) reported that feeding sulfate sulfur in a purified ration containing urea doubled the bacterial population over that observed when sulfur was omitted. Whanger and Matrone (1965) observed a greater harvest of bacterial cells when sulfur was included in a purified ration for sheep. Williams and Moir (1951) reported that methionine in natural diets increased bacterial numbers. These observations conflict with the data obtained in the studies reported here but may be explained on the basis of ration composition. No natural sulfur was available in the purified diets and in the latter report the feedstuffs were of poor quality, probably low in sulfur. Since bacteria have been shown to have a preference for organic sulfur, there may have been no increase in numbers of bacteria if inorganic sulfur had been fed. The rations fed to the fistualted steers in the present

studies were apparently adequate in natural sulfur and prevented any manifestation of effect from added sulfur.

Since the ruminant receives a large percentage of its energy requirement from the volatile fatty acids, it was felt that the phenomenon with sulfur seen in the feedlot might be reflected in the volatile fatty acid pattern in the rumen. Gzhitskii *et al.* (1961) observed that sulfate added to a urea type ration increased the total volatile fatty acids; a predominance of propionic over butyric acid was observed with no change in acetic acid. Whanger and Matrone (1966) found that total volatile fatty acids were increased three-fold and that the proportions of butyrate and higher acids were increased with the addition of sulfur to purified rations. In the present study, the addition of sulfur to an apparent sulfur adequate diet had very little influence upon the concentration of total volatile fatty acids found in the rumen. This observation was also made in the *in vitro* studies, however, there was a large increase in the total acids when sulfur was added to the sulfur-free incubation media. Although variation between the fistulated steers used in these studies was observed, sulfur additions generally resulted in an increase in the molar percent of acetate and propionate with a decrease or no change in butyrate. The acetate to propionate ratio appeared to be abnormally high in these studies (over 3:1) and was not significantly influenced by the addition of

sulfur although it did tend to be narrowed. No explanation for the wide acetate to propionate ratio can be given since these steers were on a ration which contained 70% corn.

The most consistent observation on the volatile fatty acid pattern was a reduction in the molar percent of butyrate. This reduction was noted with the fistulated steers as well as in the in vitro trials with the various levels of added sulfur. This is in contrast to the observations of Whanger and Matrone (1966) who observed an increase in butyrate production with sulfur additions to purified rations. They suggested that microorganisms existing under sulfur-free conditions do not have the necessary enzyme system to incorporate acetate into butyrate. In the present studies, the rations of the fistulated steers were not sulfur-free but the in vitro incubation media were.

In all observations of the volatile fatty acid patterns with the fistulated steers, there appeared to be a trend in increased propionate and decreased acetate production following addition of sulfur to the ration. This was noted at 20 to 30 days but was usually not observed at subsequent periods of sampling. This observation of a narrowed acetate to propionate ratio corresponds with the initial stimulation of gain observed in the feedlot with added sulfur, however, the subsequent less favorable fatty acid ratio does not. Oltjen and Davis (1965) have observed that the ratios of

volatile fatty acids may be altered with little or no effect on gain and feed efficiency being apparent.

The results obtained with the effect of sulfur upon in vitro fermentation by bacteria were in agreement with observations made by other workers. A very definite sulfur requirement in the in vitro medium for starch digestion was observed. Hunt et al. (1954), Burroughs et al. (1951) and Hubbert et al. (1958) have previously reported that sulfur is required in the in vitro medium for maximum cellulose digestion. Martin et al. (1964) observed a 95% decrease in cellulose digestion in the absence of sulfur in the medium. The present studies confirmed the work of Hubbert et al. (1958) that the microorganisms of the rumen are quite tolerant of high levels of sulfur. Little effect upon starch digestion was noted with sulfur in the medium at a level to give a S:N ratio of 1:4 as compared with the other treatments. The results from the feedlot work had indicated that sulfur at approximately this ratio gave the greatest growth response but also resulted in the most drastic depression. This suggests that the in vitro techniques are not sensitive enough or do not simulate the actual rumen condition closely enough to be of great value in studying the effect of minerals in ruminant nutrition.

The observations made in these studies have not resulted in an explanation for the initial stimulation and subsequent

depression in feedlot performance with supplemental sulfur in the ration. No observations concerning the influence of sulfur upon the protozoa population of the rumen have been made. It has been shown previously that protozoa are required for optimum performance of lambs. Therefore, the possibility exists that the effect from sulfur in these studies may have been on the protozoa. Very little is known about the mineral requirements of protozoa. Müller and Krampitz (1955) reported that radioactive sulfate was incorporated into bacteria but not into protozoa. The work of Williams and Moir (1951) indicated that methionine in the ration greatly increased the bacterial numbers but protozoa numbers were not influenced. Although these are limited observations, they would suggest that the sulfur effect in the present studies was not on the protozoa. Christiansen (1963) has observed with lambs that high concentrate ground rations and high levels of feed intake tend to decrease the protozoal population of the rumen. Since there was an increase in feed consumption with time in the trials reported here, it is likely that there was a depletion of the protozoal population in these animals. However, it appears unlikely that a reduction in protozoa numbers would account for the drastic depression in performance which was observed since there was also increased feed intake with the control and low sulfur rations. Wolf (1967) has reported improved

feedlot performance with sulfur in the ration. In his research, the response to sulfur was evident for at least 67 days following a feeding trial of 196 days. Based upon Christiansen's proposal, it is likely that the protozoal population at this time would have been very greatly reduced or eliminated from the rumen. Thus, it is unlikely that the sulfur in the ration could have stimulated the protozoa to such an extent to produce the stimulation in performance which was reported by Wolf (1967).

The presence of protozoa in the rumen has been reported to increase ammonia levels and the molar percent of butyrate (Christiansen et al., 1965; Luther et al., 1966 and Yoder et al., 1966). Some observations made in the studies reported here indicated that there was an increase in ammonia levels when sulfur was included in the ration; however the increase was not nearly great enough to be attributed to stimulation of protozoa and the increase in ammonia was not always observed. In these studies, the molar percent of butyrate was either not changed or decreased when sulfur was included in the ration. In the in vitro experiment, butyrate was decreased with increasing levels of sulfur in the medium. All of these observations suggest that the presence of sulfur in the ration was not having a stimulatory influence upon the protozoal population which resulted in the improved feedlot performance which was observed.

In concluding this discussion on sulfur, it can be pointed out that a great deal more work should be done with sulfur additions to cattle finishing rations. It has been shown in the feedlot that the sulfur requirement for maximum performance of finishing cattle may be in the range of 0.15% to 0.20% or that a sulfur to nitrogen ratio of 1:12 to 1:8 is desirable. This research has further suggested that a critical reevaluation of the mineral requirements is in order when high levels of urea and sulfur are included in the ration. No conclusive effects of sulfur upon the end products of rumen fermentation or microbial numbers have been observed. It is suggested that further trials be conducted utilizing more animals to more critically evaluate these factors in an attempt to further understand the phenomenon of the changing pattern of feedlot performance when high levels of sulfur are included in the ration.

# SUMMARY

A series of four experiments was conducted with beef cattle to determine the need for selenium in finishing rations. Because of a suspected interrelationship between selenium and vitamin E, the rations fed in this research were formulated to contain as low a content of vitamin E as was possible.

Arsanilic acid which has been used in alleviating the toxicity of selenium in seleniferous areas of the country was fed in two of these experiments in an attempt to place a stress upon the selenium nutrition of these animals. The addition of arsanilic acid appeared to depress feedlot performance. The depressed performance appeared to be partially alleviated by the addition of 200 I.U. of vitamin E per animal per day. This suggested that a portion of the selenium need may have been replaced by vitamin E. In a second trial, the presence of arsanilic acid did not appear to have any influence upon the selenium need of the animals. No response in the feedlot performance of these cattle was observed when selenium was added to the ration at the rate of 0.1 ppm.

In another attempt to elicit a response from 0.1 ppm selenium, cull red kidney beans were fed in the ration of beef heifers. Kidney beans are low in selenium and thought to contain a tocopherol inhibitory factor. Apparently no

stress was placed upon the need for selenium since its addition to the ration did not improve feedlot performance.

The addition of sulfur, an antagonist of selenium, to the ration of cattle did not appear to interfere with the utilization of the natural occurring selenium of the feedstuffs or 0.2 ppm selenium. A combination of sulfur and soybean oil did not appear to increase the selenium and/or vitamin E need of these cattle since feedlot performance was not improved with supplemental selenium.

The addition of a source of sulfur to cattle finishing rations in which urea contributed all of the supplemental protein equivalent improved feedlot performance for 127 and 83 days. During the latter portion of these trials, supplemental sulfur in excess of approximately 0.15% of the ration resulted in a depression of performance. Sulfur in the ration at less than 0.15% improved performance for the entire feeding trial. Even though supplemental sulfur did not always improve feedlot performance for the total feeding period, dressing percent and carcass weights were improved with no changed being noted in carcass grades.

Serum inorganic sulfate levels appeared to be increased with supplemental sulfur in the ration but were not greatly different among sulfur treatments. Serum sulfate levels increased up to 9 to 12 hours after feeding but were greater when sulfur was in the ration.

The effect of sulfur upon ruminal ammonia in trials with fistulated steers was variable. Generally there was no change in ammonia levels which could be attributed to the experimental treatments. In vitro trials indicated that ammonia levels were reduced in the incubation tubes with added sulfur, but there was little difference when various levels of sulfur were added.

Rumen bacterial numbers appeared to be decreased when sulfur was added to the ration but variation in numbers throughout the trials suggest that this may not have been due to the experimental treatments.

The presence of sulfur in cattle rations had little influence upon the total concentration of rumen volatile fatty acids. In vitro studies indicated that the total concentration of volatile fatty acids greatly increased when sulfur was included in the incubation medium, but little change was noted with different levels of sulfur. Although variation was noted, sulfur additions to cattle rations resulted in an increase in molar percent of acetate and propionate and a decrease or no change in butyrate. The most consistent observation was a decrease in butyrate, noted both with the fistulated steers and in in vitro trials. There appeared to be a trend in narrowing of the acetate to propionate ratio up to 20 to 30 days after the addition of sulfur to cattle rations but this was usually not observed at subsequent periods of sampling.

The addition of sulfur to a sulfur-free in vitro

incubation medium resulted in increased starch digestion, but no difference was observed among the various levels of added sulfur.

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# APPENDIX

Table 32. Experiment 727 (112 day experiment): Analysis of variance of average daily gain and feed per 100 pounds gain

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Av. daily gain | Feed/100 lb. gain |
| Total | 11 | 0.0407 | 1385 |
| Replication | 2 | 0.0139 | 3085 |
| Treatment | 3 | 0.0294 | 1245 |
| Arsanilic acid | 1 | 0.0352 | 2028 |
| Vitamin E | 1 | 0.0469 | 1365 |
| Interaction | 1 | 0.0060 | 342 |
| Error | 6 | 0.0554 | 889 |

Table 33. Experiment 727 (202 day experiment): Analysis of variance of average daily gain and feed per 100 pounds gain

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Av. daily gain | Feed/100 lb. gain |
| Total | 11 | 0.0158 | 1569 |
| Treatment | 1 | 0.0127 | 752 |
| Error | 10 | 0.0161 | 1650 |

Table 34. Experiment 734: Analysis of variance of average daily gain and feed per 100 pounds gain

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Av. daily gain | Feed/100 lb. gain |
| Total | 11 | 0.0207 | 1418 |
| Replication | 1 | 0.0617 | 9075 |
| Treatment | 5 | 0.0022 | 556 |
| Selenium | 1 | 0.0002 | 6 |
| Arsanilic acid | 2 | 0.0031 | 431 |
| Interaction | 2 | 0.0023 | 676 |
| Error | 5 | 0.0311 | 860 |

Table 35. Experiment 748 (127 day experiment - light and heavy weight cattle): Analysis of variance of average daily gain and feed per 100 pounds gain

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Av. daily gain | Feed/100 lb. gain |
| Total | 11 | 0.0152 | 1401 |
| Replication | 1 | 0.0690 | 13267 |
| Treatment | 5 | 0.0155 | 303 |
| Selenium | 1 | 0.0007 | 397 |
| Glauber's salt | 2 | 0.0380[a] | 511 |
| Interaction | 2 | 0.0006 | 46 |
| Error | 5 | 0.0040 | 125 |

[a]Significant at $P < 0.05$.

Table 36. Experiment 748 (127 day experiment - light weight cattle): Analysis of variance of average daily gain and feed per 100 pounds gain

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Av. daily gain | Feed/100 lb. gain |
| Total | 5 | 0.0139 | 178 |
| Treatment[a] | 2 | 0.0305[b] | 245 |
| A vs. B | 1 | 0.0400[b] | -- |
| B vs. C | 1 | 0.0507[b] | -- |
| Error | 3 | 0.0029 | 134 |

[a]A - control, B - 0.33 lb. Glauber's salt per day and C - 0.33 lb. Glauber's salt per day plus soybean oil.

[b]Significant at $P < 0.05$.

Table 37. Experiment 748 (82 day experiment - light weight cattle): Analysis of variance of average daily gain and feed per 100 pounds gain

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Av. daily gain | Feed/100 lb. gain |
| Total | 5 | 0.0874 | 9096 |
| Treatment[a] | 2 | 0.1504 | 20055[b] |
| A vs. B | 1 | 0.1057 | 13225 |
| B vs. C | 1 | 0.2971 | 39801[b] |
| Error | 3 | 0.0454 | 1790 |

[a]A- control, B - 0.33 lb. Glauber's salt per day and C - 0.67 lb. Glauber's salt per day.

[b]Significant at $P < 0.05$.

Table 38. Experiment 748 (127 day experiment - heavy weight cattle): Analysis of variance of average daily gain and feed per 100 pounds gain

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Av. daily gain | Feed/100 lb. gain |
| Total | 5 | 0.0057 | 40297 |
| Treatment | 2 | 0.0109 | 331 |
| Error | 3 | 0.0022 | 66941 |

Table 39. Experiment 748 (53 day experiment - heavy weight cattle): Analysis of variance of average daily gain and feed per 100 pounds gain

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Av. daily gain | Feed/100 lb. gain |
| Total | 5 | 0.0188 | 1735 |
| Treatment | 2 | 0.0232 | 1016 |
| Error | 3 | 0.0159 | 2215 |

Table 40. Experiment 759: Analysis of variance of average daily gain and serum inorganic sulfate

| Source | d.f. | Mean squares | | |
|---|---|---|---|---|
| | | Av. daily gain (83 days) | Av. daily gain (133 days) | Serum inorganic sulfate (216 days) |
| Total | 75 | 0.2715 | 0.3309 | 2.4387 |
| Replication | 2 | 1.5511 | 3.5754 | 0.3860 |
| Treatment | 4 | 0.2327 | 0.3476 | 10.3667 |
| Error | 69 | 0.2366 | 0.2359 | 2.0386 |
| Replication x treatment | 8 | 0.7740 | 1.4757 | 7.3706 |
| Sampling error | 61 | 0.1662 | 0.0733 | 1.3393 |

Table 41. Experiment 759: Analysis of variance of feed per 100 pounds of gain

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Feed/100 lb. gain (83 days) | Feed/100 lb. gain (133 days) |
| Total | 14 | 2405 | 16227 |
| Replication | 2 | 7887 | 57962 |
| Treatment | 4 | 1802 | 12016 |
| Error | 8 | 1335 | 11029 |

Table 42. Analysis of variance of serum inorganic sulfate levels of steers fed various amounts of elemental sulfur

| Source | d.f. | Mean square |
|---|---|---|
| Total | 15 | 8.2317 |
| Period | 3 | 10.8961 |
| Steer | 3 | 12.9432 |
| Treatment | 3 | 4.5453 |
| Error | 6 | 6.3871 |

Table 43. Analysis of variance of serum inorganic sulfate values at various times after feeding

| Source | d.f. | Mean square |
|---|---|---|
| Total | 29 | 3.9897 |
| Steer | 2 | 34.8109 |
| Treatment | 9 | 3.2583 |
| Sulfur | 1 | 2.4425[a] |
| Time | 4 | 6.5608[a] |
| Interaction | 4 | 0.1597 |
| Error | 18 | 0.9308 |

[a]Significant at $P < 0.01$.

Table 44. Analysis of variance of ruminal ammonia in Steer S fed a urea type ration supplemented with Glauber's salt

| Source | d.f. | Mean squares | | | |
|---|---|---|---|---|---|
| | | 1 hr. | 2 hr. | 3 hr. | 4 hr. |
| Total | 17 | 66.89 | 31.09 | 13.75 | 6.92 |
| Replication | 1 | 3.46 | 9.96 | 5.60 | 0.20 |
| Period | 8 | 133.45[a] | 62.85[a] | 24.66[a] | 12.27[b] |
| Error | 8 | 8.25 | 1.98 | 3.86 | 2.41 |

[a]Significant at $P < 0.01$.

[b]Significant at $P < 0.05$.

Table 45. Analysis of variance of ruminal ammonia in Steer S fed a urea type ration supplemented with elemental sulfur

| Source | d.f. | Mean squares | | | |
|---|---|---|---|---|---|
| | | 1 hr. | 2 hr. | 3 hr. | 4 hr. |
| Total | 19 | 44.91 | 20.97 | 14.35 | 3.61 |
| Replication | 1 | 9.22[a] | 2.59[a] | 5.49[a] | 0.89[a] |
| Period | 9 | 88.95[a] | 41.73[a] | 27.39[a] | 6.78[a] |
| Error | 9 | 4.84 | 2.25 | 2.29 | 0.74 |

[a]Significant at $P < 0.01$.

Table 46. Analysis of variance of ruminal ammonia in Steer S fed a urea type ration (Glauber's salt vs. elemental sulfur)

| Source | d.f. | Mean squares | | | |
|---|---|---|---|---|---|
| | | 1 hr. | 2 hr. | 3 hr. | 4 hr. |
| Total | 12 | 33.69 | 19.42 | 11.33[b] | 5.29 |
| Treatment | 1 | 258.28[a] | 169.53[a] | 46.76[b] | 3.78 |
| Error | 11 | 13.27 | 63.56 | 8.11 | 5.43 |

[a]Significant at $P < 0.01$.

[b]Significant at $P < 0.05$.

Table 47. Analysis of variance of ruminal ammonia in Steer B fed a urea type ration

| Source | d.f. | Mean squares | | | |
|---|---|---|---|---|---|
| | | 1 hr. | 2 hr. | 3 hr. | 4 hr. |
| Total | 15 | 11.92 | 18.53 | 25.13 | 12.16 |
| Replication | 1 | 0.18 | 9.57[a] | 15.34[a] | 7.03[b] |
| Period | 7 | 15.30 | 30.60[a] | 42.45[a] | 23.05[b] |
| Error | 7 | 10.21 | 7.74 | 9.20 | 2.00 |

[a]Significant at $P < 0.05$.

[b]Significant at $P < 0.01$.

Table 48. Analysis of variance of ruminal ammonia in Steer B fed a urea type ration supplemented with Glauber's salt

| Source | d.f. | Mean squares | | | |
|---|---|---|---|---|---|
| | | 1 hr. | 2 hr. | 3 hr. | 4 hr. |
| Total | 15 | 21.95 | 8.81 | 7.00 | 3.00 |
| Replication | 1 | 1.33[a] | 0.01[a] | 0.28[a] | 0.39[a] |
| Period | 7 | 46.11[a] | 18.79[a] | 14.57[a] | 6.29[a] |
| Error | 7 | 0.73 | 0.10 | 0.40 | 0.08 |

[a]Significant at $P < 0.01$.

Table 49. Analysis of variance of volatile fatty acid production in Steer S fed a urea type ration supplemented with Glauber's salt and elemental sulfur

| Source | d.f. | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $C_2/C_3$ | $C_2$ | $C_3$ | $iC_4$ | $C_4$ | $iC_5$ | $C_5$ |
| Total | 61 | 0.3002 | 6.8381 | 7.3824 | 0.0300 | 2.2197 | 0.1522 | 0.0430 |
| Treatment | 20 | 0.1307 | 4.0771 | 3.7528 | 0.0514 | 1.7116 | 0.2114 | 0.0421 |
| Supplement[a] | 2 | 0.6757 | 33.3842 | 20.5864 | 0.0962 | 7.5561 | 0.9034 | 0.0847 |
| A vs. B | 1 | -- | 26.7672 | -- | -- | -- | 0.6821[b] | -- |
| A vs. C | 1 | -- | 7.7400 | -- | 0.0914[b] | 8.6296 | -- | -- |
| B vs. C | 1 | 1.1867 | 64.8772[b] | 30.3790 | 0.1814[c] | 13.4301[b] | 1.7692[c] | -- |
| Time | 6 | 0.1755 | 1.8098 | 4.2022 | 0.1260[c] | 1.6234 | 0.3638[b] | 0.0572 |
| Interaction | 12 | 0.0175 | 0.3262 | 0.7225 | 0.0066 | 0.7817 | 0.0199 | 0.0200 |
| Error | 41 | 0.3829 | 8.1850 | 9.1529 | 0.0196 | 2.4675 | 0.1234 | 0.0433 |

[a]A - control, B - Glauber's salt and C - elemental sulfur.

[b]Significant at $P < 0.05$.

[c]Significant at $P < 0.01$.

Table 50. Analysis of variance of volatile fatty acid production in Steer B fed a urea type ration supplemented with Glauber's salt

| Source | d.f. | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $C_2/C_3$ | $C_2$ | $C_3$ | $iC_4$ | $C_4$ | $iC_5$ | $C_5$ |
| Total | 60 | 0.1914 | 2.1642 | 5.6424 | 0.0099 | 3.6501 | 0.1018 | 0.0427 |
| Treatment | 13 | 0.0343 | 2.1472 | 1.5433 | 0.0247 | 2.6170 | 0.2994 | 0.0215 |
| Glauber's salt | 1 | 0.1958 | 21.0573[a] | 12.5085 | 0.1733[a] | 30.3475[a] | 3.2231[a] | 0.1602 |
| Time | 6 | 0.0244 | 0.7872 | 0.8103 | 0.0221[a] | 0.5491 | 0.0977 | 0.0150 |
| Interaction | 6 | 0.0174 | 0.3555 | 0.4488 | 0.0026 | 0.0631 | 0.0139 | 0.0048 |
| Error | 47 | 0.2348 | 2.1689 | 6.7762 | 0.0058 | 3.9358 | 0.0471 | 0.0486 |

[a]Significant at $P < 0.01$.

Table 51. Analysis of variance of the effect of Glauber's salt upon viable bacterial numbers in the rumen of Steer S

| Source | d.f. | Mean square |
|---|---|---|
| Total | 39 | 66.58 |
| Replication | 4 | 57.35 |
| Period | 7 | 211.59[a] |
| Error | 28 | 31.65 |

[a]Significant at $P < 0.01$.

Table 52. Analysis of variance of the effect of elemental sulfur upon viable bacterial numbers in the rumen of Steer S

| Source | d.f. | Mean square |
|---|---|---|
| Total | 49 | 63.91 |
| Replication | 4 | 38.03 |
| Period | 9 | 266.04[a] |
| Error | 36 | 16.25 |

[a]Significant at $P < 0.01$.

Table 53. Analysis of variance of the effect of a control and Glauber's salt ration upon viable bacterial numbers in the rumen of Steer B

| Source | d.f. | Mean squares | |
|---|---|---|---|
| | | Control | Glauber's salt |
| Total | 39 | 141.27 | 68.70 |
| Replication | 4 | 52.78 | 26.08 |
| Period | 7 | 552.11[a] | 298.34[a] |
| Error | 28 | 51.13 | 17.38 |

[a]Significant at $P < 0.01$.

Table 54. Analysis of variance of the effect of various sulfur to nitrogen (S:N) ratios upon *in vitro* starch digestion (Trials 1, 2, 3 and 4)

| Source | d.f. | Mean squares | | | |
|---|---|---|---|---|---|
| | | Trial 1 | Trial 2 | Trial 3 | Trial 4 |
| Total | 14 | 275.94 | 73.63 | 343.59 | 554.06 |
| Replication | 2 | 3.10 | 1.95 | 13.80 | 18.57 |
| Treatment | 4 | 959.15[a] | 252.15[a] | 1187.19[a] | 1874.93[a] |
| Error | 8 | 2.55 | 2.29 | 4.24 | 27.50 |

[a]Significant at $P < 0.01$.

Table 55. Analysis of variance of the effect of various sulfur to nitrogen (S:N) ratios upon *in vitro* starch digestion (Combined trials)

| Source | d.f. | Mean square |
|---|---|---|
| Total | 19 | 336.19 |
| Trials | 3 | 231.25 |
| Treatment | 4 | 1288.76[a] |
| Error | 12 | 44.90 |

[a]Significant at $P < 0.01$.

Table 56. Analysis of variance of the effect of various sulfur to nitrogen (S:N) ratios upon ammonia levels after *in vitro* starch digestion (Trials 1, 2 and 3)

| Source | d.f. | Mean squares | | |
|---|---|---|---|---|
| | | Trial 1 | Trial 2 | Trial 3 |
| Total | 9 | 12.80 | 10.68 | 30.64 |
| Replication | 1 | 0.78 | 5.83 | 0.28 |
| Treatment | 4 | 26.74[a] | 20.97[a] | 68.87[b] |
| Error | 4 | 1.85 | 1.59 | 0.02 |

[a]Significant at $P < 0.05$.

[b]Significant at $P < 0.01$.

Table 57. Analysis of variance of the effect of various sulfur to nitrogen (S:N) ratios upon ammonia levels after *in vitro* starch digestion (combined trials)

| Source | d.f. | Mean square |
|---|---|---|
| Total | 14 | 16.83 |
| Trials | 2 | 1.29 |
| Treatment | 4 | 53.04[a] |
| Error | 8 | 2.25 |

[a]Significant at $P < 0.01$.

Table 58. Analysis of variance of the effect of various sulfur to nitrogen (S:N) ratios upon volatile fatty acid levels after *in vitro* starch digestion

| Source | d.f. | Mean squares | | | |
|---|---|---|---|---|---|
| | | μM V.F.A. per ml. | $C_2$ | $C_3$ | $C_4$ |
| Total | 19 | 86.96 | 14.95 | 18.39 | 1.48 |
| Trial | 3 | 164.64 | 80.25 | 93.74 | 1.65 |
| Treatment | 4 | 263.06[a] | 0.60 | 3.13 | 5.12[a] |
| Error | 12 | 8.84 | 3.41 | 4.64 | 0.22 |

[a]Significant at $P < 0.01$.